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SELECTIVE INNERVATION OF TYPES OF FIBRES IN DEVELOPING RAT MUSCLE

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SUMMARY

The technique of glycogen depletion has been used to identify the types of muscle fibres innervated by individual motor neurones in the neonatal rat. This analysis shows that neonatal motor units are highly biased in their fibre type composition, even at times when the fibres receive extensive polyneuronal innervation. This finding suggests that the innervation of muscle fibres is somehow sorted according to type during early development. This sorting does not appear to occur during the removal of the polyneuronal innervation because little, if any, increase in the bias of unit compositions occurs as the number of synapses present in the muscle is reduced 2- to 3-fold. To determine whether the sorted innervation might be explained by a selective synaptogenesis, a study was made of the type compositions of units formed by reinnervation of neonatal soleus muscle. Glycogen depletion of single units 2 weeks following crush of the soleus nerve at postnatal day 2 showed that most of them (10/12) had biased type compositions which could not be explained by a random reinnervation. The location of fibres in the reinnervated motor units suggests that the regenerating axons innervated a novel set of fibres. The differentiation of fibres into types was apparently not changed during their reinnervation. These results imply that regenerating motor neurones in the neonatal rat selectively reinnervate muscle fibre types. These and other studies further imply that the organization of fibres into motor units during normal development does not occur, as is widely believed, by a random innervation of naive fibres and their subsequent differentiation under the influence of innervation.

INTRODUCTION

Most adult skeletal muscles contain a mixture of muscle fibres of several differentiated 'types' (Burke, 1981). Because of differences in their contractile proteins, as well as in their metabolic machinery producing energy for contraction, these fibre types differ in their speed of contraction, the force they generate per cross-sectional area, and the length of time they can maintain force production. These different types of muscle fibres are organized into separate motor units. That is, the set of muscle fibres innervated by any single motor neurone are of the same type. Thus, the central nervous system, by activating particular motor neurones, can

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generate contractions in skeletal muscles which are appropriate for different motor tasks.

For a number of years the developmental mechanisms leading to this fibre type homogeneity of motor units were believed to be understood. Beginning with the experiments of Buller, Eccles & Eccles (1960*b*), it was shown that differences in the contractions of so-called 'fast' and 'slow' muscles (i.e. muscles composed of predominantly fast-twitch or slow-twitch fibres) depended upon their innervation. If the innervation to these two types of muscles was interchanged, by cutting and cross-uniting their nerves, then each 'cross-innervated' muscle assumed the contractile characteristics of the muscle previously innervated by that nerve (i.e. the fast muscle became slower and slow muscle became faster). These early experiments further implied that both fast and slow embryonic muscles began development with the same contractile properties (Buller *et al.* 1960*a*) and that these muscles diverged only gradually under the influence of their respective nerves (Brown, 1973). Further experimentation showed that most if not all of the influence exerted by a motor axon on the muscle fibres it innervated was due to the pattern of nerve impulses it conveyed to these fibres. For example, it is possible to make a fast muscle slower by implanting electrodes around the muscle nerve and stimulating this muscle with a pattern of activity resembling that of the nerve supplying a slow muscle [i.e. low-frequency stimulation (approx. 10 Hz) maintained tonically] (Salmons & Vrbová, 1969). Perhaps even more convincing were experiments in which a denervated slow muscle was stimulated directly, with an activity pattern resembling that of a nerve supplying a fast muscle (100-Hz stimuli administered phasically) for, under these circumstances, the previously slow muscle was shown to develop faster contractions (Lømo, Westgaard & Dahl, 1974). These results led to the generally held belief that the fibre type homogeneity of motor units is due to the activity imposed from a common motor neurone upon all the fibres in each unit. During development, motor neurones were believed to innervate an undifferentiated population of fibres and subsequently impose an appropriate differentiation on each fibre (see Gauthier, Lowey, Benfield & Hobbs, 1982; Vrbová, Gordon & Jones, 1978). Studies of the reinnervation of muscles supported this contention, showing that adult motor neurones display no apparent preference for reinnervating the same types of fibres which they had previously innervated. Rather, individual motor neurones were seen to reinnervate contiguous groups of fibres within the muscle (Kugelberg, Edstrom & Abbruzzese, 1970). These groups of fibres were then converted to the same type; consequently, the mosaic arrangement of types in normal muscles was replaced with a 'fibre type grouping'.

