Differences in the Neuronal Activity of Dorsal Horn Neurons between Rats Developing and Not Developing a Pain Syndrome after Sciatic Nerve Transection

G. N. Kryzhanovskii, V. K. Reshetnyak, M. L. Kukushkin, V. A. Zinkevich, and V. S. Smirnova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 119, № 5, pp. 470-474, May, 1995
Original article submitted May 25, 1994

After sciatic nerve transection, a generator of pathologically enhanced excitation is formed in dorsal spinal cord horns at the transection side in rats that have developed a pain syndrome but not in those that have not; in the latter rats, plastic changes occur instead and lead to intensified afferent inputs from tissues of the affected limb to the contralateral dorsal horns.

Key Words: *neurogenic pain syndrome; dorsal horns*

Our earlier studies showed [3,5] that the development of a neurogenic pain syndrome (PS) in rats after sciatic nerve transection is associated with the formation, in nociceptive structures, of a pathological algetic system - a new pathodynamic entity comprising altered components of the pain sensitivity system with heightened neuronal excitability and inadequate inhibitory control. We also found that such an algetic system did not form in rats without a manifest PS after sciatic nerve transection, even though afferent inputs to structures of the hemisphere contralateral to the transected nerve were increased [4,5]. To clarify the contribution of the primary relay to the changes mentioned above, we measured in the present study spontaneous and evoked neuronal activities in the spinal cord dorsal horns bilaterally in rats with and without PS after sciatic nerve transection.

MATERIALS AND METHODS

A total of 36 male Wistar rats weighing 180-220 g were used. The left sciatic nerve was transected under Hexenal anesthesia (30 mg/kg intraperitoneally) at the popliteal fossa level distal to the site of ligation, and the central end of the nerve was placed in a polyethylene capsule and left in the wound which was closed by suture. A PS was considered to have developed if autotomies were observed on the operated paw and reduced pain sensitivity thresholds were recorded in the hot plate test (the plate surface had a temperature of 55°C). On the basis of these two criteria, the rats were divided into two groups - with and without a PS. On days 5-30 after sciatic nerve transection, neuronal electrical activity in dorsal horn segments L_2-L_4 was recorded bilaterally. For this, rats under chloralose-urethan anesthesia (100 mg/kg chloralose and 1000 mg/kg urethan intraperitoneally) were each placed in a stereotaxic apparatus with rigid fixation of the head and spine, made immobile with a muscle relaxant (Myo-Relaxin), and artificially ventilated. Extracellular activity of single neurons was recorded monopolarly with glass microelectrodes having a tip diameter of 1-3 μ and filled with 2 M sodium chloride solution. The microelectrodes were each fixed in the micromanipulator of the stereotaxic apparatus and inserted to a depth of 1000 μ from the spinal cord surface per-

Laboratory of Pain Pathophysiology, Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

