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Functional reorganization of the partially denervated hindlimb extensor and flexor muscle in rat

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Abstract. In view of the neuromuscular system plasticity the functional changes induced by partial denervation are presented. The long-term effects of partial denervation of postural (*soleus* - SOL) or flexor (*extensor digitorum longus* - EDL) muscles on their EMG activity were studied in rats. The activity per motor unit was significantly higher both in the partially denervated SOL and EDL muscles. During standing or walking the EMG activity pattern of the partially denervated SOL muscle was similar to normal, while the partially denervated EDL muscle during standing exhibited abnormal tonic activity, and during locomotion its burst duration was strongly correlated to the step cycle duration. Thus, partial denervation led to an overall increase of activity of the remaining motor units in both SOL and EDL muscles, while the temporal pattern of muscle activity during locomotion was drastically altered in EDL muscle only, what indicates that partial denervation influenced the postural activity less than the phasic one.

Key words: plasticity, partial denervation, soleus, extensor digitorum longus, EMG activity

Review

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PLASTICITY OF THE NEURO-MUSCULAR SYSTEM DURING MATURATION

Plasticity in the neuro-muscular system is known to be responsible for the differentiation and preparation of the system to the demands of normal life. During development, the homogenous structure of motoneurones and muscle fibres is differentiating into the perfect system able to perform various types of movement.

In all vertebrates, the motoneurone during maturation is critically dependent on its interaction with the skeletal muscle. In newborn rats the motoneurones of different muscles are of similar sizes and only later cells of different sizes can be distinguished (Conradi 1976). The mechanisms that lead to the development of the different sizes of motoneurones and motor units (MUs) are still poorly understood.

At the early stages of postnatal development all motor units are large, i.e. individual motoneurones supply a greater number of muscle fibres in newborn animals than in adults (Redfern 1971, Bagust et al. 1973, Brown et al. 1976), and the individual muscle fibre contributes to more than one motor unit. During postnatal development the size of individual motor unit is reduced by a process of elimination of superfluous contacts, so in adults the single muscle fibre is supplied by one motoneurone only. The size of the motor unit finally depends on the number of contacts that the motoneurone is able to maintain with individual muscle fibres. The motor units can be characterized not only by their size. During maturation they develop also various types of activity, depending on the type of the muscle. During the early stages of development all motoneurones fire at relatively low rate, and are unable to sustain their firing for long periods of time. With development the motoneurones of postural muscles (e.g. soleus) become capable to sustain their activity for long periods while motoneurones of flexor muscles (e.g. EDL) start firing at higher rates (Navarrete and Vrbová 1983).

As a result of the process of developmental plasticity there are three types of motor units differentiated in adult mammals (Burke et al. 1971, 1973, 1977): tonic-slow MUs, phasic-fast fatigue resistant MUs and phasic-fast fatiguable MUs. The tonic MUs contract and relax relatively slowly while phasic MUs are usually fast contracting and relaxing. The tonic and phasic MUs differ also in their ability to withstand fatigue: the tonic (slow) units are all resistant to fatigue, whereas the phasic units can be classified into those that fatigue more readily and those that are less fatiguable (Burke et al. 1973). Muscles composed of phasic motor units (e.g. tibialis anterior or extensor digitorum longus) are involved in rapid phasic movements and rhythmic movements like locomotion. Muscles composed of tonic motor units (e.g. soleus) are also active during rhythmic movements (like locomotion), but their basic activity is related to the posture, i.e. when the animal is standing. The classification of motor units into three groups is an oversimplification, for it is known that the excitability of motoneurones and their orderly recruitment form a functional continuum without sharp distinctions (Buchtal and Schmalbruch 1980). During normal development the contact between nerve and muscle is more important for fast muscles, where the nerve induces greater changes in their contractile characteristics during this critical period than those occurring in slow muscles (Brown 1973).

