Effect of formalin on animal behaviour and spinal induction of c-Fos

Rasheed I.D.¹ and Khanna S.²

Department of Physiology, Faculty of Medicine, National University of Singapore
Block MD9, 2 Medical Drive, Singapore 117597

ABSTRACT

A hind paw subcutaneous injection of dilute formalin (1.25%, 0.1ml), a model of inflammatory pain, was used to study the spatial pattern of induction of Fos protein in the neurons of the spinal cord, Fos being a proteinous transcription factor that is expressed in neuron following synaptic excitation. Compared to injection of saline, formalin injection evoked a biphasic increase in licking and flinching of the injured paw and induced an increase in the number of Fos positive cells in the spinal cord. The highest count was obtained at the L4 level, in the superficial laminae I-II, and the deeper laminae V-VI of the ipsilateral spinal dorsal horn. Little or no Fos was induced in the contralateral spinal cord. Superficially, the Fos positive cells were concentrated medially. The spatial pattern of induction of Fos is consistent with the known functional dermatome and topographic neural organization of the spinal cord, especially in relation to nociception. Thus, noxious stimulus-induced induction of Fos serves as a useful tool to study spinal nociceptive transmission.

¹ Undergraduate Student, Life Sciences
² Associate Professor, Physiology
INTRODUCTION

Nociceptive ‘pain’ is a sensatory and emotional experience triggered by activation of specialized nociceptors and associated neurones present throughout the body. Such ‘pain’ may evoke the expression of the immediate-early oncogene c-fos which codes for the protein c-Fos. It has been suggested that c-Fos also may act as a nuclear messenger which affects the cellular milieu. Interestingly, the magnitude of this c-Fos induction is directly proportional to the magnitude of the noxious stimuli. Furthermore, analgesic drugs such as morphine can reduce the magnitude of c-Fos induction proportionally to drug dosage. Hence, the c-Fos protein is a good marker for spinal nociceptive transmission, and can be used to map the functional organisation of the spinal cord, especially with respect to nociceptive transmission.

METHODOLOGY

Two habituated rats were injected with 0.1ml of either saline or 1.25% formalin subcutaneously into their right hindpaws. The behaviour of these rats in terms of licking, lifting, and shaking of the injured paw was observed for 90 minutes and tabulated in 5 minute intervals, after which the animals were then perfused with 500ml saline, followed by 400ml 4% paraformaldehyde in phosphate buffer. Their spinal cords were extracted and alternate coronal (60 µm) sections of each of their lumbar spinal cords cut using a microtome. The sections were stained using c-Fos immunohistochemical procedures. Brown stained nuclei indicate c-Fos induction. The number of stained nuclei were counted and tabulated and from this, areas of c-Fos induction can be mapped and nociceptive nuclear locations revealed.
RESULTS AND DISCUSSION

Compared to the injection of saline, the rat injected with 0.1ml of 1.25% formalin showed a biphasic nociceptive response, yielding two peaks as shown in Figure 1. The first phase lasts from injection for about five minutes, peaking at 84 seconds of licks, lifts, and shakes of injured paw, whereas the second phase lasts from 10-35 minutes, peaking from 25-30 seconds at 104 seconds of rat nociceptive activity.

![Figure 1. Duration in seconds of licks, lifts, and shakes of injured paw (on Y-axis) per 5 minute interval (on X-axis) in the rat injected with 1.25% formalin.](image)

The highest count of c-Fos expressing nuclei was obtained at the L4 level, in the superficial laminae I-II, and the deeper laminae V-VI of the ipsilateral spinal dorsal horn. Little or no c-Fos was induced in the contralateral spinal cord. Superficially, the c-Fos positive cells were concentrated medially. The spatial pattern of induction of c-Fos is consistent with the known functional dermatome and topographic neural organization of the spinal cord, especially in relation to nociception.
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