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Failure of Regeneration of Sensory Nerve Fibers Following Neonatal Denervation and Crush Lesion in Rats

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Abstract: The effects of chronic neonatal denervation and a crush lesion on the regenerative capacity of sensory nerve fibers were studied in rats. After 50 weeks of postnatal denervation, the amplitudes of compound action potentials (CAPs) of Aαβ-, Aδ- and C-fibers were reduced to 14.3%, 18.2% and 37.4%, respectively ($P < 0.005$). Similarly, after a crush lesion, the amplitudes of CAPs were reduced to 34.3%, 38.8% and 51.6%, respectively ($P < 0.005$). The conduction velocities, thresholds and maximum strength of stimulation of nerve fibers were normal. Neurogenic plasma extravasation into the skin due to antidromic nerve stimulation was reduced to 27.5% and 30.3% after postnatal denervation and the crush lesion, respectively ($P < 0.005$). These results indicate that neonatal sensory nerve injury induces permanent neurodegeneration of peripheral sensory nerve fibers as demonstrated by the inhibition of afferent conduction property and efferent neurogenic plasma extravasation response.

Key Words: Saphenous nerve, neonatal rat, denervation, crush injury

Introduction

In adult rats, all myelinated and about 60-70% of unmyelinated nerve fibers in the peripheral nerves have been shown to successfully regenerate after chronic denervation and a crush lesion (1-12). The regenerated nerve fibers almost retain their afferent conduction property (3,13-14) and efferent neurogenic plasma extravasation response in the skin (3-5,15-18). The regeneration mechanism of peripheral nerve fibers is known to be facilitated by Schwann cells and their basal lamina that guide the growing terminals of injured nerve fibers (19-21). However, in newborns where the nervous system is not completely established it is not known whether the damaged nerve fibers are able to grow towards their proper target tissues. The aim of the present study was to investigate the regeneration ability of sensory nerve fibers after neonatal denervation and a crush lesion in rats.

Materials and Methods

Experimental animals and operations

The experiments were carried out on 4 groups of newborn (1-2 day old) white albino rats (*Rattus rattus*). During short operations under aseptic precautions, the animals were anesthetized with ether and the saphenous nerve in the mid thigh of the right hind limb was exposed. In the first group (denervated) (n = 28), the nerve was sectioned with fine scissors and the 2 stumps were positioned opposite one another. In the second group (crushed) (n = 25), the nerve was forcefully crushed several times for 30 s with watchmaker forceps. In the third group (sham) (n = 25), the nerve was just exposed without any injury or manipulation. At the end of the operations, the skin and muscles were closed in layers and the rats were allowed to recover. They were left for various postoperative periods (12, 20 and 50 weeks). A fourth unoperated (control) group (n = 5) was used to induce the normal neurogenic plasma extravasation response into the skin due to antidromic nerve stimulation for comparison with the operated groups.

Electrophysiological preparation

At 12, 20 and 50 weeks postoperatively, the rats were anesthetized with urethane (1.5 g/kg, i.p.), and the trachea and left common carotid artery were cannulated. Body temperature was maintained at approximately 37 °C using a heating blanket system under the animal, controlled by a rectal thermistor probe (Harvard Apparatus). The fur on the medial aspect of the right operated leg was clipped, the skin opened and the

saphenous nerve exposed. For electrical stimulation, a 5 mm segment of the nerve was gently dissected from the connective tissue at a distal site above the knee and placed on a pair of bipolar platinum wire hook electrodes. For electrical recording of compound action potentials (CAPs), the nerve was exposed proximally in the upper thigh, cut and placed on a similar pair of platinum electrodes. The whole nerve was covered by a warm liquid-paraffin pool made from skin flaps sutured to a metal ring. Electrical stimulation of myelinated nerve fibers was performed at 0.17-0.45 mA, 0.05 ms pulse width and 10 Hz frequency; while the unmyelinated nerve fibers were stimulated at 1.75-2.90 mA, 0.5 ms pulse width and 1 Hz frequency. CAPs were conventionally amplified, displayed on a digital storage oscilloscope (Tektronix 2232) and stored in a computer using Labview software (National Instruments).

