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MODIFICATION OF MOTONEURON SIZE AFTER PARTIAL DENERVATION IN NEONATAL RATS

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INTRODUCTION

Our previous studies (34, 33) have shown that partial denervation of the fast extensor digitorum longus muscle (EDL) by section of the L4 spinal nerve during early postnatal development reduced the number of motoneurons (MNs) supplying the muscle from 40 in control to only 6 MNs (Tyc & Vrbová 1995a, 2003). In parallel, it causes an increase in the activity of the surviving MNs in adulthood. After partial denervation at 3 days of age, the resting spontaneous activity recorded from the EDL of adult animals showed to be more than 400% higher than that in control EDL muscles. Furthermore, the typical phasic pattern of normal EDL muscles changed to a tonic pattern. This activity modification produced major changes in the remaining motor units (MUs), including a reduction of their size (34, 35) and a change towards a slow muscle fibre type i.e.: they were slow contracting and relaxing, highly fatigue resistant, had high levels of oxidative enzymes and their muscle fibres expressed slow myosin heavy chain isoforms (34). These modifications were permanent in adult animals revealing the high level of plasticity of the neuro-muscular system.

The transformation of a fast to a slow muscle indicates a change in MN activity from phasic to tonic. Whether these changes induce a modification in MN morphology is open to question. A well-known central effect of axotomy in adult animal is the increase in the excitability of the disconnected MNs (7, 15, 16). Most changes observed in MNs after axotomy are reversed after reinnervation (17, 18), thus inducing only temporary changes in MN activity unlike in young animals where firing pattern of the MN is permanently altered after nerve crush. By 1966, a relationship between the electrophysiological properties and the morphological features of the MNs, such as axon diameter and conduction velocity, size of the soma and input resistance, diameter of the somatic region and number of primary dendrites, has been established (10, 11, 12, 8, 3, 5). Moreover it has been found that the small MNs innervating slow-twitch muscle fibres are recruited more readily and fire more slowly than the large MNs innervating fast-twitch muscles, so that the MN activity pattern matches muscle fibre properties. In view of this, in our model of partial denervation converting the MU from fast to slow type and the muscular activity from phasic to tonic, a change in MN morphology can be predicted.

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The present study is part of an investigation of the effects of partial denervation on the locomotor behaviour, the electrophysiological properties and the histochemical features of the denervated fast EDL muscle (34, 35, 36, 37, 31, 32). Here, we investigate the possibility that partial denervation of a fast muscle in neonatal animals has permanent central effects on the morphology of the MNs.

MATERIAL AND METHODS

All experiments were carried out on Wistar rats under licence from the Home Office in accordance with the 1986 Animals Scientific Procedures Act. Every effort was made to minimize animal suffering.

The EDL muscle of the rat is a predominantly fast muscle, composed of 95% fast muscle fibres (27, 19), containing around 40 MUs (4, 2). In adult rats, EDL muscles are phasically active during locomotion but little activity can be seen at rest (22). Motor innervation of EDL muscle is derived mainly from 2 spinal nerves, the L4 and the L5 (1, 2). In the adult EDL, the innervation is not equally divided between these roots. The L4 spinal nerve supplies around 70-75% of the innervation and the L5 the remaining 25-30% (1). In contrast, during early stages of development, each MN supplying EDL muscle innervates a large peripheral field and the territories of L4 and L5 overlap.

Surgical procedure.

Three neonatal Wistar albino rats were operated at 3 days of age under ether anaesthesia. The day of birth was taken as day zero. The L4 spinal nerve on the left side was exposed and cut using sterile precautions at its exit from the vertebral column. In order to prevent reinnervation, about 2 to 4 mm of the spinal nerve was excised. Care was taken to avoid damage to the L5 spinal nerve. The cutaneous incision of the skin was closed and after recovering from the anaesthesia, the rats were returned to their mothers. In all experiments, the contralateral side was used as a control. In a previous experiment (34), the effect of the operation per se was tested on 3 animals. The L4 spinal nerve on the left side was exposed but not cut. The effect of the operation on the contractile properties of the muscle was tested and no differences in muscle weight, MU number, and force output were observed between sham-operated and contralateral control muscle.

Retrograde labelling and measurement of MNs.

The retrograde tracer Horseradish Peroxidase (HRP, Sigma Type VI) was prepared at 15% concentration. A solution containing 3 mg of HRP diluted to 20 μl with sterile saline (0.9% sodium chloride, Sterets Normasol, Seton Prebbles) was prepared. The HRP dose injected was function of the muscle weight. For rat weighing 100 g a normal EDL muscle weighed 50 mg, injection was 2 μl of HRP solution per muscle. For EDL muscle partially denervated at 3 days, given the muscular atrophy (78% of the control), the injected volume was reduced to 25% of the normal dose injected in control EDL.

