Characteristics of Pain Syndromes Developing in Rats upon Interaction of Two Generators of Pathologically Enhanced Excitation

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Using rat models of a neuropathic and a central spinal pain syndrome, each of which is based on the formation of a generator of pathologically enhanced excitation, the authors explored how these syndromes develop when the two respective generators of pathologically enhanced excitation interact, and found that the interaction results in accelerated development and increased clinical manifestations of both syndromes.

Key Words: *neuropathic pain syndrome; central spinal pain syndrome; generator of pathologically enhanced excitation*

The neuropathic, or "neurogenic," pain syndrome (PS) arising after traumatic injury to the sciatic nerve is closely associated with the formation of a generator of pathologically enhanced excitation (GPEE) in the dorsal horns of lumbosacral spinal cord segments on the injured nerve side [3]. This syndrome has a number of developmental and other distinctive features. On the other hand, a PS of central origin (central PS) can be brought on without sciatic nerve injury through direct exposure of the dorsal horns to substances (convulsants) that cause depolarization or impair inhibitory mechanisms of neurons [2]. Its occurrence is also linked with the formation of a GPEE in the dorsal horns under the action of convulsants. It was therefore interesting to find out how the neuropathic PS caused by sciatic nerve damage would develop when the dorsal horns of particular cord segments were acted upon additionally by a convulsant, i.e., when a second, central GPEE had formed, and also how the PS induced by the latter GPEE might in turn be affected by the neuropathic PS. Accordingly, the purpose of the present study was to investigate the mutual influences of these two hyperactive nociceptive structures in the spinal cord.

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MATERIALS AND METHODS

The study was conducted on a total of 80 rats (body weight 180-200 g), 10 rats in each series of tests. In the test rats, two PS were produced, a neuropathic PS (NPS) and a spinal "penicillin-induced" PS (PPS). The NPS was elicited by the conventional procedure [7]. Briefly, the sciatic nerve was divided under ether anesthesia at the popliteal fossa level and its proximal end was placed in a polyethylene tube with a sealed end and left in the wound, which was closed by a suture. The severity of the NPS was evaluated in scores of 1 to 11 according to the degree of autotomic damage to the deafferented limb, using our modification of a previously proposed scale [5,7]. Damage to 1, 2, 3, 4, or 5 claws was assigned scores 1, 2, 3, 4, or 5, respectively; damage to a phalanx (phalanges) on 1, 2, 3, 4, or 5 digits, scores 6, 7, 8, 9, or 10, respectively; and damage up to metatarsal bones or higher, score 11.

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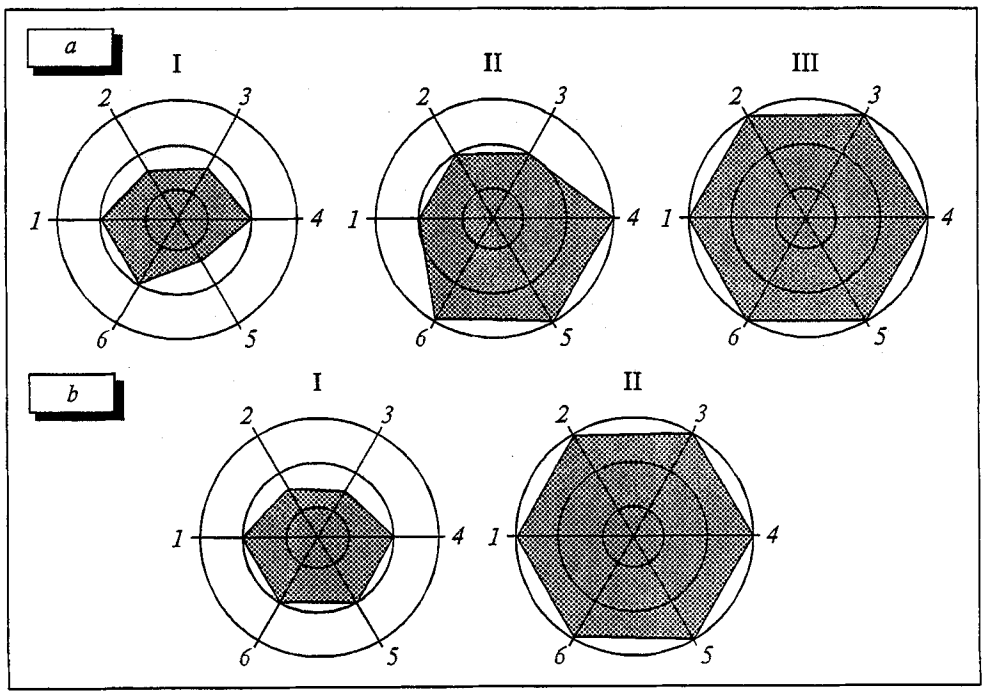

