

MATCHING OF MUSCLE PROPERTIES AND MOTONEURONE FIRING PATTERNS DURING EARLY STAGES OF DEVELOPMENT

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SUMMARY

In adults, muscle fibres match the functional requirements of the motoneurone that supplies them. During early stages of postnatal development of the rat neither muscle fibre properties, nor activity patterns of motoneurons supplying fast and slow muscles have completed their differentiation. Nevertheless, even at this early stage of development the muscles have characteristic properties that are well matched to the activity patterns of immature motoneurons. With further development differentiation of motoneurone activity and muscle fibre properties goes hand in hand. If during this period of linked differentiation, connections between the motoneurons and muscle fibres are disrupted, the development of fast muscles is permanently impaired.

INTRODUCTION

The physiological properties of mammalian skeletal muscle fibres are matched, with remarkable precision, to the activity pattern of motoneurons that supply them. Muscle fibres activated by low rates of firing contract and relax slowly, so that they achieve a smooth contraction and develop a large proportion of their maximum tetanic tension at these physiological rates of activity. Muscle fibres that are normally activated at fast rates contract and relax rapidly and develop their maximal tension at high rates of firing (for review see Vrbová, Gordon & Jones, 1978). The matching between the mechanical properties of the muscle and the type of activity it receives is so precise that even the changes of muscle fibre characteristics in response to preceding activity change appropriately to the firing pattern the muscles normally receive. So, for example, the firing rate of motoneurons to slow muscles varies very little (Navarrete & Vrbová, 1983) and in slow muscles increases in force are brought about by recruitment rather than an increase in the firing rate of motoneurons (see Freund, 1983). Correspondingly, slow twitch muscle fibres do not show a great change in their contractile properties in response to previous activity, i.e. they have small post-tetanic modifications of contractile properties (Buller & Lewis, 1965). In contrast, the rate of firing of motoneurons to fast muscles is extremely variable: in most instances they fire initially at high rates and this subsequently declines.

Key words: Development, muscle, activity.

Interestingly, fast muscles change their contractile properties in response to preceding activity and display a large post-tetanic potentiation (Brown & Von Euler, 1938; Buller & Lewis, 1965) which enables them to maintain the same force even during the irregular firing rates of fast motoneurons (Bigland-Ritchie, Jones & Woods, 1979; Hultman & Sjöholm, 1983). Thus in adults there exists a near to perfect matching of the contractile properties of muscle fibres to the functional demands of motoneurons that supply them. By virtue of this the motor unit can and does function as a true unit, and the muscle fibres are indeed just an extension of the motoneurone. Yet the muscle fibres are separate individual cells and have no means of intracellular communication with the motoneurone that supplies them. Indeed, during early stages of embryonic development the motoneurons and muscle cells develop independently, and establish connections with each other only later.

How then does the motor unit develop, and how do the muscle fibres belonging to it acquire those properties that enable them to match the requirement of the motoneurons? It could be that developing muscle cells have special characteristic markers that enable the appropriate motoneurone to recognize and contact them. Thus muscle cells may have already, even at these early stages of development, an inherent genetic programme that will express those phenotypic characteristics, most suitable for the motoneurone that has contacted them. This hypothesis, while widely accepted among some developmental neurobiologists working on chick embryos, (Lance-Jones & Landmesser, 1980) is very unlikely for the following reasons: (a) initially, even some time after innervation has taken place, the characteristic membrane and contractile properties of all muscle fibres at a comparable stage of development are similar (Gordon, Purves & Vrbová, 1977), (b) it is relatively easy by surgical manipulations to innervate embryonic muscle by inappropriate nerves (Summerbell & Stirling, 1981), and this ability of muscle to accept any alien cholinergic nerve persists in adult animals (Langley & Anderson, 1904; Weiss & Hoag, 1946) and (c) muscle fibres, embryonic or adult, have an extraordinary capacity to alter their contractile, ultrastructural and biochemical properties (Buller, Eccles & Eccles, 1960*a,b*; Ashhurst & Vrbová, 1979; Gordon, Vrbová & Willcock, 1981), and are able to alter their phenotype within a wide range in response to changes of activity (Salmons & Vrbová, 1969; Pette, Müller, Leisner & Vrbová, 1976).