Two months after partial denervation, the adult rats were anaesthetized under halothane (2% in O_2) and prepared for HRP injection. Animals were weighted before each injection to determine the amount of solution to inject. Using sterile conditions, incision of the skin was made and the muscle was gently separated from the surrounding muscles. The HRP solution was injected into the EDL muscles of both hindlimbs using a 10 μl Hamilton microsyringe. Care was taken to avoid any spread of the HRP solution. The injection area was clean and the injection was checked under dissecting microscope. The cutaneous incision was closed and after recovering from anaesthesia the animals were returned to their cage. Twenty-four to thirty hours later, the animals were deeply re-anaesthetised with chloral hydrate (4% solution, 1 ml/100 g body weight i.p.), both EDL muscles were removed and weighed and the animals perfused transcardially with 0.9% sodium chloride followed by a fixative (100 ml/100 g body weight) containing glutaraldehyde (2.5% in Millonigs phosphate buffer, pH 7.3). The sciatic nerve on the operated side was carefully dissected, traced back to the

exit of the L5 spinal nerve and examined under a dissecting microscope to verify that no reinnervation from L4 and no damage to the L5 spinal nerve occurred. The spinal cord was dissected and the control side was labelled with a thin pine placed into the right posterior horn. The spinal cord was post-fixed for 4h in the same fixative at 4 °C and cryoprotected in sucrose (30% in PBS) at 4 °C for 24-48 h. Frozen spinal cord was cut on a cryostat at 50 μm. The free floating sections were then processed, 2 to 24 h after, for HRP histochemistry using the modified Hanker-Yates method (Hanker et al., 1977) and lightly counterstained with a Nissl stain (gallocyanin).

Morphometric measurements of the labelled MNs.

The size of the cell body of a total of 204 MNs on the control side and 70 on the operated side was measured. Densely labelled MNs, the cell body of which fell entirely within the thickness of the histological section were drawn under a light microscope with a camera lucida attachment. The number of HRP-labelled MN in each ventral horn was counted. In order to avoid counting the same cell twice in consecutive sections, only those cells in which the nucleolus was clearly visible at high magnification were counted and measured. The cross-sectional area of the labelled MN was measured with a digitizing tablet linked to a computer for data analysis.

RESULTS

The transverse sections of the spinal cord of the animals injected with the HRP solution revealed the MN pool of the EDL muscles in the ventro-lateral horn. Figure 1 shows one example of the location of the HRP-labelled MNs on the right control side (marked with the pin hole in the dorsal horn) and left operated side of the spinal cord (Fig. 1A). At higher magnification (Fig. 1B, C), the histological section shows 6 heavily stained MNs with primary dendrites on the control side of the spinal cord (Fig. 1C) to be compared with the operated side (Fig. 1B) where only a single well stained MN is observed.

From the transverse sections of the spinal cord of the 3 rats, a total of 204 MNs innervating the EDL muscle on the control side and 70 MNs on the operated side were counted. MNs on both sides were densely labelled without apparent differences except in their number. Dendritic stems and first order branching were observable. The mean cell body area on the control side was 1085 $\mu m^2 \pm 21$ (± SEM, n = 204). In contrast, the mean cell body area on the operated side was 713 $\mu m^2 \pm 48$ (± SEM, n = 70). After partial denervation at 3 days, the size of the cell body area in the operated side represented 66% of the control, illustrating that the mean cell body area of the remaining MNs was significantly smaller ($p < 0.01$) compared to control. The two histograms of the distribution of the cell body area (Fig. 2) show the difference between the control MNs and the MNs in the operated side. The figure illustrates the shift in size towards smaller values of the remaining MNs (operated side) compared to the control side.

DISCUSSION

Our results show that in the adult rat, the size of the MNs innervating EDL muscles that were partially denervated at 3 days of age is around 34% smaller than the size of normal control MNs. This observation reveals that EDL MNs that survive, although programmed to become large phasic MNs, remain small at adult stage.