INNERVATION OF FIBRE TYPES IN DEVELOPING MUSCLES

Recent experimentation has shown that the above view of the initial innervation and differentiation of muscle fibres into the various types is far too simple. With the development of sensitive methods for the histochemical demonstration of the

different types of fibres, it was possible to demonstrate the presence of these types in neonatal muscles in advance of the expected times of differentiation (Brooke, Williamson & Kaiser, 1971; Riley, 1977). With the advent of antibodies directed against isoforms of the muscle contractile proteins and their use in immunohistochemistry, it was possible to demonstrate that the initial differentiation of fibres occurs very early in foetal development, beginning in the rodent a day or two after the first synapses are formed (Dhoot, 1985; Rubinstein & Kelly, 1981). While a neural determination of this early differentiation is still possible, the innervation of foetal and neonatal muscles is quite different from that in the adult animal and this difference makes such neural determination difficult to achieve. Although each adult muscle fibre is singly innervated, foetal and early neonatal muscle fibres receive a polyneuronal innervation (Van Essen, 1982). The adult, single innervation of each fibre is achieved only some days after birth (in the rodent at about 2 weeks of age) following a period in which most of the synaptic contacts initially established in the muscle are removed. Studies by Jansen and his collaborators (Brown, Jansen & Van Essen, 1976) have established that this postnatal 'synapse elimination' results from a reduction in the number of muscle fibres innervated by each motor neurone supplying the muscle (i.e. it involves a reduction in the size of motor units). Apparently, the initial polyneuronal innervation results from an initial overexuberance of the motor neurones in forming synapses in the muscle. If these overexuberant motor neurones were randomly to innervate the fibres in a muscle, then most fibres would be expected to receive convergent innervation from different types of motor neurones. This convergent innervation would considerably complicate any mechanism for the initial differentiation of these fibres on the basis of instructions from their innervation.

The polyneuronal innervation of neonatal muscles which contain differentiated fibre types raises the issue of how these early fibre types are innervated. One might imagine two possibilities. First, each motor neurone might innervate a mixed population of fibres. The type homogeneity of motor units might then emerge as a consequence of a selective synapse elimination (i.e. by the elimination of innervation placed on inappropriate fibres). Alternatively, the innervation of different types of fibres might be selective from the outset and, despite the polyneuronal innervation, each motor neurone could still confine its innervation to one type. To resolve this issue, a study of the innervation of neonatal fibres was conducted using the rat soleus muscle (Thompson, Sutton & Riley, 1984). The neonatal rat soleus contains two different types of fibres which can be easily distinguished at the beginning of the second week of life by myofibrillar ATPase histochemistry (Riley, 1977). [Using antibodies it is possible to recognize fibre types even earlier in the development of this muscle (see Rubinstein & Kelly, 1981; Dhoot, 1985).] At postnatal day 8, about 55 % of the fibres present in the muscle are 'slow' and 45 % of the fibres are 'fast' (Figs 1, 2). At 8 days each muscle fibre receives synaptic input from two or more motor neurones (Brown *et al.* 1976). About a week later in development, at day 16–17, this polyneuronal innervation has almost completely disappeared from the muscle; nonetheless, there has been no apparent change in the numbers of slow and

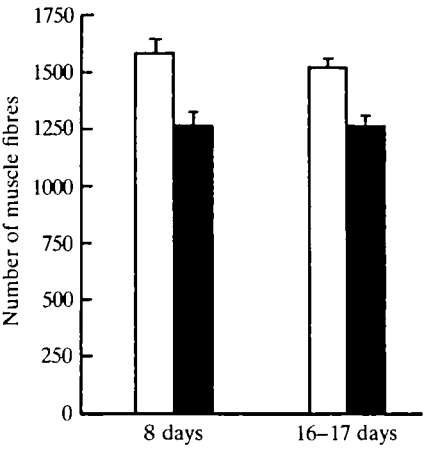

Fig. 1. Fibre type composition of neonatal rat soleus muscle at 8 days ($N = 5$) and 16–17 days ($N = 9$) after birth. Despite the dramatic change in the innervation of the muscle fibres between these two times, muscles at both ages contain approximately the same numbers of slow (open columns) and fast (filled columns) fibres. The types of fibres were determined from muscle cryostat sections reacted for myofibrillar ATPase staining after alkaline preincubation at pH 10·4. Vertical bars show S.E.M. (From Thompson, Sutton & Riley, 1984.)