Neurogenic plasma extravasation to antidromic stimulation

After the recording of the CAPs of nerve fibers, neurogenic plasma extravasation into the skin due to antidromic nerve stimulation was induced in each animal. The stimulating and recording electrodes were exchanged with each other; the stimulating electrodes were placed at the proximal cut end of the nerve, while the recording electrodes were placed at its distal exposed site. Evans blue dye (50 mg/kg) in saline was administered through the cannulated common carotid artery. After 10 min, the nerve was stimulated for 5 electrical pulses (at maximum C-fiber strength for 30 s each) at 1 min intervals. Twenty minutes later, the rats were killed by bleeding from the cannulated carotid artery and small skin pieces were excised from the skin in the medial aspect of the right leg within the innervation zone of the saphenous nerve. Background comparison of Evans blue extravasation was performed by dissecting similar pieces of skin from the corresponding skin in the contralateral aspect of the left leg in the same animal. Skin samples were placed in 3 mL of dimethylformamide (BDH) for 24 h at room temperature and the dye content (μg/g) was determined photometrically at wavelength 620 nm. Data are expressed as mean ± S.E.M., and statistical significance was considered $P < 0.05$ using Student's *t*-test.

Results

Compound action potentials

The amplitudes of the CAPs of Aαβ-, Aδ- and C-nerve fibers in the rat saphenous nerve were severely reduced after 12, 20 and 50 weeks of postnatal denervation and crush lesion as compared to those in sham operated nerves (Figures 1-3). The mean amplitudes of CAPs after postnatal denervation, the crush lesion and sham operations are shown in Figure 4. The mean amplitudes

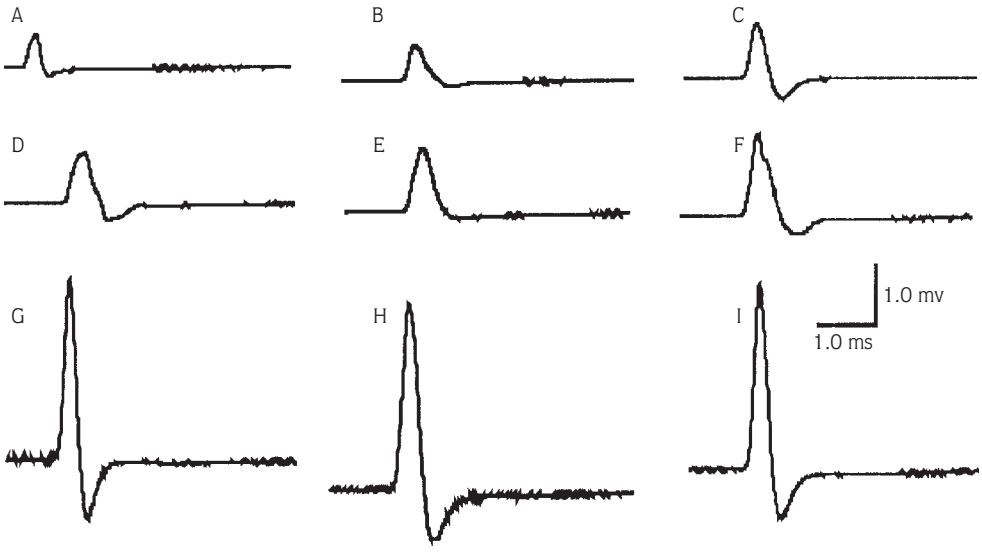

Figure 1. CAPs of Aαβ-fibers of the rat saphenous nerve after 12, 20 and 50 weeks of postnatal denervation (upper traces), crush lesion (middle traces) and sham operations (lower traces).

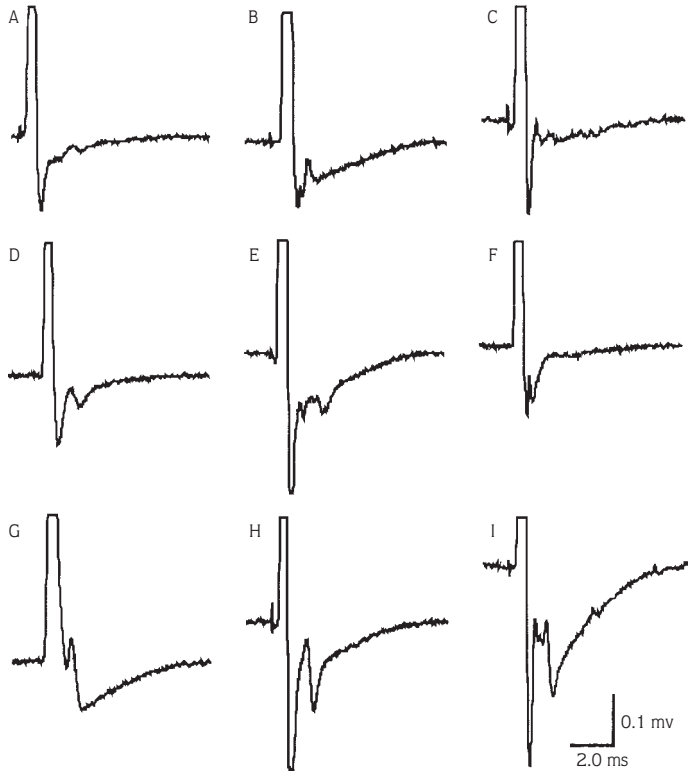

Figure 2. CAPs of Aδ-fibers of the rat saphenous nerve after 12, 20 and 50 weeks of postnatal denervation (upper traces), crush lesion (middle traces) and sham operations (lower traces).

