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Permanent Alterations of Spinal Cord Reflexes Following Nerve Lesion in Newborn Rats

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Summary

Sciatic nerve lesion in newborn rats is known to cause degeneration of a large number of axotomized motoneurones and spinal ganglion cells. Some of the surviving motoneurones exhibit abnormal firing properties and the projection pattern of central terminals of sensory neurones is altered. We report here on long-term changes in spinal cord reflexes in adult rats following neonatal nerve crush. In acutely spinalized and anaesthetized adult rats 4-6 months old in which the sciatic nerve had been crushed on one side at birth, the tibial nerve, common peroneal nerve or sural nerve were stimulated on the reinnervated and control side and reflex responses were recorded from the L5 ventral spinal roots. Ventral root responses (VRRs) to tibial and peroneal nerve stimulation on the side of the nerve lesion were significantly smaller in amplitude representing only about 15 % of the mean amplitude of VRRs on the control side. The calculated central delay of the first, presumably monosynaptic component of the VRR potential was 1.6 ms on the control side while the earliest VRR wave on the side of the nerve lesion appeared after a mean central latency of 4.0 ms that seems too long to be of monosynaptic origin. These results suggest that neonatal sciatic nerve injury markedly alters the physiological properties and synaptic connectivity in spinal cord neurones and causes a marked depression of spinal cord responses to peripheral nerve stimulation.

Key words

Neonatal nerve lesion • Spinal reflexes • Neuronal cell death • Motoneurones • Nerve conduction velocity

Introduction

Romanes (1946) was one of the first to report that peripheral nerve lesions in newborn mice lead to a considerable loss of spinal motoneurones. A more detailed study was published by Zelená and Hník (1963) in which the number and size of regenerating motor and sensory nerve fibres, and the number of muscle spindles and Golgi tendon organs was reported in the soleus muscle of adult rats following sciatic nerve crush at birth. These authors found that the number of regenerated motor fibres was reduced by 50 % and their mean diameter was decreased by 25 %. The muscle weight of reinnervated muscles in adulthood was only 40 % of that of control muscles. The muscle weight loss following neonatal nerve crush was confirmed by Vejsada *et al.* (1991) in the soleus (36 % of control muscles) and provided information about the lateral gastrocnemius (56 %) and tibialis anterior (25 %). The latter value in the tibialis anterior muscle (TA) corresponded closely to that

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reported by Navarrete and Vrbová (1983) and Lowrie *et al.* (1987) in another ankle flexor muscle, the extensor digitorum longus (EDL), which became permanently reduced to 20-30 % after neonatal nerve crush. It is interesting to note that such unsuccessful nerve regeneration and muscle reinnervation occurs in the rat only after sciatic nerve crush performed during the early postnatal critical period, since a similar lesion performed at the age of 5-6 days reduces EDL muscle weight loss only to 50 % (Lowrie *et al.* 1987). If the nerve crush is performed after the age of 10 days postnatally, nerve regeneration progresses almost normally with minimal loss of the axotomized motoneurones and good recovery of muscle weight as in adult animals (Kuno and Llinas 1970, Gallego *et al.* 1979, Lowrie *et al.* 1994). Schmalbruch (1988) came to similar conclusions in a summary of his previous work and furthermore pointed out that neonatal nerve section resulted in more serious loss of motoneurones and dorsal root ganglion cells than neonatal sciatic nerve crush.

The situation after neonatal nerve crush in rats is even less satisfactory as far as muscle sensory functions are concerned. Zelená and Hník (1963) found that although the number of sensory nerve fibres in the regenerated soleus nerve was only reduced by 12 %, their mean fibre diameter was 50 % smaller. Bondok and Sansone (1984) reported an even greater loss of sensory neurones in spinal ganglia L4 and L5 after neonatal sciatic nerve crush, which predominantly afflicted the largest neurones. The neonatal period is apparently critical for the development and maturation of muscle stretch receptors for which the presence of primary afferents is essential. Zelená and Hník (1963) reported that the reinnervated soleus muscle contained virtually no muscle spindles (2.4 on the average) in comparison with 15 in the normal muscle and only an occasional Golgi tendon organ (0.2 on the average) as compared with 14 in control muscles. The spindles occasionally found were dwarfish in size and atypical in structure containing mostly only one intrafusal muscle fibre instead of the usual four. The remaining myelinated sensory fibres ended in the soleus muscle as free sensory endings (Zelená and Hnik 1963). The function of the atypical spindles was later studied and these were found to respond to muscle stretch by a dynamic and static response, but developed considerably slower frequency responses than normal spindles (Paleček *et al.* 1989). The conduction velocity of regenerated nerve fibres innervating these atypical spindles was 50 % slower on the average than that of normal primary afferents. In a subsequent report, the number of atypical spindles was found to be between 0 to 2 (1.1±0.3, mean±S.E.M.) in the soleus and 1 to 2 in the TA muscle (1.6±0.3) (Vejsada *et al.* 1991).