fast fibres (Fig. 1). To ascertain how individual motor neurones distribute their terminals to the two fibre types present in this muscle, the technique of glycogen depletion developed by Kugelberg (1976) was employed to mark the fibres in the muscle innervated by a single motor neurone. Briefly, the ventral roots were teased into fine filaments until one was isolated which contained only a single motor axon to the soleus muscle. This axon was then stimulated repetitively under conditions of anoxia so that the fibres activated by this stimulation were depleted of their glycogen. Serial sections were made of each such muscle. One section was stained for glycogen, using the periodic acid/Schiff's reagent (PAS) procedure, and an adjacent section was stained with the myofibrillar ATPase procedure to identify the two types of fibres. The same fibres could be recognized in adjacent sections. By comparison of the two sections, the type identity of each fibre lacking stain for glycogen could be determined (Fig. 2). In this manner, it was possible to determine the composition of a number of units at 8 days, during the period of polyneuronal innervation, and at 16–17 days, by which time most of the fibres are singly innervated. These procedures revealed that at each age, motor units were composed predominantly of either slow fibres or fast fibres (Fig. 3). At 8 days, each unit contained 2–3 times the number of fibres it contained at 16–17 days, showing anatomically what had previously been deduced from the amplitudes of the contractions of single units: that during synapse elimination there is a reduction in the number of fibres innervated by each motor neurone. At neither age were the motor units completely homogeneous. At both ages, the slow motor units contained about 20–30 % fast fibres, and the fast motor units contained less than 10 % slow fibres. Thus, the level of precision of innervation of the fibre types did not seem to be markedly improved by the synapse elimination