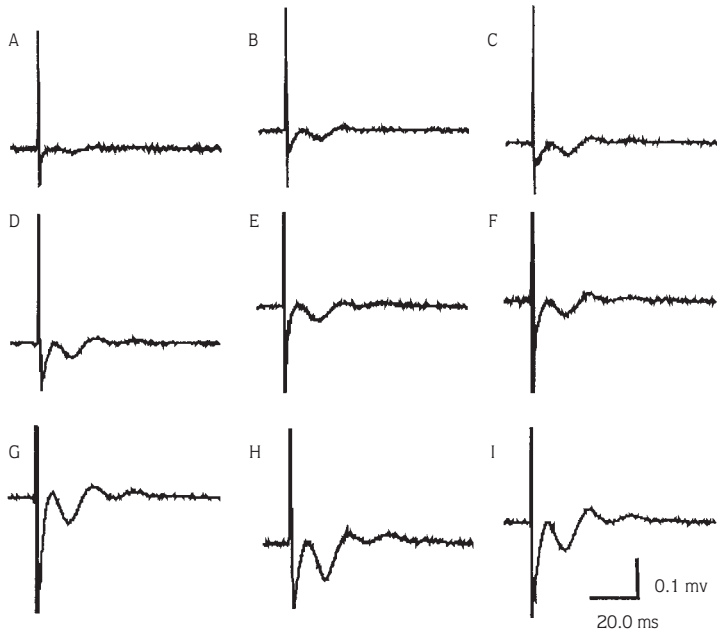

Figure 3. CAPs of C-fibers of rat saphenous nerve after 12, 20 and 50 weeks of postnatal denervation (upper traces), crush lesion (middle traces) and sham operations (lower traces).

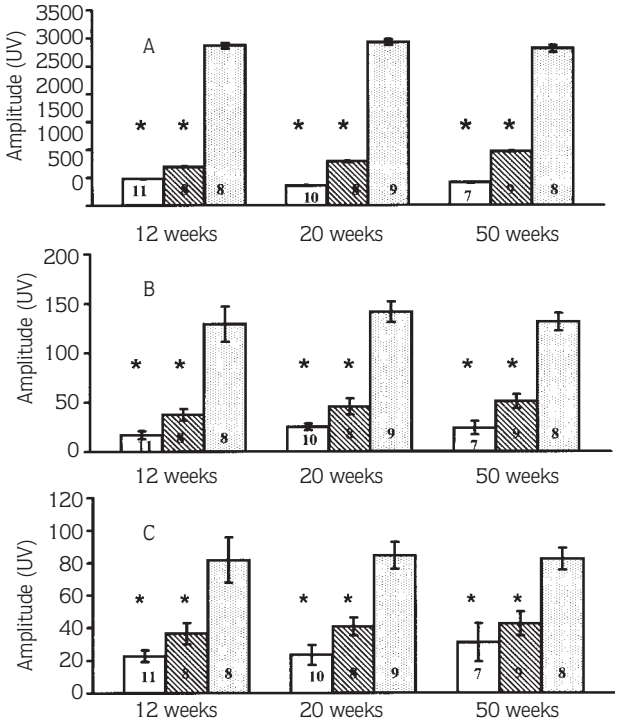

Figure 4. Mean amplitudes of the CAPs of Aαβ-fibers (A), Aδ-fibers (B) and C-fibers (C) of the rat saphenous nerve after 12, 20 and 50 weeks of postnatal denervation (open columns), crush lesion (hatched columns) and sham operations (stippled columns). Standard error bars are shown.