In the present report we studied reflex ventral root responses to peripheral nerve stimulation in order to elucidate in more detail the actual changes occurring in the connectivity of flexor and extensor spinal cord circuits in this situation. Some of the results were partly published elsewhere in preliminary form (Hník *et al.* 1997).

Method

Animals and experimental procedure

Ten newborn rat pups (day 0) of the Sprague-Dawley strain of either sex were anaesthetized by hypothermia on an ice-cooled Petri dish (see Eriksson *et al.* 1994, Li *et al.* 1994, Iwasaki *et al.* 1995). The sciatic nerve on the right side was crushed with a fine watchmakers forceps in midthigh and the slit in the skin was closed with an atraumatic needle suture. The pups were then returned to their mothers. After a period of about 10 days, during which the denervated paw was flaccid, dorsiflexion and plantar flexion of the ankle returned, although the toe spreading reflex was missing.

At the age of 4 to 6 months, the rats were anaesthetized by an intraperitoneal injection of chloralose (Abbott, U.K.) in a dose of 60 mg/kg which was supplemented during the experiment with approximately 30 % of the initial dose when necessary. The animals were then spinalized between T8-T10 and the spinal cord and roots were disclosed by laminectomy L2-L6. The posterior tibial nerves (T), common peroneal (CP) and sural (S) nerves were dissected free on both sides, transected peripherally and stimulated *via* bipolar platinum electrodes with single pulses of 0.2 ms duration and supramaximal intensity for eliciting a reflex response in the central stump of ventral roots L5. This segment provides the major motor innervation of the lower leg muscles (Vejsada and Hník 1980). The ventral root responses were recorded with bipolar platinum electrodes. The nerves, roots and spinal cord were immersed in paraffin oil and the temperature of the pools, formed by skin flaps, was maintained at 37 °C by radiant heat. The heart rate, respiration and rectal temperature were monitored throughout the experiment and served as indicators for supplementing anaesthesia.

Evaluation of ventral root responses (VRRs)

In each animal, the VRRs to single stimuli applied to individual nerves on the operated side was compared with the responses on the control, non-operated side. We evaluated the latency of the first VRR component to stimulation of each nerve. Furthermore, we measured the total duration of the VRR polysynaptic component in response to single stimuli applied to the nerves. The duration of the compound action potential in the dorsal roots was also evaluated to determine the dispersion of the conduction velocity of afferent nerve fibres. The maximum conduction velocity of motor nerve fibres present in the three nerve studied was also determined by stimulating the cut central nerve stumps and recording from the peripheral stump of L5 ventral roots. Finally, the central intraspinal delay was calculated by recording the cord dorsum potential at the entry of the L5 dorsal root into the spinal cord and the response in the L5 ventral root. The conduction time necessary for the action potential due to the largest motor fibres to reach the proximal electrode on the ventral root was subtracted to obtain the intraspinal delay.

From these data, mean values ± S.E.M. were calculated for each of the above VRR parameters obtained from five to eight animals and compared with the control side.