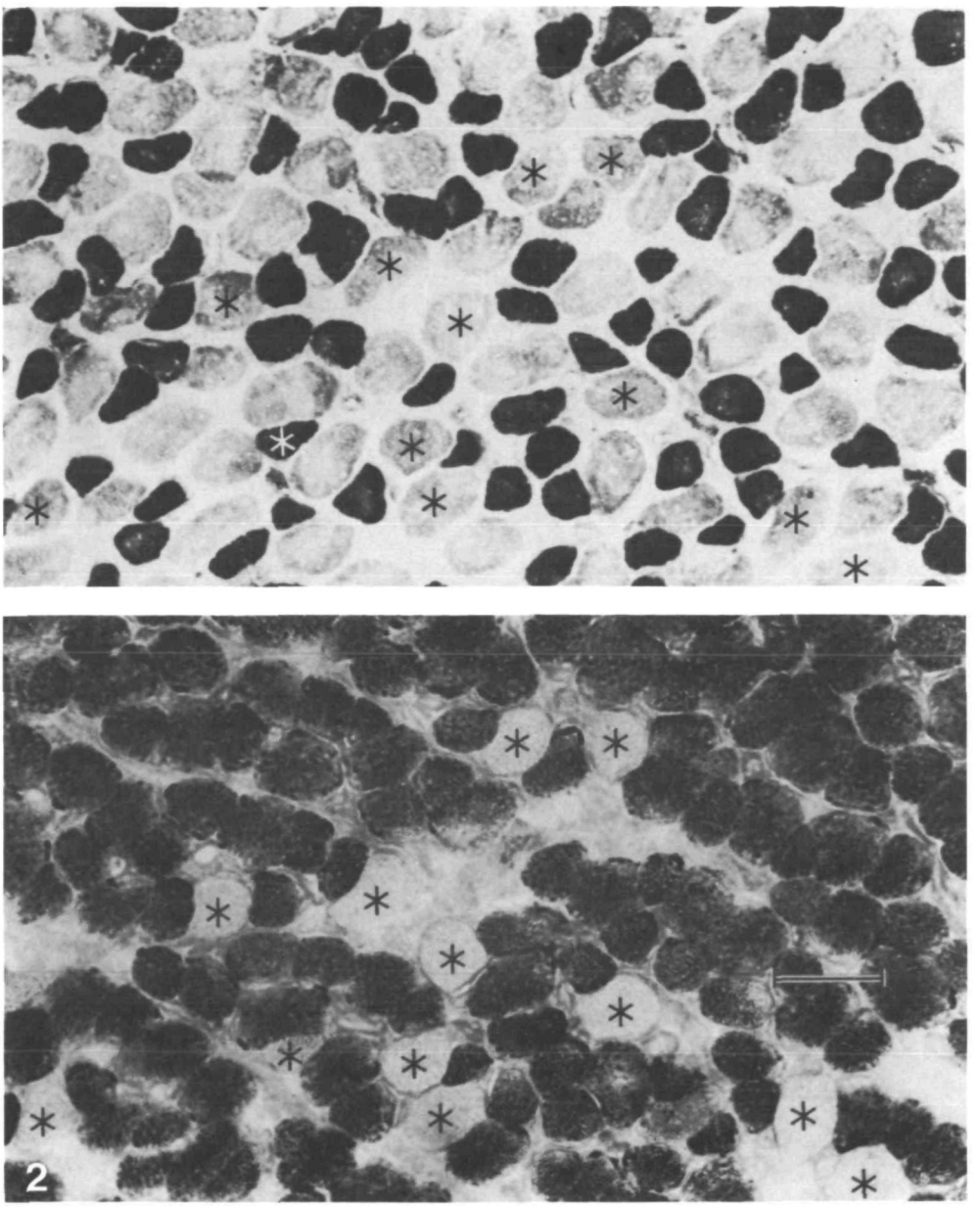

Fig. 2. Fibre types in a portion of a single motor unit in an 8-day-old rat soleus muscle identified by the technique of glycogen depletion. Frozen cross-sections of the muscle have been stained for myofibrillar ATPase activity following alkaline preincubation (top) and for glycogen (bottom). In the ATPase stain, slow fibres are lightly coloured and fast fibres are darkly coloured. Asterisks mark glycogen-depleted fibres and the corresponding fibres stained for ATPase. All but one of the glycogen-depleted fibres are of the slow fibre type. Scale bar, 25 μm. (From Thompson, Sutton & Riley, 1984.)

occurring during this period. The implication of these findings is that the polyneuronal innervation of the fibre types in the muscle is ordered (Fig. 4). Apparently there are two types of motor neurones present in the muscle and each of these confines most of its innervation to one type of fibre. This means that the polyneuronal innervation of each fibre is by motor neurones which are largely of the same type. Somehow prior to 8 days of development, the innervation of the rat soleus

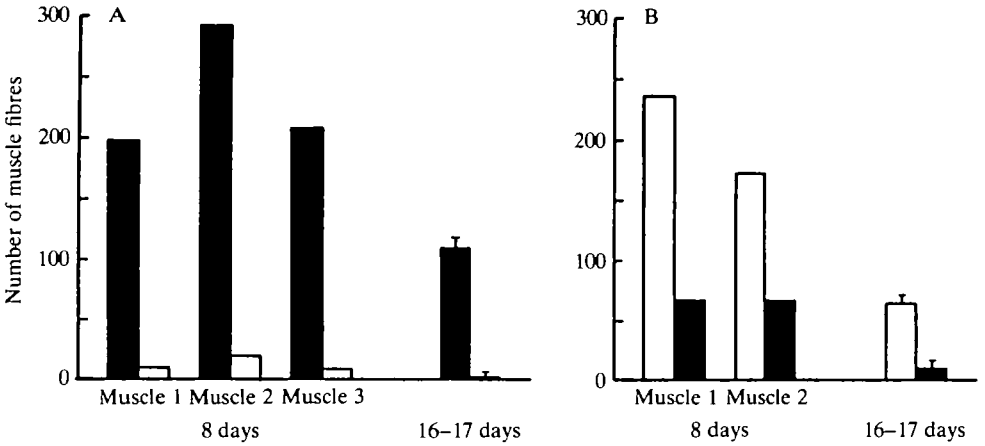

Fig. 3. Fibre type compositions of single motor units from 8-day- and 16- to 17-day-old rat soleus muscles. (A) Motor units composed predominantly of fast fibre types. The number of fast (filled columns) and slow (open columns) fibres in each of three units depleted at day 8 and the average number of fast and slow fibres in four units depleted at day 16–17 are shown. (B) Motor units composed predominantly of slow fibre types. The number of slow (open columns) and fast (filled columns) fibres in each of two units depleted at day 8 and the average number of fast and slow fibres in five units depleted at day 16–17 are shown. Vertical bars indicate S.E.M. in both A and B. Fig. 1 shows the fibre type compositions of the whole soleus muscle at these two ages. (Modified from Thompson, Sutton & Riley, 1984.)

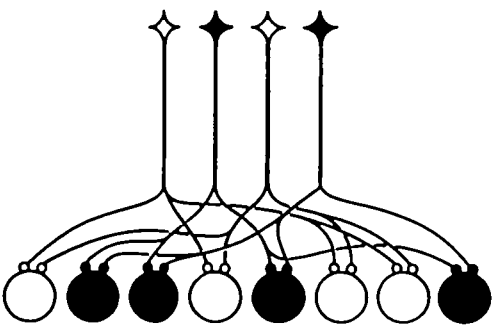

Fig. 4. Diagram interpreting the innervation of fibre types in 8-day-old rat soleus muscle. The diagram depicts four motor neurones and eight muscle fibres and their synaptic connections. Shading is used to illustrate that there are two types of muscle fibres and two types of motor neurones which innervate them. Most of the polyneuronal innervation results from innervation of each type of muscle fibre by motor neurones of a complementary type. The diagram exaggerates the precision of the innervation. (From Thompson, 1986.)

muscle has become sorted by fibre type. Similar findings have been made in the soleus muscle of the neonatal rabbit (Gordon & Van Essen, 1985). Furthermore, the glycogen depletion procedure has been employed to investigate the fibre type composition of motor units in a typical fast-twitch muscle, the extensor digitorum longus (EDL) muscle of the rat (Balice-Gordon & Thompson, 1986). At 6–7 days, EDL motor units are also highly biased in their fibre type composition, despite the presence of extensive polyneuronal innervation.

