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# Expression of Fos protein in the rat central nervous system in response to noxious stimulation: effects of chronic inflammation of the superior cervical ganglion

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## Abstract

The aim of this study was to investigate the possible interactions between the nociceptive system, the sympathetic system and the inflammatory process. Thus, the superior cervical ganglion of rats was submitted to chronic inflammation and Fos expression was used as a marker for neuronal activity throughout central neurons following painful peripheral stimulation. The painful stimulus consisted of subcutaneously injected formalin applied to the supra-ocular region. Fos-positive neurons were identified by conventional immunohistochemical techniques, and analyzed from the obex through the cervical levels of the spinal cord. In the caudal sub-nucleus of the spinal trigeminal nuclear complex, the number of Fos-positive neurons was much higher in rats with inflammation of the superior cervical ganglion than in control rats, either sham-operated or with saline applied to the ganglion. There was a highly significant difference in the density of Fos-positive neurons between the inflamed and control groups. No significant difference was found between control groups. These results suggest that the inflammation of the superior cervical ganglion generated an increased responsiveness to painful stimuli, which may have been due to a diminished sympathetic influence upon the sensory peripheral innervation.

**Key words**
- Autonomic nervous system
- Pain
- Sympathetic system
- Trigeminal complex

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The sympathetic nervous system consists of a complex neural network that is involved not only in the control of involuntary functions to maintain homeostasis, but also in the modulation of sensory processes, notably the generation or maintenance of pain states (1,2). In normal tissue, the sympathetic nervous system may not markedly contribute to the function of the nociceptive system (3). Instead, the influence of the sympathetic innervation upon the nociceptive C fibers seems to be evident in inflamed tissues or chronic nerve damage (4-6). Several mechanisms could be involved in sympathetic-sensory interactions in inflamed tissue. It has been reported that catecholamines and bradykinin might contribute to hyperalgesia in models of inflammation (7,8). They might

act in concert with several inflammatory cellular mediators such as prostaglandins, interleukins (9), and nerve growth factors (10), and sensitive neurons might influence inflammatory processes through the release of neuropeptides such as substance P and calcitonin gene-related peptide. These peptides may play an important role in the modulation of peripheral pain triggered by an inflammatory agent, generating sensitization of the nociceptors (11,12). To better understand the possible interactions between the nociceptive system, the sympathetic nervous system and the inflammatory processes, we studied the response of trigeminal neurons to peripheral nociceptive stimulation in animals previously submitted to chronic inflammation of the superior cervical ganglion (SCG). We used the expression of the Fos protein as a marker of neuronal activity following noxious stimulation (13,14). In the present investigation, we emphasized the study of the caudal subnucleus of the trigeminal spinal tract nucleus (Sp5C), which has