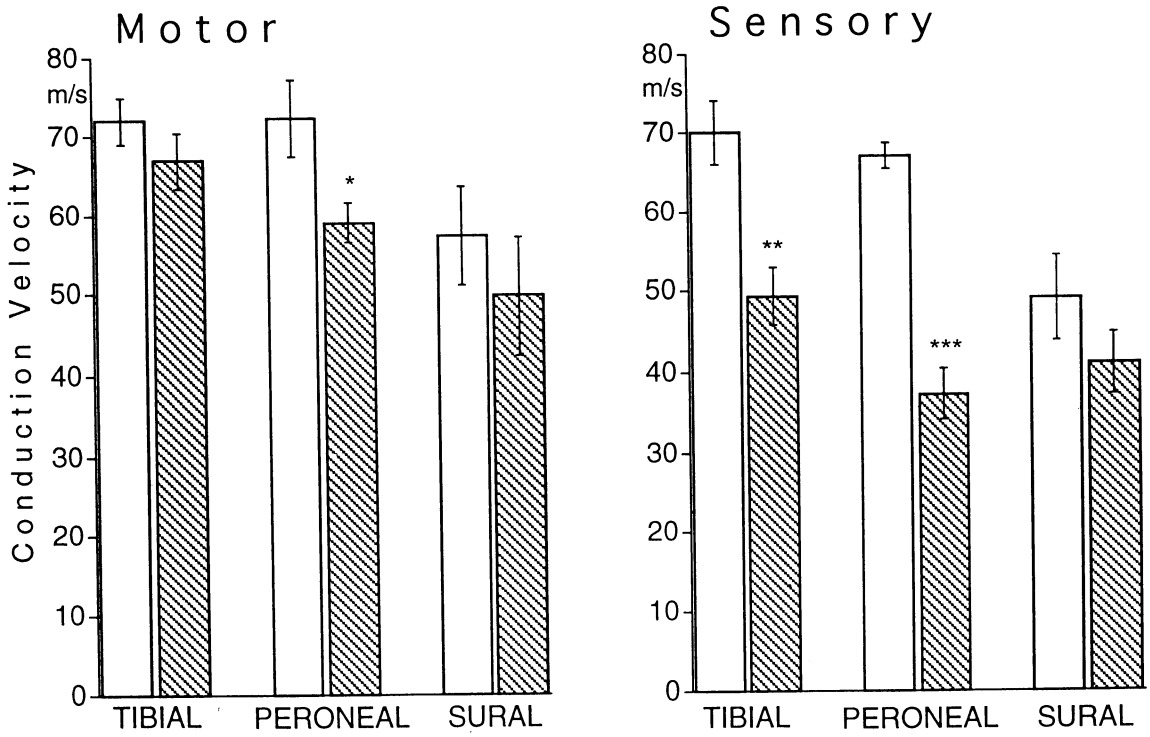

Fig. 1. *Conduction velocities (in m/s) of fastest motor and sensory nerve fibres in the tibial, common peroneal and sural nerves on the control side (open columns) and reinnervated side (hatched columns) in adult rats after neonatal sciatic nerve crush. The conduction velocity of motor fibres was only significantly slower in the peroneal nerve on the reinnervated side (* $p<0.05$). The fastest sensory nerve fibres conducted at a slower rate as compared to the control side (** $p<0.01$) in the tibial nerve and even more slowly in the peroneal nerve (*** $p<0.001$). The difference in the sural nerve was not statistically significant. Data are means ± S.E.M.*

Results

General locomotor activity

Rats with unilateral neonatal sciatic nerve crush were kept in the same cage with their mothers until weaning, when they were kept apart according to sex. On cursory inspection, their locomotor activity appeared normal, the only difference observed was the smaller girth of the shank and calf musculature due to considerable muscle atrophy. As has already been mentioned above, the toe spreading reflex could not be elicited on the side of the nerve crush.

Maximum conduction velocity in motor and sensory nerve fibres in the regenerated nerve

The conduction velocity (CV) of motor nerve fibres was measured by stimulating the central stump of each nerve and recording from the peripheral stump of

the cut L5 ventral root on both sides. This procedure was also used for assessing the CV in fastest sensory fibres by recording from the site of entry of L5 dorsal root into the spinal cord. As can be seen from Figure 1, the fastest conducting fibres in each nerve were those on the control side. However, the CV values in motor nerve fibres were only significantly slower in the peroneal nerve, while those in the tibial nerve and sural nerve were not significantly altered. A marked slowing down of sensory nerve fibres was found in the tibial nerve ($p<0.01$) and peroneal nerve ($p<0.001$), but again this was not significant in the sural nerve (Fig. 1). We also measured the duration of discharges in the dorsal root L5 to stimulation of the three nerves studied.