Fig. 1. Development of the PPS in rats after hind limb denervation. *a*) PPS in the group where a GPEE was set up at the T_{4-5} level: *I*) control rats with intact sciatic nerve; *II*) and *III*) rats with the hind limb denervated 3 and 14 days before the onset of the PPS, respectively. *b*) PPS in the group where a GPEE was set up at the L_{4-5} level: *I*) control rats (those with intact sciatic nerve); *II*) rats with the hind limb denervated 3 days before the onset of the PPS. The radius vectors denote characteristics of the PS as follows: *1*) frequency of attacks of pain; *2*) duration of one attack; *3*) intervals between attacks; *4*) response to provocation; *5*) vocalization; *6*) motor activity. The magnitude of the PS, i.e., the magnitude of its manifestations, was rated as follows: 0) no symptoms; 1) slight; 2) moderate; 3) severe. In these circular diagrams each circle corresponds to one score, counting from the center.

The PPS was produced by setting up a GPEE in lumbar (L_{4-5}) or thoracic (T_{4-5}) spinal cord segments on the left through application of an agar plate (1×0.4×10 mm) containing benzylpenicillin sodium (5000 IU) to the spinal cord surface at the L_{4-5} or T_{4-5} level following laminectomy under ether anesthesia [1]. The penicillin dose was so selected that the PPS was of medium severity initially (no more than score 2).

Signs of the PS were assessed on a 3-point scale. Score 1 was assigned to rats developing 2-3 attacks of pain in 5 minutes lasting 3-4 sec each with intervals of 95-100 sec between the attacks, which were each accompanied by a weak short squeak and motor excitation (1-2 short runs in the cage). Rats developing 2-4 attacks per minute lasting 12-17 sec with 6-10-second intervals between the attacks accompanied by sharp squeaking and high motor activity (running with jumps) throughout the attack were assigned score 3.

RESULTS

Sciatic nerve transection was followed by motor disturbances in the form of peripheral paresis, whose manifestations declined in five days. Eight days posttransection, however, the rats became restless, kept emitting unprovoked squeaks, developed clonic twitchings of the deafferented paw, and tried to lick and bite it; in addition, autotomies (bitten-off claw tips on 2 or 3 digits) were observed in 1 or (less often) 2 rats out of 10 in each group. After 14 days, autotomies assigned score 4 were present in 4 rats out of 10. By day 25, claws had been bitten off from all digits and pieces of tissue had been gnawed away from the interphalangeal joints in 7 rats out of 10. The autotomies occurred in a paroxysmal manner and were preceded by squeaking, restlessness, and clonic twitchings of the operated paw. Thus, the GPEE did not manifest itself clinically during the first 5 days.