POSSIBLE MECHANISMS FOR SORTING THE INNERVATION OF FIBRE TYPES

On first examination, this 'sorted innervation' in polyneuronally innervated muscle might be taken to indicate a selective synaptogenesis between predetermined types of motor neurones and muscle fibres. However, this result says nothing about how this innervation pattern arises. In fact, it is exactly what would be required if motor neurones were to determine the types of the fibres they innervate. Some kind of order to the innervation would be necessary so that each fibre could receive coherent instructions for its differentiation. A number of other possibilities exist for creating such an innervation.

For example, one alternative to a selective synaptogenesis is that the motor neurones recognize each other rather than the fibres which they innervate. Perhaps the first motor neurone to innervate a naive, undifferentiated, muscle fibre excludes further innervation by any but corresponding types of motor neurones. This type of arrangement would create a sorted innervation whereby motor neurones could impose differentiation on muscle fibres.

Another possibility is that there is not a selective synaptogenesis but rather a selective synapse elimination. It is possible that motor neurones do not initially form selective connections with only the appropriate types of muscle fibres, but that recognition events subsequent to initial synaptogenesis result in the survival of only the appropriate connections. It is not yet possible to exclude this mechanism since the above study was conducted 8 days after birth, by which time a great deal of synapse elimination has already occurred (Brown *et al.* 1976).

Yet another set of possibilities arises from the manner in which muscle fibres are generated. The myotubes giving rise to the fibres in each muscle are generated at slightly staggered times of development. One generation, the so-called 'primary' myotubes, arise first followed by so-called 'secondary' myotubes (see Rubinstein & Kelly, 1981). In some muscles there is evidence suggesting that these different generations of myotubes differentiate into distinct types of muscle fibres (see Rubinstein & Kelly, 1981). This raises the possibility that, if different types of motor neurones grow into the muscle at times corresponding to these waves of myogenesis, then they might be constrained to innervate separate populations of muscle fibres, which may or may not have already become committed to some particular differentiation. By invoking this kind of timing hypothesis, it is even possible to resurrect ideas that the motor neurones are initially undifferentiated and that they undergo a 'myotypic' specification (Weiss, 1937) according to the predetermined type

of muscle fibre they come to innervate. Since each motor neurone innervates many fibres in the muscle, such a myotypic mechanism would be unlikely if more than one type of fibre were available for innervation at the time each set of motor neurones enters the muscle. However, if sets of motor neurones enter the muscle at times corresponding to the genesis of each type of fibre, then it becomes possible to limit contact of motor neurones to one type.

It is clear that an essential component to understanding the early innervation of muscle fibres is a knowledge of the origin of muscle fibre diversity. Recent studies of the differentiation of muscle fibre types in the chick embryo have provided some surprising insights. These studies have shown that at least the initial stage of differentiation into fibre types is probably intrinsic to the fibres and occurs independently of the nervous system. First, removal of the neural tube prior to the establishment of neuromuscular junctions results in aneural muscle fibres which nonetheless undergo differentiation into fibre types (Butler, Cosmos & Brierley, 1982; Phillips & Bennett, 1984). Second, if neuromuscular junctions are allowed to form in the presence of curare, so that the muscle is totally paralysed, differentiation continues (McLennan, 1983; Crow & Stockdale, 1986; Sohal & Sickles, 1986). Third, manipulations causing muscles to become innervated by inappropriate motor neurones [forcing innervation of a transplanted wing bud by lumbar motor neurones (Laing & Lamb, 1983) or of brachial muscles by transplanted thoracic spinal cord (Butler, Cauwenbergs & Cosmos, 1986), or rotating the lumbar spinal cord so that leg muscles become innervated by novel motor pools (Vogel & Landmesser, 1987)] do not apparently change the characteristic fibre type compositions present in particular fast, slow or mixed muscles. Lastly, Miller & Stockdale (1986) have shown that myoblasts isolated from early chick limb buds are committed to one of three lineages of fibre types. Upon induction of myotube formation in culture, each lineage participates in the generation of myotubes of a different fibre type. These results, if they apply to mammalian muscle, would require that whatever mechanism sorts the innervation of fibre types plays no role in the differentiation of the muscle fibres. However, a similar role for cell lineage in the differentiation of mammalian fibre types has yet to be demonstrated. The observation that adult chicken muscles are much more resistant to fibre type conversion upon cross-innervation than are mammalian muscles (Vrbová *et al.* 1978) suggests that the differentiation of avian fibre types might be more rigidly preprogrammed.