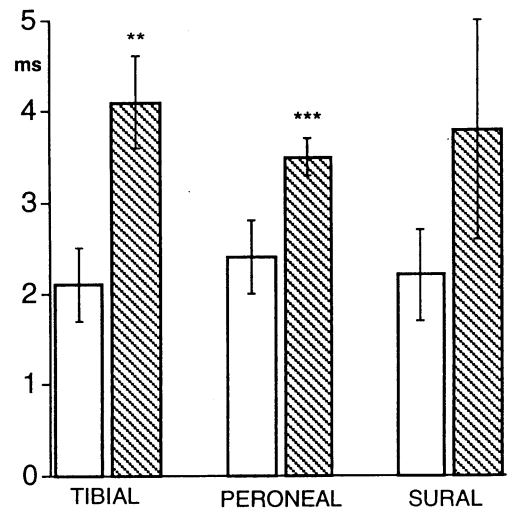

Fig. 2. *Duration of discharges in dorsal root L5 recorded close to its entry into the spinal cord. The dispersion of the responses to single volleys applied to each of the nerves on the reinnervated side was significantly prolonged for $p<0.01$ to stimulation of the tibial nerve and for $p<0.001$ to that of the common peroneal nerve as compared to the control side. The differences to sural nerve stimulation were not statistically significant. For other legend see Figure 1.*

Duration of discharges in intact L5 dorsal roots to single stimuli to the individual nerves

The duration of afferent volleys reaching the spinal cord were significantly prolonged on the reinnervated side as compared to the control side, with the exception of the responses from the sural nerve (see Fig. 2). The greater dispersion of afferent discharges arriving at the entry of dorsal roots L5 is apparently due to differences in conduction velocity of regenerated sensory nerve fibres after neonatal nerve crush, characterized by a marked reduction in the number of the largest group I afferents (see also the Introduction).

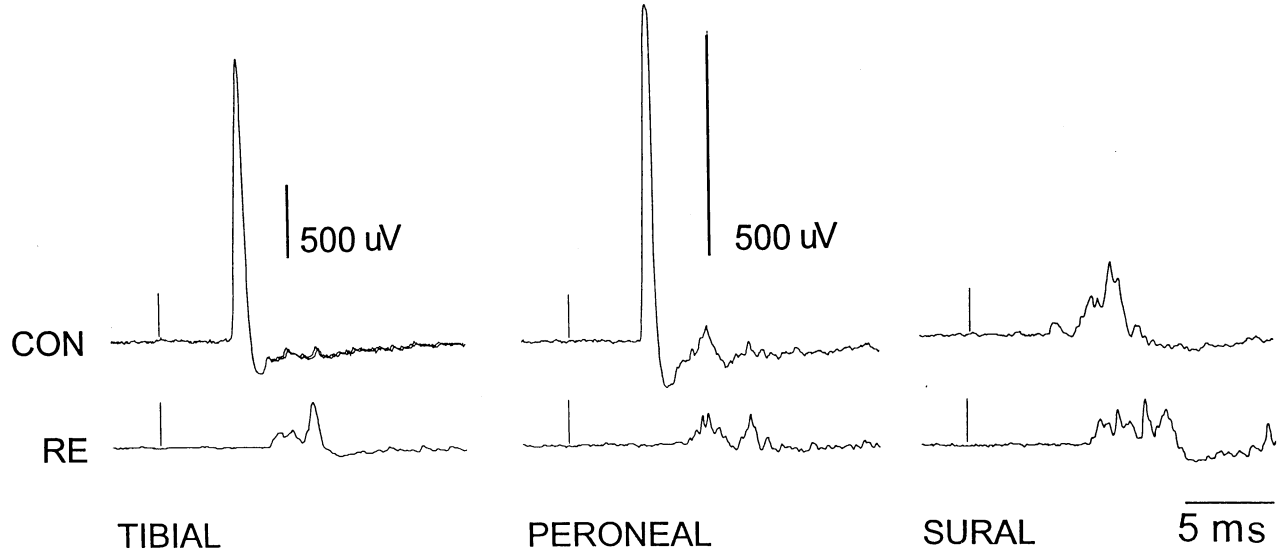

Fig. 3. *Representative ventral root reflex responses (VRRs) to single nerve volleys applied to the central stump of the tibial nerve, common peroneal nerve and sural nerve on the control side (CON) and reinnervated side (RE) in adult rats after neonatal sciatic nerve crush. Arrows indicate the stimulus artifact. VRRs were recorded from the central stump of ventral root L5. The voltage calibration for Per and Sur nerves is common for both nerves. The time calibration applies to all six records.*