Rather than a specific recognition during development it is more likely that the muscle adjusts to the particular activity pattern of the motoneurone.

In order to find out more about the developmental events that induce within the muscle fibres those properties that match best the requirements of the motoneurone it is important to analyse the changes of activity patterns of developing motoneurons in relation to the properties of muscle fibres supplied by these cells.

The first spontaneous movement in the rat, at embryonic day 15½, consists of lateral flexion of the head (Angulo y González, 1932; Windle & Baxter, 1936). Increased motility then progresses in a cranio-caudal direction. Generalized mass movements can be noticed from day 16 onwards, but these involve mainly the head, neck and forelimbs (Narayanan, Fox & Hamburger, 1971). In addition to these generalized movements, flexion of individual limbs has also been noted (Narayanan *et al.* 1971).

The frequency at which these localized movements occur increases with the age of the embryo.

The position of the embryo *in utero* is completely different from that after birth. Its limbs are in flexion and held close to the trunk, and the locomotor system has to be adjusted to fulfil this function, hence it is mainly flexion of limbs that is noted. *In utero* there is obviously no need for the embryo to cope with gravity, so before birth antigravity muscles will probably be less well developed than flexor muscles. Thus even at this stage, several days before birth, muscles are heterogeneous and their heterogeneity may express their different function, and reflect different stages of maturity within the same group of muscles.

POSTNATAL DEVELOPMENT OF PHYSIOLOGICAL PROPERTIES OF SOLEUS AND EXTENSOR DIGITORUM LONGUS (EDL) MUSCLES

Close (1964) followed the changes of contractile properties of rat soleus and EDL muscles during postnatal development. He confirmed that in rats, just like in other mammals (Buller, Eccles & Eccles, 1960*b*), both future slow and fast muscles are slowly contracting and relaxing at birth, and with age the contractions of both types of muscles become faster. The increase of speed of contraction continues in EDL, but the contraction of soleus becomes slower after the initial increase in speed. Close (1964), using measurements of force-velocity relations, established that at birth the speed of shortening of soleus and EDL is similar, and that while it increases in EDL with development, in soleus no change is noticed. This finding differs from changes of the time course of the isometric twitch contraction which becomes faster with age. Since the force-velocity relation of the soleus does not change with age, it is likely that in this muscle the increase in speed is brought about by an improved mechanism of Ca^{2+} uptake that takes place during development, whereas the increase in speed of the isometric contraction of EDL is brought about by a true change of the contractile machinery (Close, 1964; Vrbová, 1980). This seems to be the case in cat muscles as well, for in these too soleus is much slower at birth than the fast FHL muscle and it has a much higher tetanus/twitch ratio (Buller & Lewis, 1965). Fig. 1 summarizes measurements of time to peak tension of the isometric twitch contraction obtained from soleus and EDL muscles of rats during the first 3 weeks after birth. The early difference between EDL and soleus probably reflects the relatively advanced developmental stage of EDL in relation to soleus, a pure antigravity muscle that before birth is not required to perform any function.

Both types of muscles are two to three times slower at birth than 3 weeks later. Moreover, soleus is unable to maintain tension for long periods of time in response to high frequency nerve stimulation (N. S. P. Handysides, R. Navarrete & R. A. D. O'Brien, in preparation). Thus the mechanical properties are very different from those of adult muscles, and would be a poor match for activity patterns generated by motoneurons from older animals. It was therefore of particular interest to establish whether these immature muscles are activated by an activity pattern that matches this developmental stage.

CHANGES OF ACTIVITY PATTERNS OF MOTONEURONES TO SLOW AND
FAST MUSCLES WITH DEVELOPMENT

In adult animals the firing pattern of individual motoneurones depends on their synaptic connections and their size (Eccles, Eccles & Lundberg, 1957; Henneman, Somjen & Carpenter, 1965). Coordinated movement results from the orderly