PLASTICITY OF NEURO-MUSCULAR SYSTEM IN ADULTS

Even in adult animals some aspects of plasticity are present. One example of the ability of the adult neuromuscular system to adjust to altered functional demands is reflected in changes that skeletal muscles display when innervated by an alien nerve. The functional and biochemical properties of skeletal muscle are determined by its innervation. On reinnervation a denervated muscle will become fast or slow, strong or weak, fatigue resistant or easily fatiguable, depending on the type of innervation it receives (Buller et al. 1960, Kugelberg et al. 1970, Luff and Webb 1985). The most convincing example that illustrates the effect of innervation on muscle properties was provided by experiments in which the nerve from a slow muscle was transferred and made to reinnervate a fast muscle and *vice versa.* The experiments of cross-innervation led to the transformation of the cross-reinnervated muscles. The effect of the nerve on muscle properties could be achieved by altering the activity pattern imposed on the reinnervated muscle. After the cross-reinnervation, the nerve continues to transmit its original activity pattern to the new muscle it now supplies (Sperry 1941), and this novel for the reinnervated muscle activity caused clear transformation of muscle fibres.

The dependence of muscle fibres on the activity imposed by the motoneurone was confirmed in experiments in which the activity pattern to a given muscle was modified without interfering with the muscle's innervation. This was achieved either by reducing the normal tonic activity to the slow soleus by abolishing segmental afferent input (Vrbová 1963 a,b) or by increasing the activity to fast muscles by imposing tonic activity onto them by chronic, low frequency electrical stimulation. These procedure induced the "inactive" soleus muscle to become fast contracting (Vrbová 1963, Salmons and Vrbová 1969) and the chronically stimulated fast muscles to become slow contracting and acquiring all the biochemical characteristics of a slow muscle (Salmons and Vrbová 1969, Pette and Vrbová 1994).

Another striking example of the adult skeletal muscle's dependence on its innervation is the finding that when the muscle is deprived of its innervation, it atrophies and its muscle fibres finally degenerate. However, if denervated muscles are reinnervated before they degenerate a considerable degree of recovery can occur (Vrbová et al. 1995). Following partial denervation, when some of the axons supplying the muscle are interrupted, the severed axons and their terminals degenerate. After some delay the fine nerve processes - called sprouts - appear at nerve terminals and nodes of Ranvier of the remaining intramuscular nerves, and begin to contact vacated end-plates. Gradually, these sprouts become myelinated and those that do not contact endplates usually disappear (Brown et al. 1981). Thus, by axonal sprouting motoneurones are able to expand their peripheral field, and increase the innervation ratio of the motoneurone.

FUNCTIONAL REORGANIZATION OF HINDLIMB EXTENSOR AND FLEXOR MUSCLE AFTER PARTIAL DENERVATION

The new situation, where a motoneurone is induced to occupy a larger than usual peripheral field provides a good model for the study of neuronal plasticity and for examining the response of the nervous system to this novel situation. Here we present results that demonstrate the functional reorganization of motoneurones to hindlimb extensor and flexor muscle after partial denervation when their motor unit territory was enlarged.

Two muscles each typical of a particular function were chosen: the extensor digitorum longus - a predominantly fast muscle belonging to the group of physiological flexors, and the soleus muscle - a representative of the group of slow extensor muscles. The SOL as well as the EDL muscles in the rat are innervated mainly by two spinal roots: L4 and L5, occasionally the L3 root can make a small contribution to the innervation of EDL. In adult rat, the innervation to SOL and EDL muscles is not equally divided between these roots. The majority of the motor innervation to SOL is contained in the L5 ventral ramus, while that to EDL in the L4 ventral ramus. Thus, partial denervation of the SOL achieved by section of the L5 ventral ramus deprives it of 50-70% of its motor innervation. The EDL was partially denervated by section of the L4 ventral ramus which supplies 60-80% of EDL's innervation.

SOLEUS - EXTENSOR (TONIC) MUSCLE

It is known that in young rats partial denervation of SOL muscle results in the persistence of the expanded peripheral field of the remaining motor units (Fisher at al. 1989). The adaptive changes occur in response to the increased functional demands on those motoneurones whose axons exit through the L4 ramus. Our question was: Is the EMG activity of expanded remaining motor units of the SOL muscle also affected by partial denervation?