0007-4888/95/0005-0452$12.50

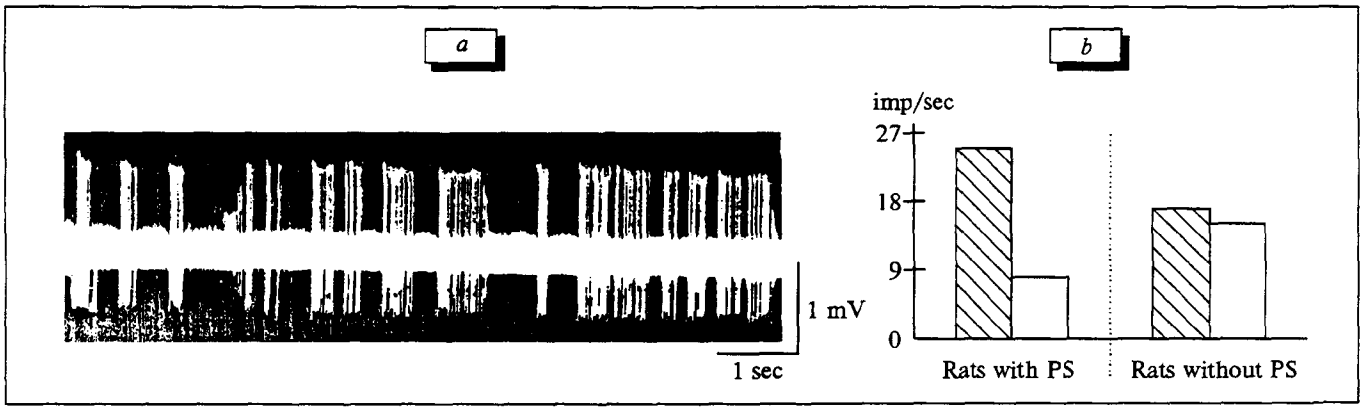

Fig. 1. Background neuronal activity in dorsal spinal cord horns 2 weeks after sciatic nerve transection. *a*) background neuronal firing pattern in the dorsal horn of segment L_4 on the transection side in a rat with PS; *b*) background neuronal firing frequency in rats with and without PS. Dark bars: neuronal activity on the transection side; white bars: neuronal activity on the contralateral side.

pendicular to it midway between the medial dorsal artery and the site of entry of the dorsal roots into the cord [13]. Neuronal activity was delivered, via a cathode follower, to the input of a VC-9 broadband amplifier and then taperecorded. Each neuron was tested as follows: after the background activity of the neuron had been recorded for 1-3 min and the depth to which the microelectrode was inserted had been noted, weak and strong mechanical and electrical stimuli were applied to both hind limbs and to the tail and changes in neuronal firing activity were recorded. For mechanical stimulation, the hair coat was gently touched with a glass rod or the skin was pricked with a needle or pinched with pincers. Electrical stimuli were 0.1-msec current pulses of 0.1 to 20 mA delivered via bipolar needle electrodes placed 5 mm apart. If the neuronal firing activity changed, we waited until the original background activity was restored and then proceeded to test the next neuron. A neuron that altered its activity in response to a weak mechanical or electrical stimulus but not to a strong stimulus was classified as one of low threshold. A neuron increasing its firing frequency in response to a strong mechanical (prick or pinch) or electrical stimulus was classed among neurons of a broad dynamic range. High-threshold neurons were considered to be those that responded only to mechanical or electrical stimuli sufficiently strong to activate A-Δ and C afferents [1].

In addition to extracellular activity of individual neurons, the total evoked potential (EP) in response to electrostimulation of both hind limbs through bipolar steel electrodes was recorded in each animal from the dorsal horns of segment L_4 bilaterally with monopolar glass microelectrodes (tip diameter 10-15 μ) filled with 2 M NaCl and inserted 200-300 μ deep to the spinal cord surface. This electrostimulation was carried out in symmetrical points above the nerve transection site using 0.1-msec rectangular pulses of 3-5 mA - current strengths that exceeded 2- to 3-fold the thresholds for generation of EPs. The recorded potentials were fed to the input of the VC-9 broadband amplifier and then averaged for 10 stimulus presentations using a specialized computer.

RESULTS

In rats that had developed PS, a total of 86 neurons were identified in the dorsal horns (46 on the left and 40 on the right). Of these, 28 left neurons and 21 right ones responded to peripheral stimuli of different strengths. The spontaneous (background) firing by dorsal root neurons on the side ipsilateral to the damaged nerve occurred at a significantly higher mean frequency ($p<0.01$) than that on the contralateral side (Fig. 1, *b*). Another feature of spontaneous neuronal activity on the side of nerve damage was the occurrence of discharges in the form of rhythmic bursts that gradually transformed into a prolonged (80 to 3200 msec) high-frequency tonic discharge (Fig. 1, *a*). Moreover, a significantly larger number of neurons were identified per recording track for each rat on the damaged nerve side than on the contralateral side. Thus, the activity of 5 to 9 neurons could be recorded in one track on the former side but only the activity of 1 or 2 neurons in the symmetrical track on the latter.