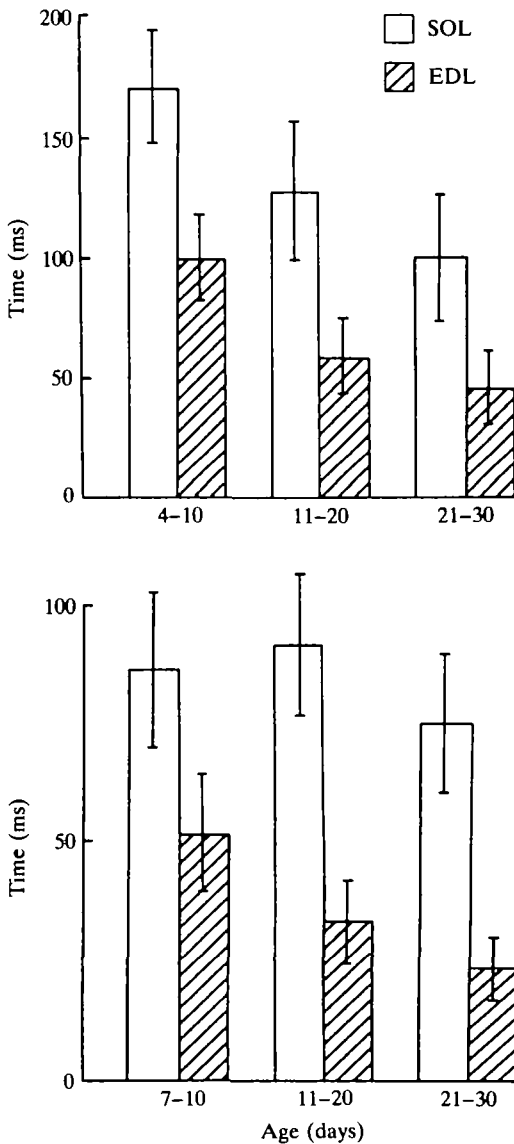


Fig. 1. Top panel: time to peak twitch tension of soleus (SOL) and extensor digitorum longus (EDL) muscles from rats of different ages. Vertical bars represent S.D. Lower panel: mean interspike intervals of single motor units from soleus and extensor digitorum longus muscles from rats of different ages.

recruitment and finely controlled rate of firing of these motoneurons (Adrian & Bronk, 1929). Motoneurons can be arguably divided into those that are recruited during postural movements and fire at low rates for prolonged periods of time, and those that are responsible for fast, phasic movements and fire at high rates for brief periods of time (see Granit, 1972). In addition, the order of recruitment and the duration of motoneuron firing are related to size, small motoneurons are recruited first and fire for long periods of time whereas large motoneurons are recruited later and do not maintain their firing (Henneman *et al.* 1965). These control mechanisms thus ensure the execution of coordinated movement of the adult individual.

In rats under 10 days of age, forward locomotion is accomplished mainly by the forelimbs, the hindlimbs showing a very poor postural support function at this stage (Stelzner, 1971; Navarrete & Vrbová, 1983). Postural stimuli produce non-graded righting responses involving uncoordinated displacement of all limbs. This differs from the response of adults where balance is maintained by selective activation of hindlimb postural muscles without necessarily displacing the limbs. Nociceptive stimulation of very young animals elicits strong generalized responses involving all limbs in contrast to the localized flexor reflex observed in adults. The basic behavioural pattern of the adult rat, particularly the precise coordination of the limbs during locomotion and the graded postural responses to tilting, are first seen in animals aged 18–21 days (Navarrete & Vrbová, 1983).

This poor coordination of newborn animals is perhaps not surprising since at this stage of development the connections between the motoneurons and their afferent inputs are not yet mature and all the motoneurons are small (Mellström & Skoglund, 1969; Ford & Cohan, 1968). Thus the main control mechanisms are not yet fully developed. Evidence is accumulating to show that at this stage different classes of motoneurons cannot be distinguished from each other by their particular patterns of activity. Bursian & Sviderskaya (1971) recorded EMG activity from the gastrocnemius muscle of newborn rats and kittens and found that the mean discharge frequency of motor units was low. The adult gastrocnemius is a mixed muscle, composed of different types of motor units, therefore these results could mean either that only slow motor units are active at this early stage of development, or that both presumptive slow and fast motor units fire at low rates in neonatal animals. In order to compare the activity pattern of developing slow and fast units, it is necessary to record activity from muscles of more or less homogeneous populations of motor units. To approach this requirement the soleus muscle can be used for the study of development of slow type motor units and EDL for that of fast motor units.