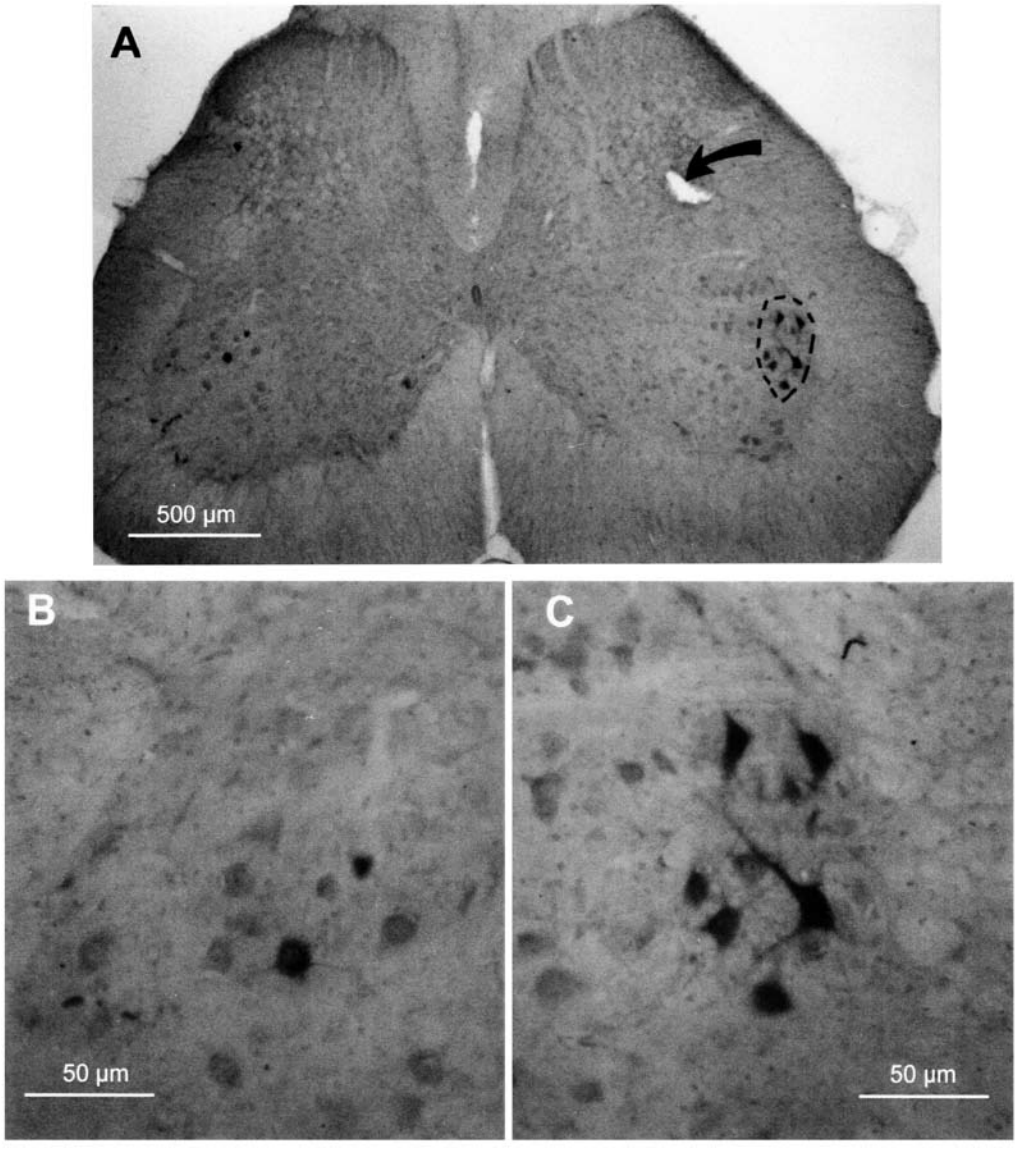

Fig. 1. - *HRP labelled EDL MNs following partial denervation.*
A: Photomicrograph of a 50μm transverse section of the spinal cord of a rat. The left EDL muscle of this animal was partially denervated at 3 days. The right side was left as the control and is identified by the hole in the dorsal horn (arrow). The MNs in the EDL pool are located in the ventro-lateral horn. B, operated side and C, control side at higher magnification.

The role of the muscle in the regulation of the morphology of the MNs during development has received little attention. Only a few studies have addressed the question. The effects of disconnection of neuromuscular contacts on MN morphology have been assessed by peripheral nerve-crush, rhizotomy, partial denervation and dorsal root section perfor-