The partial denervation of one hindlimb was performed on rats at 5 days after birth. The EMG activity of both SOL muscles was investigated during exploratory behaviour or during regular locomotion along a runway at different times after operation (6 days, 16 days and 6 months) (Sławińska et al. 1995).

The aggregate EMG activity which was determined by counting all EMG signals crossing the noise level and expressed as a number of counts per minute, was lower in the partially denervated SOL than that in the contralateral control muscle. The aggregate EMG activity was lower in the partially denervated SOL muscle at all stages, although between 11 and 21 day of age there was a large (3-4 fold) developmental increase of the overall amount of EMG activity both in the control soleus muscle and in the partially denervated muscle, as it is seen in normal developmental conditions.

To estimate the average activity per motor unit in the partially denervated or contralateral soleus muscle, the total aggregate EMG activity recorded in the muscle was divided by the number of verified motor units (Sławińska et al.1995). It is interesting, that the partially denervated soleus muscle had only 30-50% of its normal complement of motor units and the amount of activity per motor unit was higher than that in the control muscle.

During spontaneous behaviour the partially denervated soleus exhibited a typical tonic activity pattern, similar to normal SOL muscle. When the rat was walking, the activity of both operated and control SOL muscles was alternating in a manner close to the typical pattern of ankle extensor, but the burst duration of partially denervated muscle was significantly shorter. Analysis of EMG activity of both SOL muscles showed a significant linear relationship between the EMG burst duration and the step cycle duration. However, the slope of this relationship for operated and contralateral soleus muscles was different. The difference was due to the fact that the burst duration in the operated muscle was shorter than in the control muscle.

Summarizing, partial denervation of the soleus muscle in young animals leads to an overall increase of activity of the remaining motor units but does not alter too much their temporal pattern during locomotion. After partial denervation the changes in the activity of motor units of SOL muscle during locomotion were rather minor. It means that partially denervated soleus can function relatively well even when it is innervated by a reduced number of motoneurones.

EXTENSOR DIGITORUM LONGUS - PHYSIOLOGICAL FLEXOR (PHASIC) MUSCLE

In the case of SOL muscle it was found that after partial denervation in young rats, the size of MUs was bigger than in muscle of normal adult animals. Thus, it seems that the soleus motoneurones can maintain their expanded neonatal peripheral field (Fisher et al. 1989). In contrast, when EDL muscle was partially denervated at 3 or 5 days, its MUs were even smaller than normal MUs (Tyč and Vrbová 1995). It is not clear why the remaining MUs of partially denervated EDL muscle are unable to maintain their expanded peripheral field if the injury was performed at an early stage of 3 days after birth. It could be that the severely reduced innervation is not sufficient to bring about the full maturation and development of EDL muscle fibres. It is also possible that the increase in the activity of the remaining MUs leads to a reduction of their peripheral field. When EDL muscle was partially denervated at 18 day after birth when all MUs have already reached theirs adult size, the remaining MUs were able to increase their size almost twice (Connold et al. 1992, Tyč and Vrbová 1995). This increase in size of MUs shows that the L5 motoneurones supplying the EDL muscle could enlarge their peripheral field by sprouting.

In view of these findings we asked: How the EMG activity of remaining EDL motor units is affected by partial denervation performed on animals at various age?

The experiment was carried out on two groups of animals: in one group the partial denervation was performed on animals at an early age - 3 days, and in the other at 18 days after birth. Two to 3 months later the EMG activity of both EDL muscles was investigated.