REINNERVATION OF DEVELOPING FIBRE TYPES

One way to discover whether neonatal motor neurones in the mammal have the capacity for selectively innervating muscle fibre types is to examine what selectivity motor neurones display in reinnervating a neonatal muscle. While it is clearly the case that mammalian motor neurones reinnervate adult fibre types nonselectively (see Dum, O'Donovan, Toop & Burke, 1985*a*; Foehring, Sypert & Munson, 1986), such might not be the case for the neonate. Observations of differences in the selectivity of reinnervation by embryonic *vs* adult neurones have been reported (Farel &

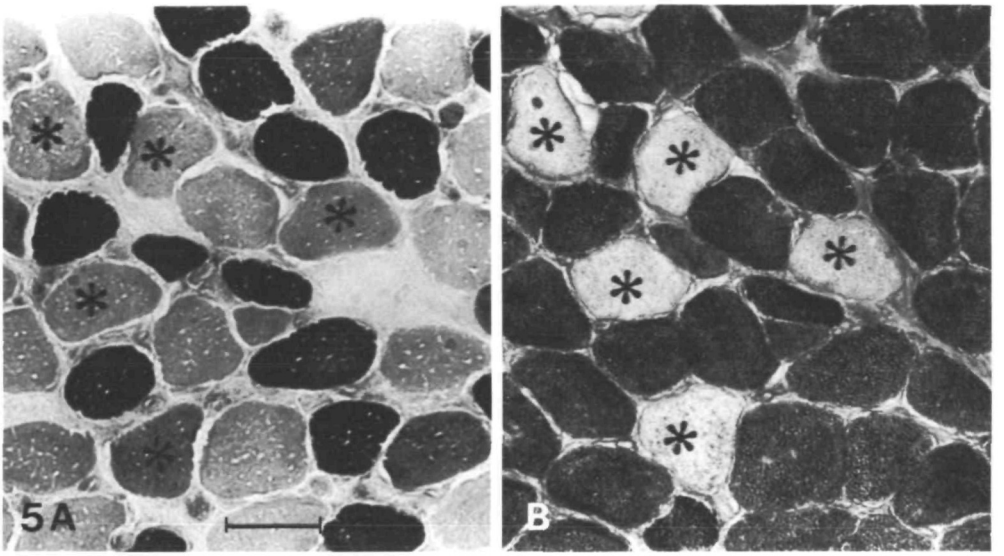

Fig. 5. Fibre types in a portion of a 16-day-old motor unit formed by reinnervation subsequent to crush of the soleus nerve at postnatal day 2. Fibres innervated by a single motor neurone have been identified by the technique of glycogen depletion. (A) Section stained for myofibrillar ATPase and (B) section stained for glycogen. In the ATPase stain, slow fibres are lightly coloured and fast fibres are darkly coloured. Asterisks are used to mark the depleted fibres in each section. All of the depleted fibres shown here are of the slow fibre type. Throughout the muscle this unit contained 65 slow and 15 fast fibres. Scale bar, 25 μm. (From Soileau & Thompson, 1985.)

Bemelmans, 1986). Furthermore, a selective innervation of fibre types is not without precedent, as it appears to occur in lower vertebrates [frogs (Elizalde, Huerta & Stefani, 1983), *Xenopus* (Nudell & Grinnell, 1985) and snakes (Wilkinson & Lichtman, 1985)]. A study of the reinnervation of newborn rat soleus muscle was therefore conducted (Soileau & Thompson, 1985, 1986). The soleus nerve was crushed at the point where it enters the muscle in 2-day-old animals. Reinnervation of these muscles occurred very quickly. Signs of reinnervation by a few motor neurones were first detected at 5 days; by 12 days, almost all of the motor neurones had regenerated and almost all of the fibres in the muscle had become reinnervated.

Initial experiments showed that despite the denervation and reinnervation, 14- to 18-day-old muscles contained the same two fibre types present in normal muscle and that, moreover, these types were distributed in the same mosaic pattern as in the normal muscle (Fig. 5). Glycogen depletion was then utilized to examine the fibre types innervated by a single motor neurone in each of 12 reinnervated muscles at 14–18 days (see, for example, Fig. 5). In each experiment, the single motor axon, isolated by teasing ventral root filaments, was selected randomly. The results of these glycogen depletions showed that all of the motor units contained a mixture of fibre types; however, seven of the 12 units were composed of greater than 80 % of one type (Fig. 6). This is far from what would have been expected of a random innervation in which each muscle should have generated motor units whose fibre type compositions