When receptive fields of neurons were examined, 9 neurons (32.1%) with broad receptive fields responding to mechanical stimulation of the left hind limb, sacrum, and tail were detected on the side of damage, and 2 of these were simultaneously

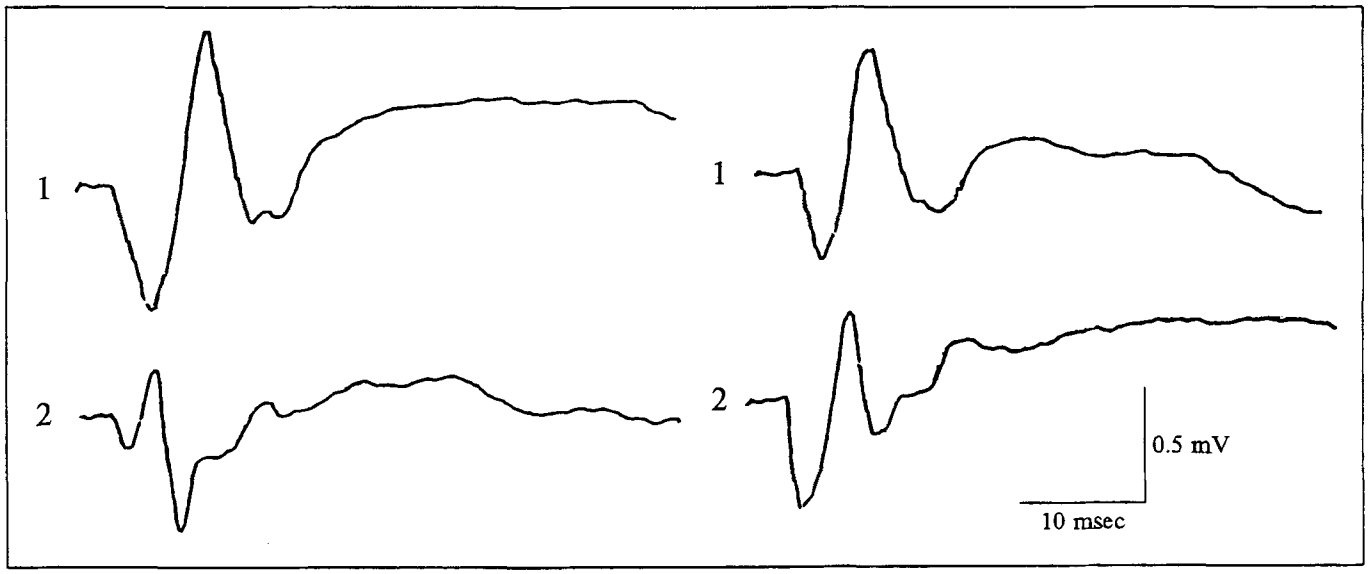

Fig. 2. Ipsilateral (*a*) and contralateral (*b*) EP in dorsal horns of a rat that developed a PS after sciatic nerve transection. Here and in Fig. 3: *1*) EP upon stimulation of the paw with transected sciatic nerve; *2*) EP upon stimulation of the contralateral paw.

activated from the right and left sides. In contrast, only 1 neuron with a broad receptive field was identified on the side contralateral to the cut nerve, and this neuron was activated upon stimulation of the entire skin surface of the right hind limb and the tail root.

Moreover, rats with PS tended to have a decreased proportion of low-threshold neurons (7.1%) and an increased proportion of neurons with a broad dynamic range (35.7%) on the damaged nerve side as compared to the contralateral side (41.6% and 8.3%, respectively). Also, the frequency of firing by neurons that responded to strong mechanical or electric stimuli was higher on the damaged nerve side, and the firing frequency remained increased for 7-18 sec after stimulation was stopped.

In the rats that had not developed PS, 32 neurons were identified in the dorsal horns (18 on the left and 14 on the right); of these, 8 left neurons and 9 right ones responded to peripheral stimuli. There was no significant difference in