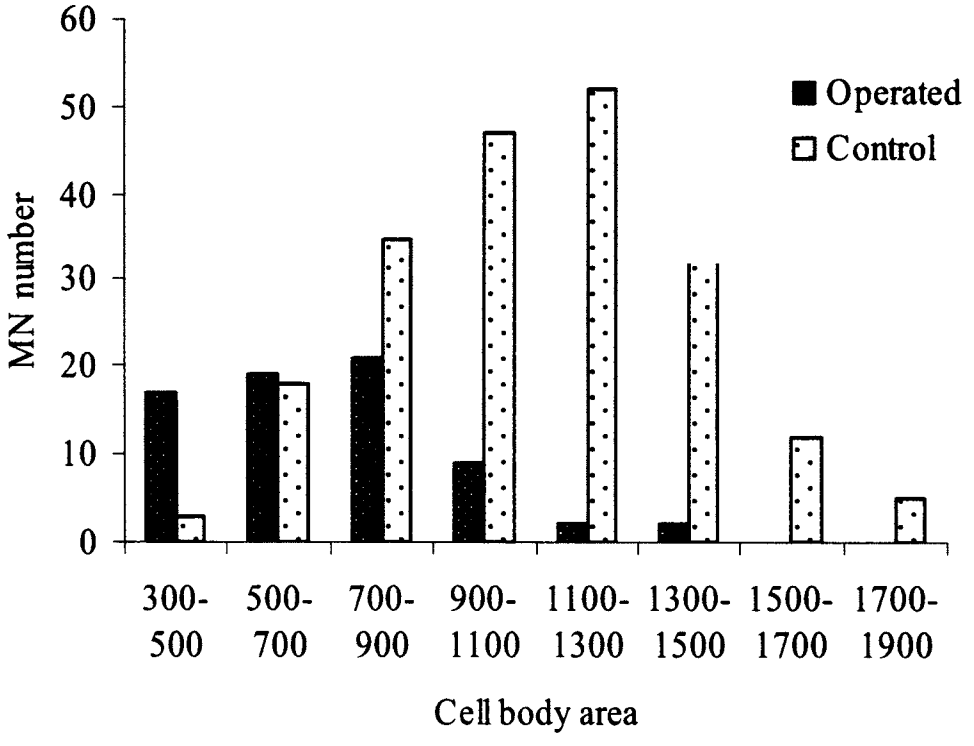

Fig. 2. - *Effect of partial denervation on the MN size.*
The two histograms show the size distribution of the MN cell body area (abcissae in μm^2) in control (n = 204) and operated sides (n = 70).

med at birth or at different times after birth. For example, using Sholl method (29), it was found that the total dendritic length was smaller than in control MN in the transverse plane whereas no significant change was observed in the longitudinal plane (23, 24) although a compensatory increase in total dendritic density in sectors which were not sampled could have occurred. These central effects were detected following nerve-crush but not after blockade of neuromuscular transmission alone (25, 26). The results obtained with different muscles and with various experimental procedures for measuring the size of the MNs are difficult to compare, providing no conclusive evidence.

Electrophysiological studies have shown that the total surface area of the motoneuronal membrane of slow MUs was 22% smaller than that of the fast MUs in adult (5). Furthermore, the branching of dendritic trees was less complex in the slow MUs than in the fast ones (6). In our experimental situation, the size of the soma of the surviving EDL MNs is similar to the size of the small MNs of the slow MU described by Cullheim et al. (5, 6), even though a drawback of our results is that only the soma of the EDL MNs is measured giving no information about the spatial extension and the complexity of their dendritic trees.

However, the relationship i.e. a small soma, a small dendritic field observed in different types of MNs (40, 14, 28, 38, 39) is so strong that the somatic area can be used to estimate the total extend and the surface area of the dendrites (21, 13). Thus, our measurements of the somatic diameters and surface area are a good prediction of the overall size and complexity of the MNs.

An interesting difference is observed between the effects of partial denervation performed at the same stage of development on the slow Soleus muscle and on the fast EDL muscle. In the Soleus muscle, 20% of the MNs survive and are capable of inducing a four time expansion of the MU size while no effect on the size of their soma is observed (20). The increase in size of the MU does not seem to interfere with the activity pattern of the remaining MNs which remains tonic (30, 31). In contrast, partial denervation of the EDL muscle induces a reduction of the MU size and a complete transformation of the MU activity from fast to slow (34, 32). The difference between the responses of the two muscles is an increase in the MU activity in the EDL muscle. It may be caused by the fact that, after partial denervation, the incoming afferent inputs converge to a smaller number of MNs since the injured MNs have died. This increase in MU activity enhances the withdrawal of the nerve terminals, thus inducing a reduction of the peripheral field which may, in turn, restrict the MN growth. The surviving MNs remain small in size according to the size principle (10, 11) stating that tonic and most active MNs are small.

SUMMARY

Our previous studies have shown that partial denervation of extensor digitorum longus muscle (EDL) in the rat at 3 days of age causes an increase in the activity of the intact motoneurons. The originally phasic pattern of activity of EDL became tonic after partial denervation. These modifications of motoneuron activity were associated with the change in the phenotype of the muscle from fast to slow contracting and with a conversion of the muscle fibres from a fast to a slow type. The present study investigates whether the size of the cell body of the active EDL motoneurons change in parallel with the altered muscular activity. The study involved partial denervation of rat EDL muscle by section of the L4 spinal nerve at 3 days of age. Then the remaining motoneurons from L5 spinal nerve supplying the EDL muscle were retrogradly labelled with horseradish peroxidase two months later. The results show a reduction in motoneuron size in parallel with an increase in activity of the motoneurons after partial denervation of EDL muscle.

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