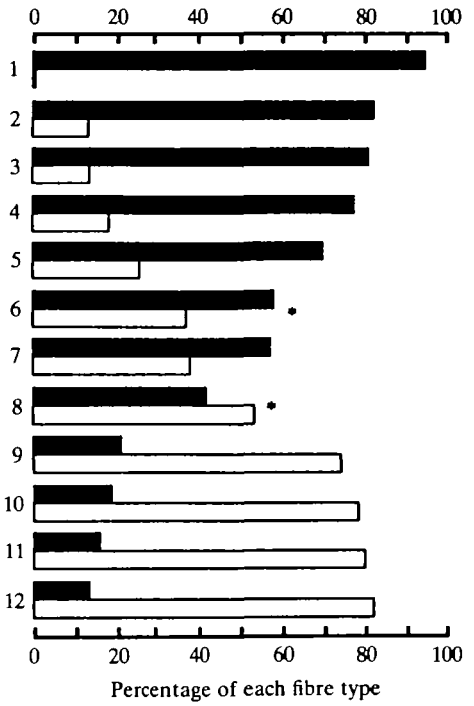

Fig. 6. Fibre type compositions of 12 single, reinnervated motor units in 14- to 18-day-old rat soleus muscles. Muscles were reinnervated following crush of the soleus nerve at postnatal day 2. The units have been arbitrarily arranged from top to bottom in order of increasing slow fibre content. The two units marked with asterisks had fibre type compositions which were not significantly different from that expected of a random reinnervation. The 12 muscles from which these units were taken contained an average of 54 ± 1% slow fibres. (From Soileau & Thompson, 1986.)

mirrored that of the muscle as a whole (i.e. 54% slow fibres, 46% fast fibres). A binomial test was employed to determine the probability of obtaining the observed unit compositions by chance. For each unit of N depleted fibres, the probability of obtaining a unit composed of *at least* the observed number of fibres of the more prevalent fibre type was calculated. The probability of an axon innervating each type of fibre was assumed to be given by the prevalence of that type in the muscle. This analysis revealed that, for all but two of the reinnervated units, the type composition was one which would be expected by chance in fewer than 5 in 1000 cases. Thus, upon reinnervation of neonatal muscles, most motor units formed have fibre type compositions which are highly biased towards one or the other of the two types present in the muscle.

EXPLANATIONS FOR THE BIASED FIBRE TYPE COMPOSITION OF MOTOR UNITS FORMED BY REINNERVATION OF NEONATAL SOLEUS

The biased type composition of the motor units formed by reinnervation suggests a selective reinnervation of fibre types. However, before such a conclusion can be made a number of other alternative possibilities need to be considered.

One explanation of the bias to neonatal reinnervation is that motor neurones regenerate down the Schwann tube/basal lamina pathways left behind by their degenerating distal axons and are mechanically funnelled back down to the fibres they had previously innervated. Such a mechanism appears to account for much of the selectivity of reinnervation of peripheral muscles following nerve crush in the adult frog (Westerfield & Powell, 1983). However, the location of the fibres in the reinnervated motor units argues against this explanation. Normal motor units in the soleus muscle of the rat have fibres scattered throughout the muscle (Kugelberg, 1976). In contrast, the reinnervated motor units were restricted to smaller regions of the muscle, and the fibres, while not usually adjacent, were clustered in close proximity (Fig. 7) (Soileau & Thompson, 1985). Apparently, the regenerating neonatal motor axons reinnervate a novel set of muscle fibres.

A further consideration is the constancy of the differentiation of the muscle fibres. If the differentiation changes during reinnervation, e.g. as a result of reprogramming by novel innervation, then the fibre type composition of units at 14–18 days would not indicate the initial choices made by the regenerating axons. To examine this question, two monoclonal antibodies to the myosin heavy chain, one of which binds to the population of slow fibres in soleus and a second which binds to the population of fast fibres, were used to examine the type composition of soleus muscles at the time of denervation, as a consequence of denervation, and during the course of reinnervation (Soileau & Thompson, 1986). Use of these antibodies in immunohistochemistry revealed that two populations of fibres stained differentially at 2 days (the time the denervations were performed in the reinnervation experiments) and that