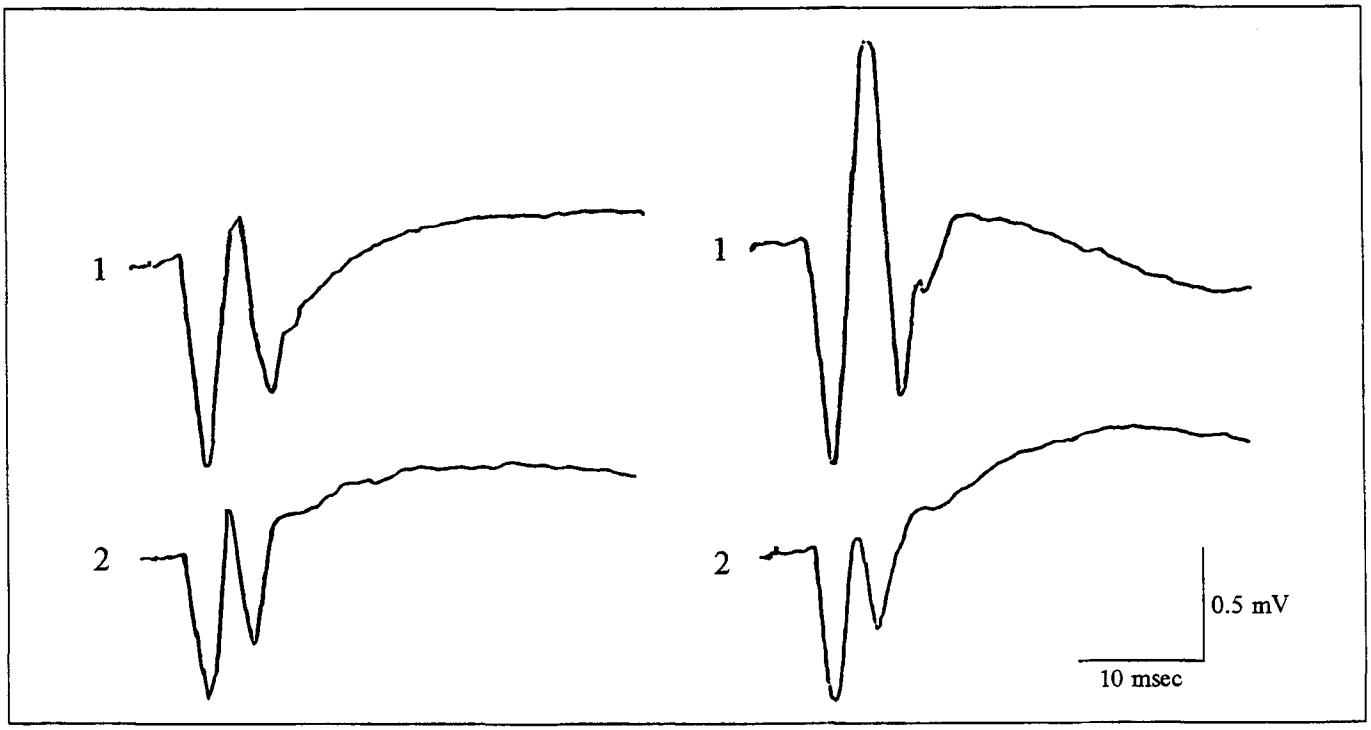

Fig. 3. Ipsilateral (*a*) and contralateral (*b*) EP in dorsal horns of a rat that did not develop a PS after sciatic nerve transection.

background neuronal firing frequencies between the sides ipsilateral and contralateral to the damaged nerve (Fig. 1, *b*). However, background neuronal firing was significantly lower on the damaged nerve side ($p<0.05$) and significantly higher ($p<0.05$) on the contralateral side than in rats with PS (Fig. 1, *b*). In addition, no high-frequency bursts were observed in rats without PS while the duration of afterdischarges by neurons responding to peripheral stimuli was substantially lower (3-8 sec) than in rats with PS (7-18 sec). Rats with and without PS did not differ significantly in the sizes of receptive fields.

In rats of both groups, the amplitudes of ipsilateral EPs in response to stimulation of the limb with the cut nerve (Fig. 2, *a*, *1* and Fig. 3, *a*, *1*) were higher than those of contralateral EPs arising in response to stimulation of a symmetrical point on the opposite limb (Fig. 2, *a*, *2* and Fig. 3, *a*, *2*). Rats that did not develop PS (Fig. 3, *b*, *1*), unlike those that did (Fig. 2, *b*, *1*), showed considerably increased EP amplitudes in the contralateral dorsal horns upon hind limb electrostimulation, the increase being more than 1.5 times greater than that in EP amplitudes on the ipsilateral side.