It is well known that in the adult soleus muscle only low frequency (7–25 Hz), tonic EMG activity can be recorded. Moreover even at rest, a few motor units maintain regular discharges for several minutes in the absence of any apparent external stimulus. Recruitment of previously silent motor units occurs on postural activation or during spontaneous locomotion, and is accompanied by a very limited increase in the firing rate of the previously active units (Vrbová, 1963; Navarrete & Vrbová, 1983).

In newborn rabbits and in rats of up to 12 days of age, the EMG activity of the soleus muscle is strikingly different from the adult. Motor units do not sustain

discharges for more than a few seconds and during spontaneous or reflex activity, several motor units are activated synchronously at very low frequencies (3–10 Hz) for a short time. Both postural and nociceptive stimuli produce intermittent activity resembling the phasic pattern of adult fast muscles particularly in the youngest animals.

At about 12 days of age the soleus muscle undergoes a fundamental change in its pattern of activity. At this time, tonic discharges from single motor units are first detected in the resting animal and during the third week there is a progressive increase in the proportion of tonic motor unit activity and a greater regularity in the discharge of individual motor units. After 3 weeks the basic pattern of activity in the soleus muscle resembles that of the adult.

In adult animals, no EMG activity can be recorded from the fast muscles at rest (Vrbová, 1963; Navarrete & Vrbová, 1983). During movement, only phasic short-duration discharges of motor units at high frequency (15–77 Hz) can be recorded. In the EDL of newborn animals the EMG activity is also phasic but unlike in the adult, the firing frequency of the majority of its motor units is relatively low (Fig. 1). Although even in the youngest group of animals some motor units reach high instantaneous firing rates during strong activation of the muscle, the vast majority of motor units fire at low frequencies.

Fig. 1 also shows that between 11–20 days of age, the mean firing rate increases and by 20 days most motor units have high mean discharge rates comparable to the adult (see Navarrete & Vrbová, 1983). Thus during early stages of development both types of motoneurons fire at relatively low rates, and are unable to sustain their firing for long periods of time. With development, soleus motoneurons become capable of sustaining their activity for long periods while motoneurons to EDL start firing at high rates. In addition to these developmental changes in the pattern of activity, there is a several-fold increase in the aggregate activity of both types of units during spontaneous movement. The extent of this increase is illustrated in Fig. 2, which also shows that the aggregate EMG activity in the older animals is greater in soleus than in EDL.

Thus the activity patterns of motor units are not specialized in newborn animals. This lack of differentiation is probably due to the immaturity of afferent inputs to motoneurons of newborn animals. It is known for example that in rats, corticospinal fibres reach the lumbosacral segments of the spinal cord only between 9 and 14 days of age (Hicks & D'Amato, 1975; Donatelle, 1977) and the absence of the tonic stretch reflex (Skoglund, 1960; Bursian, 1973) shows that the proprioceptive inputs onto motoneurons are also not yet fully functional at birth.

IS THERE A MATCHING OF MUSCLE PROPERTIES TO MOTONEURONE FIRING PATTERNS AT THIS STAGE?

Even during early postnatal development the contractile properties of EDL are different from those of soleus, in that EDL contracts and relaxes faster. Consistent

with this, the frequency of firing of motor units to EDL is also faster than that to soleus (Fig. 1). Fig. 1 also shows that the increase in firing frequency of EDL motor units is matched by an increase in the speed of contraction of this muscle. The situation is somewhat different for the soleus; this muscle is slow in the newborn but during the first 3 weeks of life becomes considerably faster, so that by 3 weeks it achieves the values of EDL at birth. Yet, soleus is activated throughout this time by a low frequency activity. It is clear from the experiments of Close (1964) that even though the time course of the isometric contraction changes in soleus, there is no change in the force-velocity relationship and thus the low frequency activity is probably not altering the contractile machinery of the soleus, but affecting if anything at all, the development of a different intracellular compartment. The difference between the nature of the postnatal changes of contractile properties of EDL and soleus is also illustrated by their different response to denervation. If soleus is denervated at birth, it continues to increase its speed of contraction, until it reaches, albeit more slowly, the value characteristic for a 20- to 30-day-old rat. On the other hand, neonatal denervation of EDL prevents the usual increase in the speed of contraction (Brown,