On days 3, 7, 14, 21, or 28 after the denervation, a second GPEE was set up in the thoracic (T_{4-5}) or lumbar (L_{4-5}) spinal cord segments. In control rats (those with intact limbs), penicillin application to the dorsal surface of thoracic segments was followed in 20 min by shrill squeaking of short duration and running from one corner of the cage to another, after which the animals assumed a frozen posture and started, in silence, to lick the skin at the hip joint level and in the abdominal region and to comb out the fur on the

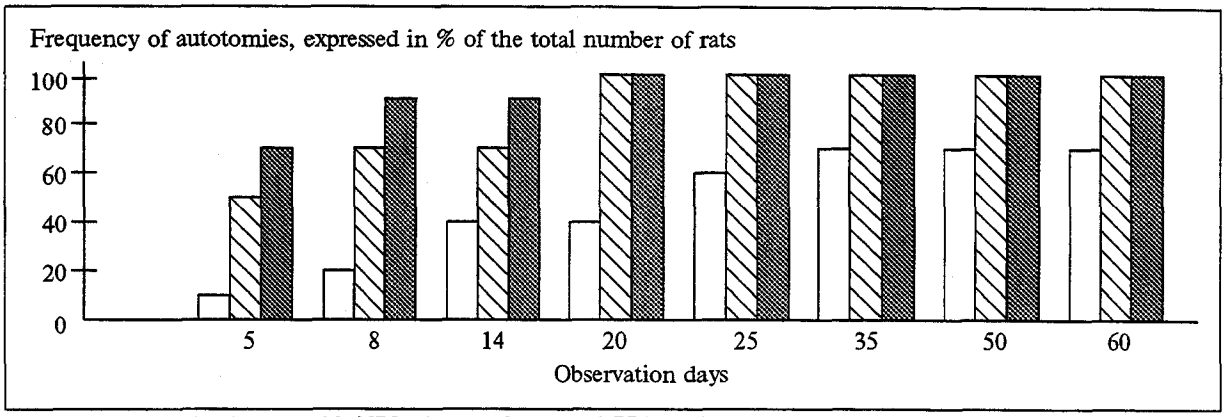

Fig. 2. Frequency of autotomies in rats with NPS after production of PPS in thoracic or lumbar spinal cord segments. Ordinate: frequency of autotomies, expressed in % of the total number of rats. Here and in Fig. 3, white bars represent control rats with the NPS, while hatched and dark bars represent rats in which the PPS was produced at the T_{4-5} or L_{4-5} level, respectively, 3 days after hind limb denervation.

sides of the body; none of them was seen trying to lick peripheral areas of the shin or foot. Attacks of pain in these control rats occurred at a frequency of 2-3 per 3 min and lasted 5-6 sec each for a total of 1.5-2 h (Fig. 1, *a*, *I*). The control group where penicillin was applied to the dorsal surface of lumbar segments began experiencing pain within 10-15 min; the attacks, lasting 5-6 sec each, occurred at a frequency of 2-3 per minute for a total of 2 h and were accompanied by squeaking and rapid darting about the cage; the animals licked their fur and bit the skin in the thigh region on the side of penicillin application (Fig. 1, *b*, *I*).

The group of test rats in which the PPS was produced in the thoracic cord segments 3 days after hind limb deafferentation began experiencing attacks of pain 15 min after penicillin application. The attacks were accompanied by pronounced motor excitation, multiple upright postures, and shrill squeaking throughout the attack. The animals were seen licking the surface of the thigh, biting off pieces of tissue from it, and combing out the side fur. Attacks occurred at a frequency of one per minute and lasted 7-8 sec each for a total of 2-2.5 h (Fig. 1, *a*, *II*). In the groups where a PPS was produced 14, 21, or 28 days after hind limb deafferentation, attacks of pain appeared 10 min after penicillin application, occurred at a frequency of 3-4 per minute, lasted 10-12 sec each for a total of 3 h, and were accompanied by continuous squeaking and running about, interrupted only for the purpose of biting off tissue from the denervated limb (Fig. 1, *a*, *III*). It should be noted that the rats with the PPS produced on the indicated days after limb denervation were mainly biting off the tips of their digits, which is more characteristic of animals with an NPS.

The behavior of rats with the PPS elicited in the dorsal part of lumbar cord segments 3 days after limb denervation was similar to that of rats in which penicillin was applied to the dorsal surface of thoracic segments 14 days postdenervation (Fig. 1, *b*, *II*).