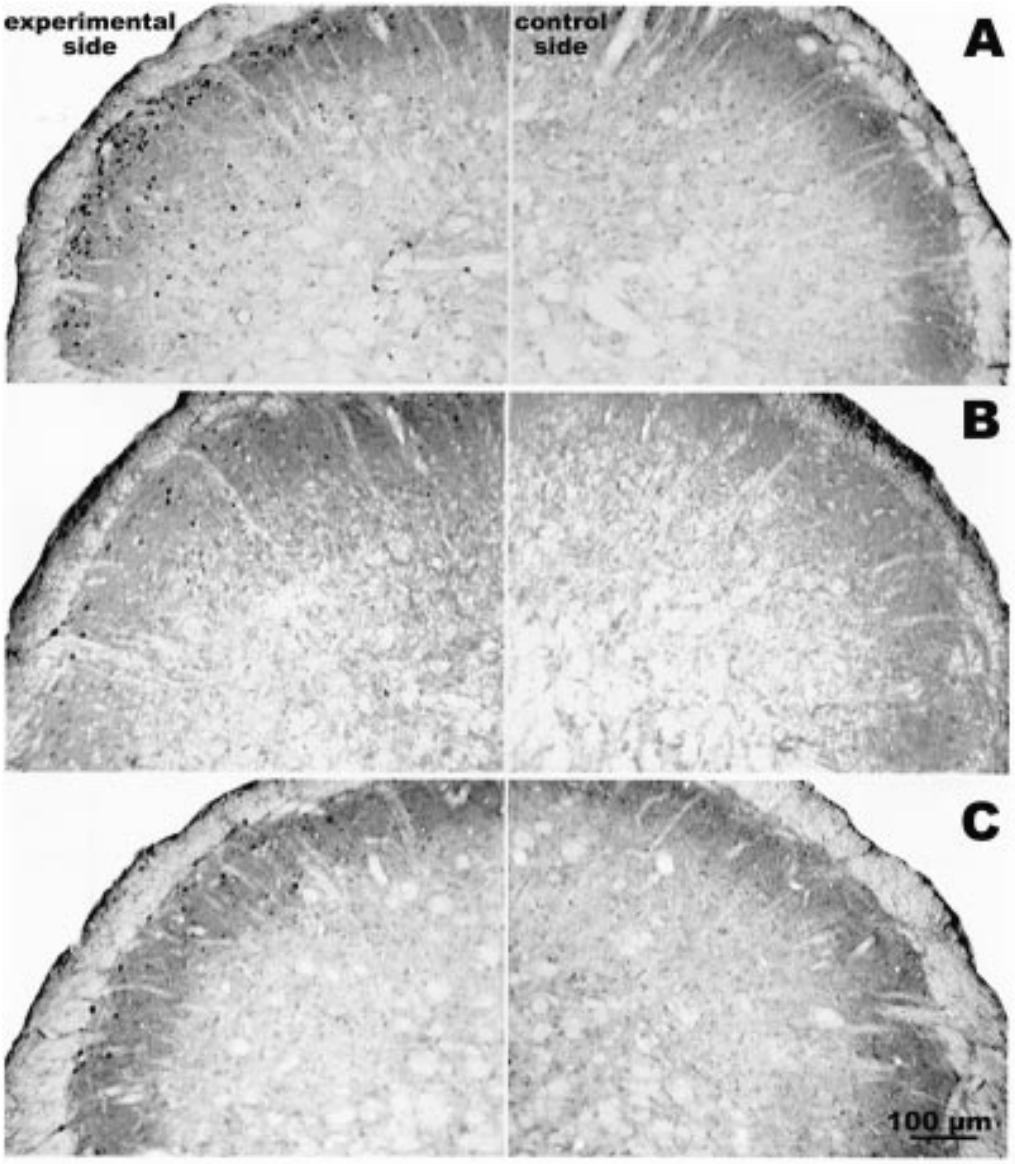


Figure 1 - Digital images of transverse sections of the rat brain at the level of the obex in the different experimental groups (*A*, rats with inflammation of the superior cervical ganglion (SCG); *B*, rats with saline applied around the SCG; *C*, sham-operated rats) submitted to supra-ocular formalin injection. Fos-positive nuclei are visible in the caudal subnucleus of the spinal trigeminal tract, basically restricted to the side ipsilateral to the noxious stimulation.

been considered to be part of the system processing pain signals and thermal information carried by cranial and upper cervical afferents (15).

Adult male albino Wistar rats (*Rattus rattus*) were used in this study. They were divided into three groups depending on the treatment of the SCG (N = 3 per group). One group was submitted to inflammation of the left SCG, the second group had a saline solution applied to the left SCG, and the third group was sham-operated. Animals were anesthetized with chloral hydrate (35 mg/100 g body weight, *ip*). Chronic inflammation of the SCG was obtained by depositing a piece of absorbable gelatin sponge (Gelfoam, Upjohn, Kalamazoo, MI) impregnated with 10 µl of a solution of the Calmette-Guerin bacillus (Instituto Butantã, Brazil) on the surface of the ganglion (16). After 15 days, painful stimulation (subcutaneously injected 5% formalin, 150 µl) was applied to the left supra-ocular region. After 90 min, the rats were deeply anesthetized and perfused with saline solution followed by 4% paraformaldehyde. Transverse sections of the brainstem and cervical cord (30 µm) were processed by previously described immunohistochemical methods (17-19). A rabbit polyclonal antiserum that recognizes the Fos protein (Ab-5, 1:1,000; Oncogene Science Inc., LaJolla, CA) was used. Incubations with the Fos antiserum and a biotinylated secondary antiserum (1:200; Jackson Labs, West Grove, PA ) were carried out at room temperature. The main control for immunostaining specificity was the omission of the primary antibodies from the procedure. Sections were then incubated with the avidin-biotin-peroxidase complex (ABC Elite Kit, Vector Labs, Burlingame, CA) and reacted with diaminobenzidine. The sections (18 sections/experimental group) were analyzed with a microscope coupled to an image analysis system. Statistical analysis (ANOVA and Tukey-Kramer's *post hoc* test) was performed to compare the densities of Fos-positive neurons in different groups.

Table 1 - Amount of Fos-positive neurons in the Sp5C of the different groups.

Values are reported as the average number of labelled neurons per section ± SEM from each side of the caudal subnucleus of the trigeminal spinal tract nucleus in the cervical spinal cord per animal group. *P<0.0001 compared to the sham-operated and saline groups (ANOVA, Tukey test). The results for the control groups were statistically similar.

| Experimental groups | No. of Fos-positive neurons/section |
|---|---|
| Inflamed | |
| Stimulated side | 339 ± 31* |
| Control side | 36 ± 10 |
| Saline | |
| Stimulated side | 130 ± 12 |
| Control side | 50 ± 10 |
| Sham operated | |
| Stimulated side | 142 ± 16 |
| Control side | 30 ± 4 |

Labeled neurons were observed in the Sp5C and upper cervical cord levels following supra-ocular noxious stimulation in all experimental groups. The labeling was basically restricted to the stimulated side. Thus, the contralateral side was considered as a control for the methodology. At other cervical levels, stained neurons were almost absent. Fos-positive neurons were more evident in the inflamed group than in the saline or sham-operated control groups. Labeled cells were distributed throughout the extension of the Sp5C in the inflamed group, and predominated in Rexed laminae I and II (20). A few nuclei were seen in laminae III-V (Figure 1). The same laminar distribution was observed in control groups, although the labeled cells were present in much reduced numbers and sometimes concentrated in the dorsal and/or ventral aspects of the nucleus. Table 1 presents the data about the densities of Fos-positive cells in the different groups. Anatomo-pathological analysis showed that the SCG was structurally disorganized, with

interstitial and peri-ganglionary infiltration, confirming the chronic inflammation.

These results suggest a participation of the sympathetic nervous system in the processing of nociceptive signals. The increased numbers of Fos-positive neurons suggest that chronic sympathetic inflammation amplified the response of central neurons to painful stimulation. Although several possibilities have been raised to characterize the influence of the sympathetic system upon the transmission of nociceptive information, we suggest that inflammation may have generated a functional ablation of the SCG. Accordingly, the adrenergic peripheral influence might have been diminished, leading to an increase of substance P at peripheral terminals, and thus to hyperactivity of C-fibers (21). Although further experiments are needed to directly address this hypothesis, this study may provide basic information about the interactions between sympathetic and sensory nerves. This knowledge may contribute to the elucidation of the physiopathology of chronic pain and hyperalgesic states.

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