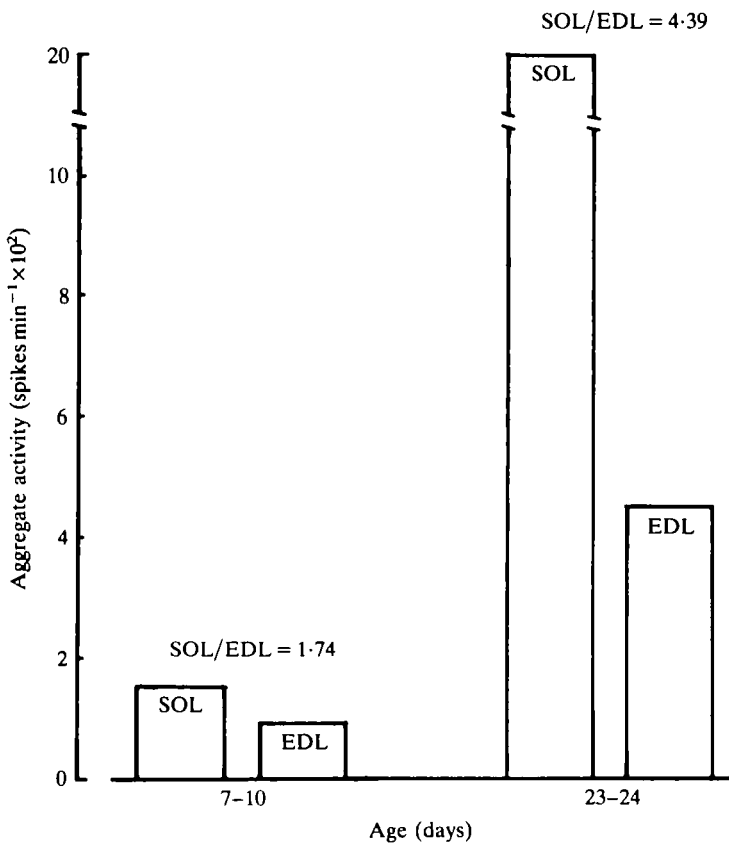


Fig. 2. A block diagram showing aggregate EMG activity recorded from soleus (SOL) and extensor digitorum longus (EDL) muscles of rats of different ages during unrestrained locomotion.

1973). These results indicate that the high frequency activity to EDL is indeed bringing about the change in contractile properties of this muscle, whereas the small amounts of low frequency activity to soleus may have a different function that is not yet clear. This activity may then alter the sensitivity of the muscle membrane to acetylcholine (ACh), for this event takes place during the same developmental period. Another function of this activity is doubtless the induction of the normal development of the neuromuscular junction (see O'Brien, Ostberg & Vrbová, 1978) which then enables the motoneurons and muscle to function as a unit.

Thus in the rat, the changing pattern of motoneurone activity during development affects mainly the contractile properties of fast muscles, and alters them so as to match them to the high frequency activity that these motoneurons transmit to these muscles during later development. It is not certain whether this developmental event (i.e. the induction of the matching properties of muscle) can take place only during this critical period of development, or whether the motoneurons and muscles maintain their ability to develop together normally also later in life.

EFFECTS OF TEMPORARY DENERVATION DURING EARLY POSTNATAL LIFE ON MUSCLE DEVELOPMENT

When in 5- to 6-day-old rats the sciatic nerve is crushed it grows back to its original target muscles within 10–15 days after the injury. In adults after a similar operation, complete recovery of function of the reinnervated muscles takes place. A strikingly different result is obtained when the injury is inflicted on animals during the first week of life. Although all the axons reach the muscle and are guided to their original target the recovery of function is very selective (Lowrie, Krishnan & Vrbová, 1982). Slow muscles, such as soleus, recover almost completely, whereas fast muscles such as EDL, or tibialis anterior (TA) regain only about half the tension of the unoperated control muscles (Fig. 3). This poor recovery of the fast muscles is due to a selective absence of their largest glycolytic fibres, for these muscles, although supplied by their usual number of motoneurons, lack their complement of glycolytic fibres and contain only oxidative fast, fatigue-resistant fibres. The dramatic loss of muscle fibres occurs after reinnervation has taken place. Table 1 shows that at the time when the nerves reach the muscle, all muscle fibres are present, and disappear only later, several days after reinnervation has taken place (Lowrie & Vrbová, 1984). The sharp decline of muscle fibre numbers following reinnervation (20–30 days after nerve crush) suggests that the fibres may have been actively destroyed. When the nerve is crushed at 5 to 6 days the muscle is isolated from all nervous influence and muscle differentiation is retarded. This retardation is much more obvious for fast than slow muscles, since the slow muscles continue to become faster, as though they were innervated, while the increase in speed of fast muscles is arrested by the temporary denervation (Brown, 1973). Meanwhile the motoneurons, although disconnected from their target, probably continue to develop their central connections and differentiate. Upon reinnervation the immature fibres from fast muscles that were not yet induced by the small amounts of relatively low frequency activity typical for animals of that age to