Ventral root responses

Representative VRRs to stimulation of individual nerves, the posterior tibial, common peroneal and sural nerve, on the control and reinnervated side are shown in Figure 3. While the VRRs on the control side had a clear-cut monosynaptic component, which was followed by a smaller and longer-lasting polysynaptic response, the VRRs on the reinnervated side in response to tibial, peroneal and sural nerve stimulation were much smaller in amplitude compared with the control side. Furthermore, the first potential wave appeared with a much longer latency and the amplitude of this fastest reflex reponse was, as a rule, smaller than the subsequent components of the response. The mean amplitude of the maximum VRR potential was only 12.5 % of the control side to stimulation of the tibial nerve, 16 % to peroneal nerve stimulation and 38.9 % to that of the sural nerve (see Fig. 4)

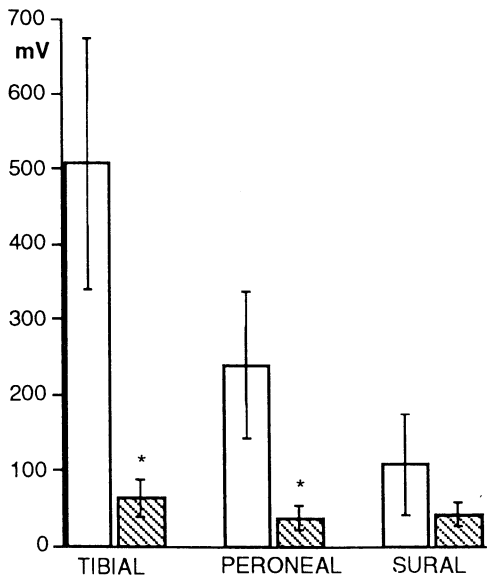

Fig. 4. *The maximum amplitude of VRRs in the tibial, common peroneal and sural nerves on the control side (open columns) and the side of neonatal nerve crush (hatched columns). The asterisks represent the statistical significance of differences between control and reinnervated side for $p<0.05$. The differences in the sural nerve were not statistically significant.*

Central synaptic delay in the spinal cord

In order to assess the actual duration of transmission of VRRs within the spinal cord, the time taken from the entry of the respective nerve volleys into the spinal cord was assessed by recording cord dorsum potentials at the entry of L5 dorsal roots. The recording electrodes placed on the ventral roots L5 and the distance from the exit of the ventral roots to the proximal recording electrode was measured and the conduction time required for the first component of the VRR to reach the proximal recording electrode was subtracted. The central synaptic delay plus conduction within the spinal cord was then calculated. As can be seen in Figure 5, the central delay of VRRs to tibial nerve and common peroneal nerve was longer on the reinnervated side by 166% and 160%, respectively. The central delay of the response to stimulation of the sural nerve was also longer (see Fig. 5), but this difference was not statistically significant.

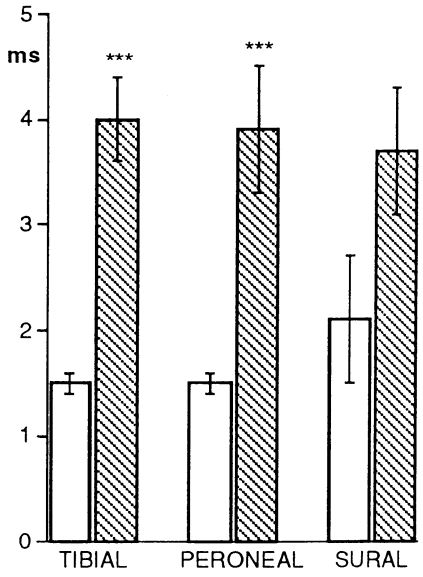

Fig. 5. *Central delay of VRRs in the spinal cord calculated as the difference between arrival of the peripheral nerve volley at the entrance of L5 dorsal root into the spinal cord and the first component of the VRR response minus time for conduction in the central stump of the L5 ventral root. The central delay on the reinnervated side was greatly prolonged in response to both tibial and common peroneal nerve (for $p<0.001$), but was not statistically significant for sural nerve stimulation. For other legend see Figure 1.*