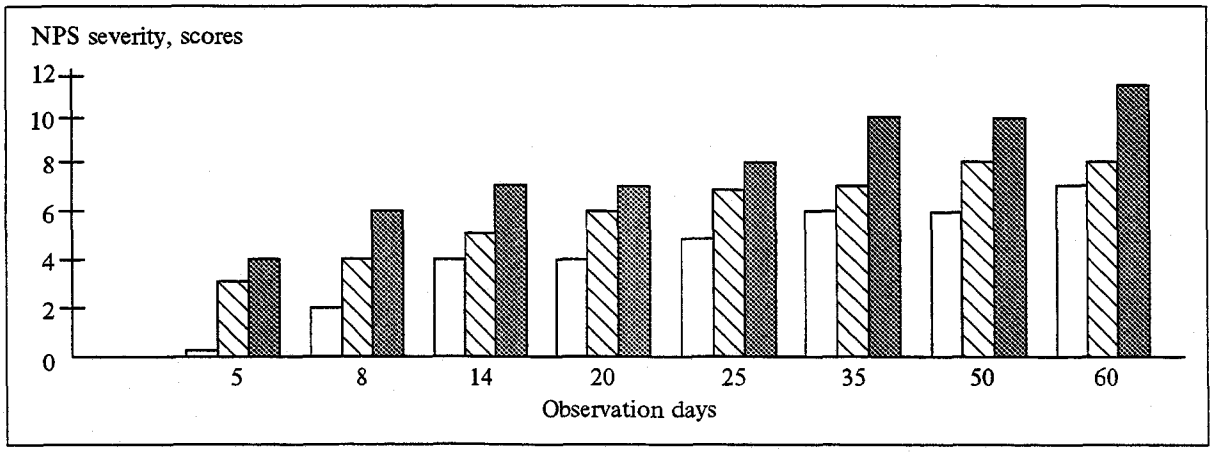

Fig. 3. Evolution of NPS in rats following production of the central spinal pain syndrome in thoracic or lumbar spinal cord regions.

After the PPS in rats with the NPS had cleared, these animals were followed up to see how the latter syndrome would develop further. It was then found that in the two groups of 10 rats where the PPS had been produced in the thoracic (T_{4-5}) and lumbar (L_{4-5}) cord segments 3 days after limb denervation, the NPS had reached score 3 in 5 rats of the former group and score 4 in 7 rats of the latter group as soon as day 3. By day 14, this syndrome was well marked in all rats regardless of the site of PPS production (Fig. 2), although at any given time it was more severe in the group where the PPS had been elicited in the lumbar cord segments (Fig. 3). In the groups with PPS produced in the thoracic or lumbar segments 7, 14, 21, or 28 days after limb denervation, the NPS developed 1 or 2 days after clearance of the PPS and was at its height 7 days later.

It has been shown that if a focus of relatively weak epileptic activity is created in some area of the cerebral cortex and a new, more powerful focus is then set up in another cerebral cortical area, the activity of the primary focus will increase under its influence [4]. Similarly, we found in the present study that when two independent GPEE were present in the nociceptive system of the spinal cord, activation of one of them resulted in heightened activity of the other. This was manifested in increased expression of the PBS elicited in the presence of the NPS as well as in increased expression of the latter syndrome itself. The longer the time during which the NPS lasted before the PPS was produced, the more pronounced were the clinical manifestations of both syndromes as a result of enhanced hyperactivity in the nociceptive neurons. Our results agree with those reported by Seltzer *et al.* [6] who showed that strychnine, a blocker of glycine receptors, intensified autotomy when injected subdurally 10 min prior to sciatic nerve transection. An important determinant of the interaction between the two GPEE is the distance that separates them. If both GPEE have been set up in the same structures, they will potentiate each other even in the early period of their formation, and this will be reflected in augmented clinical manifestations of the two respective syndromes. If, on the other hand, the two generators are far apart, then the deficit of inhibition in the pool of GPEE neurons and neuronal excitability should reach a higher level for the two GPEE to influence each other.

Thus, as this study indicates, two independent GPEE set up in the nociceptive system of the spinal cord can augment each other's activity, thereby aggravating the pathological process for which they are responsible.

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