of the CAPs of myelinated nerve fibers after the crush lesion were significantly higher than those after denervation, $P < 0.005$ (for Aαβ-fibers) and $P < 0.01$ (for Aδ-fibers). However, the mean amplitudes of the CAPs of unmyelinated C-fibers after the crush lesion were not significantly different from those after denervation, $P > 0.25$. The means of the conduction velocities of nerve fibers are presented in Table 1. The variations in conduction velocities were different for each type of nerve fiber and were seen equally in all 3 groups of operated nerves. The means of the conduction velocities of the CAPs of Aαβ-nerve fibers were not significantly different from those after the sham operations ($P > 0.1$). However, the means of the conduction velocities of the CAPs of Aδ-nerve fibers ranged from $P > 0.1$ to $P < 0.005$ compared to those after the sham operations. In contrast, the means of the conduction velocities of C-nerve fibers were significantly different from those after the sham operations ($P < 0.005$). The thresholds and maximum strength of stimulation of all nerve fibers after chronic neonatal denervation and the crush lesion were normal, as shown in Tables 2 and 3, respectively.

Neurogenic plasma extravasation

Neurogenic plasma extravasation of Evans blue into the skin due to antidromic nerve stimulation was highly

Table 1. Conduction velocities (m/s) of the CAPs of Aαβ, Aδ- and C-nerve fibers of the rat saphenous nerve after 12, 20 and 50 weeks of postnatal denervation, crush lesion and sham operations. Mean ± S.E.M.

		Aαβ-fibers	Aδ-fibers	C-fibers
Denervation	12 weeks	62.63 ± 28.65 (11)	7.25 ± 0.70 (11)	0.76 ± 0.04 (11)
	20 weeks	44.56 ± 1.71 (10)	11.41 ± 0.65 (10)	1.02 ± 0.05 (10)
	50 weeks	48.94 ± 3.12 (7)	10.71 ± 0.99 (7)	1.63 ± 0.08 (7)
Crush	12 weeks	49.53 ± 4.20 (8)	10.04 ± 0.55 (8)	0.98 ± 0.03 (8)
	20 weeks	51.39 ± 1.76 (8)	10.18 ± 0.61 (8)	0.90 ± 0.03 (8)
	50 weeks	61.73 ± 8.86 (9)	13.25 ± 1.24 (9)	2.09 ± 0.11 (9)
Sham	12 weeks	52.50 ± 4.03 (8)	11.74 ± 1.24 (8)	1.03 ± 0.06 (8)
	20 weeks	53.24 ± 2.23 (9)	12.00 ± 0.67 (9)	1.21 ± 0.06 (9)
	50 weeks	65.51 ± 3.25 (8)	12.70 ± 0.7 (8)	2.12 ± 0.09 (8)

Table 2. Thresholds (mA) of the CAPs of Aαβ-, Aδ- and C-nerve fibers of the rat saphenous nerve after 12, 20 and 50 weeks of postnatal denervation, crush lesion and sham operations. Mean ± S.E.M.

		Aαβ-fibers	Aδ-fibers	C-fibers
Denervation	12 weeks	0.198 ± 0.01 (11)	0.23 ± 0.01 (11)	1.86 ± 0.03 (11)
	20 weeks	0.19 ± 0.01 (10)	0.22 ± 0.01 (10)	1.76 ± 0.02 (10)
	50 weeks	0.19 ± 0.03 (7)	0.22 ± 0.01 (7)	1.75 ± 0.03 (7)
Crush	12 weeks	0.20 ± 0.01 (8)	0.25 ± 0.01 (8)	1.76 ± 0.02 (8)
	20 weeks	0.20 ± 0.01 (8)	0.24 ± 0.01 (8)	1.75 ± 0.02 (8)
	50 weeks	0.20 ± 0.01 (9)	0.24 ± 0.01 (9)	1.77 ± 0.02 (9)
Sham	12 weeks	0.21 ± 0.01 (8)	0.27 ± 0.02 (8)	1.81 ± 0.02 (8)
	20 weeks	0.20 ± 0.01 (9)	0.25 ± 0.01 (9)	1.77 ± 0.01 (9)
	50 weeks	0.20 ± 0.01 (8)	0.25 ± 0.01 (8)	1.77 ± 0.01 (8)

Table 3. Maximum stimulation strength (mA) of the CAPs of Aαβ-, Aδ- and C-nerve fibers of the rat saphenous nerve after 12, 20 and 50 weeks of postnatal denervation, crush lesion and sham operations. Mean ± S.E.M.