Discussion

It has been shown in the present report that ventral root responses (VRRs) to stimulation of any of the three peripheral nerves (tibial, common peroneal and sural) on the side of neonatal sciatic nerve crush are much

reduced in amplitude and their central latency is prolonged. This concerns both the reflex arc to extensor (stimulation of the tibial nerve) as well as to the flexor muscles (stimulation of the common peroneal nerve). The responses to sural nerve stimulation in general followed this pattern, but the changes were not as marked. Actually, the fact that VRRs can be elicited by stimulation of the sural nerve is not so surprising since in the rat, contrary to man, the sural nerve contains an anastomotic branch joining the lateral plantar nerve and contains a small number of motor nerve fibres innervating the abductor digiti quinti, flexor digiti V brevis and lumbrical muscles (Peyronnard and Charron 1982), representing only 3 % of motoneurones contained in the motoneurone pool supplying the sciatic nerve in the rat (Swett *et al.* 1986).

Peripheral nerve lesions in adult animals lead only to a temporary decrease in the amplitude of monosynaptic excitatory postsynaptic potentials (EPSPs) (Eccles *et al.* 1959, Kuno and Llinas 1970) and this depression tends to return to normal within 8 weeks after sciatic nerve crush (Gallego *et al.* 1979). However, nerve crush in 1-day-old rats has been reported subsequently to reduce monosynaptic EPSPs in corresponding motoneurones of the injured medial gastrocnemius nerve, or these motoneurones exhibited no EPSPs at all in adult rats (Miyata *et al.* 1986). This has been ascribed to a permanent reduction of the monosynaptic input in adulthood after neonatal nerve crush. The present results seem to fit in with this interpretation. It is unlikely that the first component of the VRRs to tibial and peroneal nerve stimulation is monosynaptic in origin since 1) the central transmission time in the spinal cord on the side of neonatal nerve crush is considerably delayed and 2) it is practically never the largest component of the response.

Despite the markedly reduced amplitude of VRRs on the side of previous neonatal sciatic nerve crush, which is apparently due to altered intraspinal connectivity, locomotion appeared normal in these rats. However, in an EMG study, Navarrete and Vrbová (1984) reported abnormal EMG activity in the extensor digitorum longus muscle (EDL), a flexor of the ankle, at rest. This was subsequently confirmed in the tibialis anterior muscle (TA, another flexor muscle) by Vejsada *et al.* (1991). Furthermore, we previously reported that the reinnervated TA muscle was activated abnormally during locomotion, with an appropriate burst during the swing phase and an „extensor-like" burst during the stance phase of each step, while the TA muscle of the control side only fired during the swing phase. Besides this, the reflex EMG responses to plantar flexion or dorsiflexion of the ankle were very weak or absent on the reinnervated side. This was ascribed to the fact that both the soleus muscle and TA muscles were almost devoid of spindles (Vejsada *et al.* 1991). These results are in agreement with the present findings concerning the considerable depression of VRRs.

There is, however, a discrepancy with the findings of Navarrete *et al.* (1990) who reported that stimulation of branches of the injured but regenerated sciatic nerve elicited ipsilateral reflex EMG responses in the reinnervated EDL muscles that were about three times larger than those from the uninjured side. These authors thus concluded that the reflex responsiveness of the injured flexor motoneurones is enhanced after neonatal sciatic nerve section. At present, it is difficult to explain the conflicting results of Navarrete *et al.* (1990) with our findings. It is unlikely that the difference between their findings and the present results is due to the general anaesthetic employed. Chloralose used in our experiments is known not to depress reflex activity in the spinal cord but, on the contrary, to enhance monosynaptic reflexes (Shimamura *et al.* 1968) and the responses on the control, non-operated side were certainly unaffected. Another possibility of explaining the discrepancy between our results and those of Navarrete *et al.* (1990) could concern the spinal cord transection in our experiments. However, this did not affect VRRs on the control side and it is unlikely that it would have a depressing effect on the side of neonatal nerve crush. Nevertheless, this controversy would deserve further investigation. On the basis of our own results we feel entitled to conclude that the spinal cord connectivity following neonatal nerve crush in rats is clearly grossly depressed. This concerns both extensor and flexor motoneuronal pools.

Acknowledgements

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Reprint requests

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