Thus, as is evidenced by the results presented above, substantial neuronal hyperactivation occurred on the sciatic nerve transection side in animals that had developed a PS. The hyperactivation was manifested in greatly elevated background neuronal firing frequencies, bursts of neuronal activity, pronounced afterdischarges following the delivery of peripheral stimuli, and increased EP amplitudes. Taken together, these findings indicate that a generator of pathologically enhanced excitation formed on the transected nerve side in rats with PS [2]. Similar changes in the neuronal activity of dorsal horns were observed by other authors in various animal models of neurogenic PS [6,7,10,12,13] as well as in a patient with chronic deafferentation pains [11]. Of special note are the increased EP amplitudes we recorded from the contralateral horns of rats without PS when tissues of the affected limb were stimulated. This increase in EP amplitudes was not associated with pathological neuronal hyperexcitability (neither epileptiform activity nor reduced thresholds for EP generation were observed in such rats), but was probably due to plastic processes leading to enhanced afferent inputs from receptors of the limb with the cut nerve to neurons of the contralateral dorsal horn as a result of the activation of previously inactive synapses. The presence of such connections is confirmed by both morphological [8] and electrophysiological [9] studies. Increased afferent inputs from the affected paw are therefore received by the contralateral dorsal horns in rats that do not develop a PS. Previously we observed a similar enhancement of afferent inputs to the somatosensory cortex of the ipsilateral normal hemisphere in rats without clinical signs of a neurogenic PS [5]. In such rats, a generator of pathologically enhanced excitation activity was recorded from the dorsal horns on the nerve transection side, whereas neuronal activity in the somatosensory cortex of the contralateral hemisphere remained within normal limits, indicating that the absence of a clinically manifest PS in these animals could be attributed to their failure to develop a pathological algetic system. The enhancement of afferent inputs we noted in this study on the side contralateral to the damaged limb may be presumed to have resulted in elevated activity of the overlying healthy projection structures of the brain, which precluded the formation of a pathological algetic system.

REFERENCES

1. L. V. Kalyuzhnyi, *Regulation of Pain Sensitivity: Physiological Mechanisms* [in Russian], Moscow (1984).
2. G. N. Kryzhanovskii, *Determinant Structures in Nervous System Pathology* [in Russian], Moscow (1980).
3. G. N. Kryzhanovskii, V. K. Reshetnyak, M. L. Kukushkin, and V. S. Smirnov, *Byull. Eksp. Biol. Med.*, **113**, № 3, 234-236 (1992).
4. G. N. Kryzhanovskii, V. K. Reshetnyak, M. L. Kukushkin, *et al.*, *Byull. Eksp. Biol. Med.*, **114**, № 9, 229-231 (1992).
5. G. N. Kryzhanovskii, V. K. Reshetnyak, M. L. Kukushkin, *et al.*, *Byull. Eksp. Biol. Med.*, **115**, № 5, 458-459 (1993).
6. D. Albe-Fessard and M. C. Lombard, in: *Advances in Pain Research and Therapy*, (J. J. Bonica *et al.*, eds.), Vol. 5, New York (1983), pp. 691-700.
7. H. Asada, W. Yasumo, and Y. Yamaguchi, *Pain*, Suppl. 5, S22 (1990).
8. J. L. Culberson, D. E. Haines, D. L. Kimmel, and P. B. Brown, *Exp. Neurol.*, **64**, № 6, 83-89 (1979).
9. C. C. Hunt and N. Kuno, *J. Physiol. (London)*, **147**, 347-384 (1959).
10. J. D. Loeser and A. A. Ward, *Arch. Neurol.*, **17**, 629-636 (1967).
11. J. D. Loeser, A.A. Ward, and L. E. Write, *J. Neurosurg.*, **29**, № 1, 48-50 (1968).
12. M. C. Lombard and J. M. Besson, *Pain*, Suppl. 5, S21 (1990).
13. J. Palecek, V. Palecekova, P. M. Dougherty, *et al.*, *J. Neurophysiol.*, **67**, № 6, 1562-1573 (1992).