		Aαβ-fibers	Aδ-fibers	C-fibers
Denervation	12 weeks	0.27 ± 0.02 (11)	0.36 ± 0.03 (11)	2.77 ± 0.24 (11)
	20 weeks	0.23 ± 0.01 (10)	0.28 ± 0.02 (10)	1.92 ± 0.06 (10)
	50 weeks	0.21 ± 0.01 (7)	0.29 ± 0.03 (7)	1.91 ± 0.05 (7)
Crush	12 weeks	0.24 ± 0.02 (8)	0.33 ± 0.03 (8)	2.08 ± 0.10 (8)
	20 weeks	0.21 ± 0.01 (8)	0.28 ± 0.01 (8)	1.89 ± 0.03 (8)
	50 weeks	0.22 ± 0.01 (9)	0.28 ± 0.01 (9)	1.92 ± 0.03 (9)
Sham	12 weeks	0.24 ± 0.01 (8)	0.37 ± 0.02 (8)	2.12 ± 0.07 (8)
	20 weeks	0.24 ± 0.01 (9)	0.33 ± 0.01 (9)	2.06 ± 0.06 (9)
	50 weeks	0.22 ± 0.01 (8)	0.33 ± 0.02 (8)	1.94 ± 0.04 (8)

significantly reduced after 12, 20 and 50 weeks of postnatal denervation and crush lesion, $P < 0.005$. Figure 5 shows the mean amounts of Evans blue dye (μg/g) extracted from the skin in the neonatal denervation, crush lesion, sham and unoperated (control) nerves.

Discussion

The present study showed that chronic neonatal denervation and a crush lesion to the rat saphenous nerve induced permanent degeneration of myelinated and unmyelinated nerve fibers, as demonstrated by severe reductions in the amplitudes of the CAPs of nerve fibers and inhibition of neurogenic plasma extravasation into the skin due to antidromic nerve stimulation. In adult rats (3,18) and rabbits (14) the transectioned peripheral nerve fibers were able to regenerate and retain their normal afferent conduction property and efferent neurogenic plasma extravasation response. However, in adult cats the regeneration of nerve fibers was almost

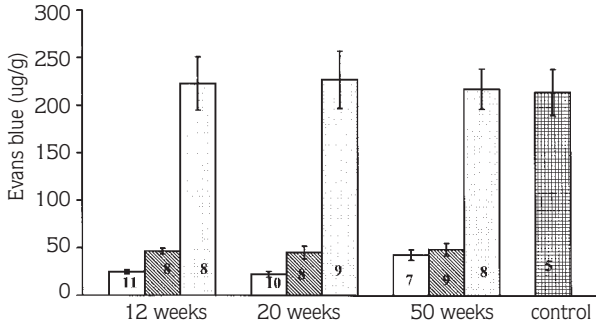

Figure 5. Neurogenic plasma extravasation of Evans blue dye (µg/g) into the rat skin due to antidromic stimulation of the saphenous nerve after 12, 20 and 50 weeks of postnatal denervation (open columns), crush lesion (hatched columns), sham operations (stippled columns) and unoperated- control (cross hatched column). Standard error bars are shown.

complete after a nerve lesion, but not after transection (19). It is known that Schwann cells play a role in the regeneration of peripheral nerve fibers that guide them to restore their original innervation pattern (20-21). Unlike in adults, the failure of the regeneration of peripheral sensory nerve fibers after neonatal injury found in the present study was probably due to the incomplete functional and anatomical development of nerve cells and their neuroglia Schwann cells during the early postnatal period. In the light of the present results, it seems that the regeneration ability of myelinated (but not unmyelinated) nerve fibers after a crush injury was slightly higher than that after denervation. This may support the above idea concerning the role of Schwann cells in forming guidance tubes for the growing terminals of the injured myelinated nerve fibers towards their proper targets. The variations in the conduction velocities of nerve fibers may reflect the degree of sprouting of different nerve fibers after injury depending on the diameter of the nerve fibers. For example, the conduction velocities of the CAPs of the injured C-nerve fibers were significantly less than those in sham operated nerves ($P < 0.005$), but not for the conduction velocities of Aαβ-nerve fibers ($P > 0.1$). However, the conduction velocities of Aδ-nerve fibers ranged between these 2 extremes, being from $P > 0.1$ to $P < 0.005$.

The failure of nerve fiber regeneration after neonatal injury is reflected in the severe reduction in the neurogenic plasma extravasation response in the skin. This property is a good indication of the extent of unmyelinated nerve fiber regeneration of the polymodal nociceptor class, but not for other unmyelinated or myelinated nerve fibers (3, 22). In conclusion, the present study showed that exposing the peripheral sensory nerve fibers to traumatic injury during early postnatal stages induces permanent degeneration of nerve fibers as demonstrated by a severe reduction in afferent conduction property and a loss of neurogenic plasma extravasation response.

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