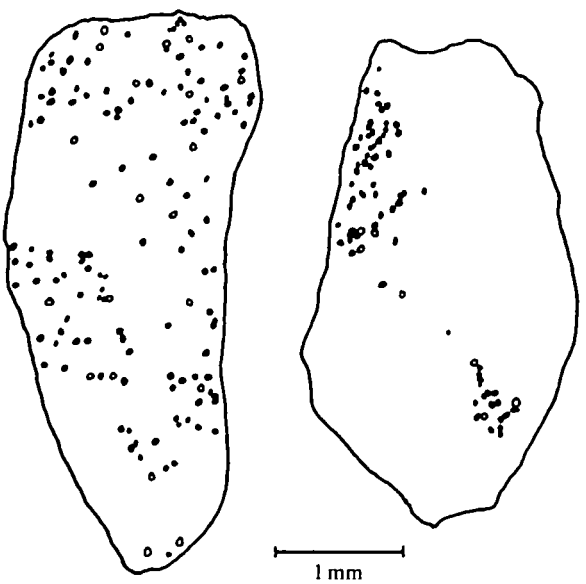

Fig. 7. Example of the location of fibres contained in single motor units in a normal 16-day-old rat soleus muscle (to the left) and in a reinnervated muscle (to the right). The outline of the muscle cross-section has been traced and the position of each depleted fibre indicated.

during the subsequent 2 weeks of development, there was no evidence that fibre types were being interconverted. Denervation of 2-day-old soleus muscles by resection of the soleus nerve (to ensure that no reinnervation occurred) did not result in immediate dedifferentiation of the fibres; two distinctly different fibre types remained in the muscle for at least 10 days. Since reinnervation of the muscles was complete by 10 days after nerve crush, it is unlikely that the fibres had lost their differentiation prior to reinnervation. This was confirmed by immunohistochemical examination of the fibres during the course of reinnervation. At all times, two distinct types of fibres were seen. Only a small minority of fibres (less than 3 %) stained strongly with both antibodies. This result argues that if fibre type interconversions occur during reinnervation, these interconversions are very rapid, so that few fibres with an intermediate myosin expression are seen at any point in time.

Taken together, these results argue that neonatal soleus motor neurones reinnervate the two types of fibres present in the muscle in a selective manner. This apparent selectivity may explain the segregation of innervation of fibre types in the soleus during normal development.

CONCLUSIONS

Apparently, the process of innervation of developing skeletal muscle fibres is much more interesting than was previously believed. Rather than the differentiation of fibre types being due solely to the nervous system, there are now indications of an intrinsic component to the early fate of each fibre. The innervation of fibres is not random. Rather, particular types of motor neurones can be identified by the type of fibre they innervate, and the polyneuronal innervation of muscle fibres is largely a convergence from like types of motor neurones. The reinnervation experiments imply that neonatal motor neurones have the capacity to recognize and preferentially innervate particular types of muscle fibre.

Two questions about these findings immediately come to mind. How does one reconcile the plasticity of fibres in the adult animal with the suggestion that their type in the foetus may be determined by intrinsic mechanisms? Second, why the difference in selectivity of adult and neonatal reinnervation?

Neither question has yet been answered. As pointed out above, it is still possible that mammalian fibre types differentiate according to the innervation they receive, provided there is some mechanism which sorts the innervation by the different types of motor neurones to different populations of naive myotubes. It is also possible that, even if foetal and neonatal fibres are predetermined, their differentiation can be altered by changing their innervation or activity. This question has yet to be fully explored. However, various recent experiments imply that there are limitations to the plasticity of adult muscle fibres. For example, the fibre types in some muscles are resistant to change upon cross-innervation (see Dum *et al.* 1985*b*). In several cases, the completeness of the transformation of adult fibre types is questionable (see Buller, 1983). Westgaard & Lømo (1987) have proposed that each fibre type has an 'adaptive range' within which it can be altered by its innervation or by a change in its

activity. There seems to be no intrinsic incompatibility to fibres having a predisposition to some line of differentiation and also having the capacity to alter this intrinsic course upon innervation by a neurone other than the appropriate one.

Differences in adult and neonatal reinnervation are traditionally explained by arguing that mechanisms ensuring the correct guidance of growing axons and the connection of these axons to the correct synaptic targets are operative only during a limited time of development. There are many possible mechanisms of this type. An example would be that innervation slowly induces the formation of an endplate on each muscle fibre and that this endplate, upon denervation and reinnervation, promotes synapse formation by any type of motor neurone. There is evidence supporting aspects of this general hypothesis. McMahan and his collaborators (Nitkin *et al.* 1983) have shown that innervation promotes the production of a specialized basal lamina at the endplate; this endplate basal lamina persists upon denervation and serves as the site on the fibre which is preferentially reinnervated. Indeed, this basal lamina can induce synapse formation even in the absence of the muscle fibre. Additionally, there is evidence suggesting that the endplate, and presumably the basal lamina as well, is much more labile in newborn muscle fibres (Brown *et al.* 1976). Whether induction of an endplate basal lamina, or some other mechanism, explains the difference in selectivity of reinnervation of adult and neonatal muscle fibres awaits further experimentation.

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