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RESEARCH ARTICLE

Preemptive Analgesia with Acupuncture Monitored by c-Fos Expression in Rats

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Abstract
Pain behavior and awareness are characterized by heightened alertness and anxiety, which begin to disappear as soon as the curative process starts. The present study aimed to quantify c-fos expression in rat spinal cords and brains after a surgical stimulus and with preoperative or postoperative acupuncture. Animals were randomly divided into preoperative and postoperative groups and were then further divided into control, manual acupuncture (MA), or electroacupuncture (EA) groups. Expression of c-fos was quantified using immunohistochemistry. The collected data were analyzed using the *t* test at a 5% probability level. Presurgery and postsurgery spinal cord c-fos expressions were similar in all of the treatment groups. In the control rats, c-fos expression was higher before surgery than after surgery, contradicting the expected outcome of acupuncture and preemptive analgesia. After treatment, the expression of c-fos in the brains of the

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rats in the MA and the EA groups was reduced compared with that of the rats in the control group. These findings suggest that acupuncture used as preemptive analgesia in rats is a useful model for studying its application in human treatment.

1. Introduction

Pain is an uncomfortable sensorial and emotional experience associated with an actual or psychogenic lesion, and an important mediator of response to harmful agents. Pain control can involve a multimodal approach that includes pharmacological and nonpharmacological techniques, promoting relaxation and reduction of the painful stimulus [1]. Among the nonpharmacological techniques with recognized analgesic effects are manual acupuncture (MA) and electroacupuncture (EA), which activate the sensitive-discriminative system to stimulate a suppressive pain response [2,3]. Pain control can be started prophylactically, and the preemptive administration of opioids or local anesthetics can reduce postoperative pain, hyperalgesia, and morphine intake [4,5]. This suggests that an analgesic intervention before surgery will produce a better outcome than the same intervention after surgery [6].

Pain response may influence the cellular genome, causing important changes in gene transcription and protein synthesis. Genes that show a rapid change in expression after neuronal activity are known as early or immediate response genes. The structural events that occur in the nervous system after a pain stimulus are related to the activation of these genes, which include proto-oncogenes such as *c-fos* and *c-jun*. The identification of c-fos expression in the spinal cord and brain is a reliable method for assessing the efficacy of analgesic treatments [7].

The repetition of a stimulus is capable of reducing c-fos expression [8,9]. Mapping of the brain areas associated with the analgesic effects of acupuncture by means of c-fos expression in either anaesthetized or restrained animals has helped to elucidate its mechanisms of action [7].

This study aimed to quantify c-fos expression in the spinal cord and brain of rats after a painful stimulus in order to evaluate the preoperative and postoperative effects of acupuncture.

2. Materials and methods

2.1. Animal treatments

Thirty-six adult male Wistar rats, from the Animal Department of Universidade Federal de São Paulo (UNIFESP), Brazil, weighing 210—235 g were maintained five to each cage in order to avoid stress caused by overcrowding, under normal temperature and controlled light, and with food and water provided *ad libitum*. All experimental protocols were approved by the Animal Care and Use Committee of UNIFESP and were in accordance with National Institutes of Health guidelines on animal care.

Animals were distributed randomly to control, preoperative, and postoperative groups, with 12 animals in each group, and then subdivided into MA and EA groups, with six animals in each group. All animals were anesthetized with 45 mg/kg sodium thiopental until no motor sensibility could be observed. The preoperative groups were submitted to MA or EA at 100 Hz frequency in the following points: Stomach 36, Kidney 1, and Bladder 67, bilaterally (Fig. 1). The needles were removed after 20 minutes and surgery was started. An approximately 1-cm long incision was made in the plantar region of the left paw using a scalpel blade. After this, superficial and deep tissues were dissected. After 60 minutes, animals were sacrificed and perfused. In the postoperative groups, animals were stimulated for 20 minutes, at the same points after surgery, and then sacrificed and perfused.

Rats were euthanized by injecting 75 mg/kg sodium thiopental intraperitoneally, and perfusion was started 60

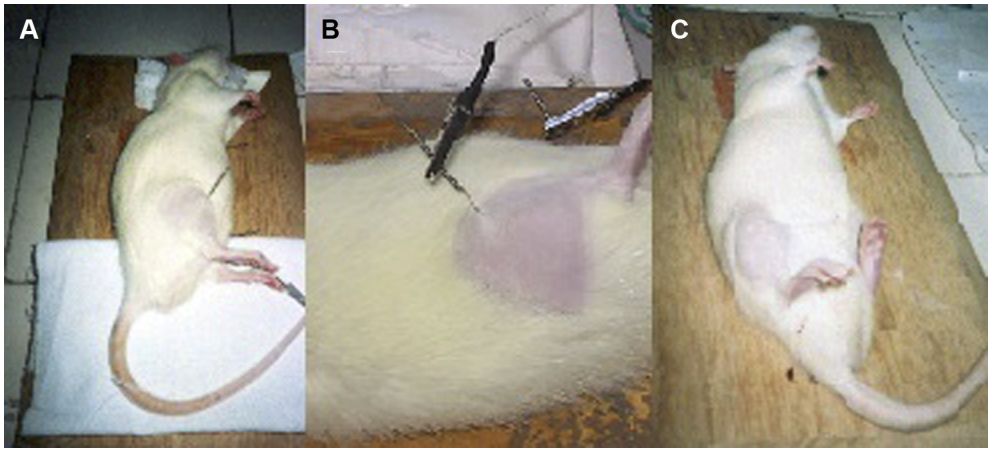

Figure 1 Manual acupuncture (MA) and electroacupuncture (EA) in rats. (A) MA in E36. (B) EA in E36. (C) EA in B67 (Ting).