The EDL muscle belongs to the group of dorsiflexor muscles of the ankle. When rat is standing, EDL muscle is not activated and its EMG signal is almost flat. When the rat is walking the EMG activity is characterized by the rhythmic bursts which correspond to the swing phase of the step cycle. The partial denervation of the EDL muscle in both groups of animals induced changes of burst profile which could be either much longer than in normal animals, or the muscle was activated twice during the step cycle, what produced double bursts in EMG activity. Moreover very often the partially denervated EDL was activated during standing. The burst duration of the EMG activity of the EDL muscle recorded in adult rats during regular locomotion is constant and independent of the step cycle duration. In the case of partially denervated animals the burst activity duration of unoperated EDL muscle was constant for various step cycle duration as it was in normal EDL, while the duration of the burst activity of partially denervated EDL was correlated to the step cycle duration (Tyč, Sławińska and Vrbová, in preparation).

The aggregate EMG activity were higher in partially denervated EDL muscle (Tyč and Vrbová 1995). It means that the motor units in the partially denervated EDL muscle have increased the frequency of their activity markedly, because the number of remaining MUs was lower than in the control muscle. This change was much greater when the partial denervation was carried out at an early stage of development (3 days) than that in even slightly older animals.

This part of our experiments can be summarized: partial denervation of the fast EDL muscle leads to an overall increase of activity of the remaining motor units (similarly as it was in the partially denervated soleus muscle), but the temporal pattern of EMG activity was more affected (in opposition to the slow soleus muscle): additionally tonic-like activity pattern was observed in normally phasic muscle during locomotion as well as during standing.

COMPARISON OF THE EFFECTS OF PARTIAL DENERVATION ON HINDLIMB EXTENSOR AND FLEXOR MUSCLES

Although the analysis of total EMG activity seems to give different results for the SOL and EDL muscles, estimation of single unit activity showed that in both cases partial denervation of rat hindlimb muscles caused an increase of the activity of the remaining motor units in both fast and slow muscles. The factors responsible for the increase of overall activity of individual motor units are unclear. In the case of EDL muscle the partial denervation caused a complete transformation of fast to slow muscles fibres (Tyč and Vrbová 1995). The extent of the muscle fibres transition from fast type II to type I seems to be proportional to the extent of increased activity. In the case of soleus muscle it could be that the frequency of firing of each motor unit increases or/and motor unit are recruited more often. This increase in activity is accompanied by the fact that in partially denervated SOL muscle, 2-3 months after injury, all the muscle fibres were of the slow type I, whereas in control SOL muscles about 20-30% of fibres were fast (Connold and Vrbová 1990).

In the case of EDL muscle the change in overall EMG activity was much greater when the partial denervation was carried out during early postnatal development. The difference between the animals operated on at 3 days and 18 days might be related to the greater plasticity of motor function in the younger animals, which responded to injury by a much more pronounced change. Particularly, that EDL muscle operated on at 3 days of age is characterized by a smaller force and size of MUs than in those operated on at 18 days (Tyč and Vrbová 1995).

The temporal pattern of EMG activity during locomotion was only slightly affected in the partially denervated soleus but very different in the fast EDL, no matter whether the partial denervation was carried out at 3 or 18 days of age. The characteristic feature of this difference was the presence of tonic activity during locomotion as well as during standing. Our results indicate that increase in tonic activity was much more pronounced in animals operated at 3 days of age, showing once more that the plasticity of the young motor system is greater than that in even slightly older animals.

In comparison to partially denervated fast EDL muscles the slow SOL muscles were less affected by partial denervation. This indicates that postural function is less perturbed by partial denervation than phasic activity. In this respect, our findings seem to be consistent with those that a crush lesion of the sciatic nerve in newborn rats markedly alters the EMG activity pattern in the fast flexor tibialis anterior whereas that of the slow extensor SOL is much less affected (Vejsada et al. 1991). In addition, it was shown (Vejsada et al. 1991) that the basic EMG activity pattern of reinnervated lateral gastrocnemius muscle, a fast ankle extensor (Ariano et al. 1973), is relatively well preserved. This seems to be consistent with the nature of postural function which although less precise is involved in all motor functions. Therefore, the postural system seems to be protected from perturbation (soleus, gastrocnemius lateralis), whereas the more precise phasic activity can be readily modified (extensor digitorum longus, tibialis anterior).

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