change their properties, may not be able to withstand the now much larger amounts of high frequency activity imposed upon them by the fast, older motoneurons. In contrast, the soleus muscle fibres to some extent continued their differentiation, and at the time of reinnervation acquired contractile properties similar to those of innervated muscles. Most important, soleus muscle fibres are activated by motoneurons that fire at low frequencies, thus matching the slow time course of contraction of their target muscle fibres and therefore the mismatching is not as great

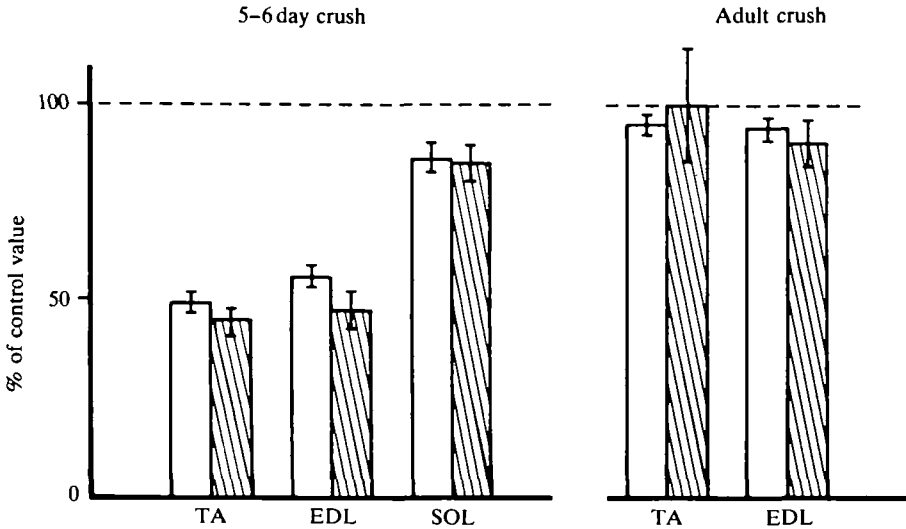


Fig. 3. Muscle weights (white bars) and tetanic tensions (hatched bars) of reinnervated tibialis anterior (TA), extensor digitorum longus (EDL) and soleus (SOL) muscles are expressed as a percentage of control, unoperated values. Note that the recovery of the fast muscles in the adults is nearly complete, while that in animals which received nerve crush at 5-6 days is very poor. *N* for adults nerve crush = 4, 2 months after nerve crush; *N* for 5-6 day crush = 10-19, 1-11 months after nerve crush. Vertical bars represent S.E. of the means.

Table 1. Changes in muscle fibre numbers in control (Cont), reinnervated (Op) and denervated EDL muscles

Age in days	18			26			35		
	Op	Cont	Op/cont (%)	Op	Cont	Op/Cont (%)	Op	Cont	Op/Cont (%)
Reinnervated	4200	4450	97	2900	3650	79	1250	3400	37
	4350	4650	94	3250	4400	74	1350	4300	31
Denervated	4750	4550	104	3900	4250	92	4000	3800	105
				4700	4350	108	4100		

The nerve injury was inflicted at 5-6 days after birth.

as in fast muscles. Thus, interrupting the interaction between skeletal muscle fibres and motoneurons during the period of development when both the activity pattern of motoneuron and muscle properties are changing together, produces a mismatching that then leads to a permanent impairment of fast muscles. Thus it appears that during normal development the contact between nerve and muscle is more important for fast muscles, where the nerve induces a change in their characteristics during this critical developmental period.

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