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Anxiety-like symptoms induced by morphine withdrawal may be due to the sensitization of the dorsal periaqueductal grey

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ABSTRACT

Withdrawal from morphine leads to the appearance of extreme anxiety accompanied of several physical disturbances, most of them linked to the activation of brainstem regions such as the *locus coeruleus*, ventral tegmental area, hypothalamic nuclei and periaqueductal grey (PAG). As anxiety remains one of the main components of morphine withdrawal the present study aimed to evaluating the influence of the dorsal aspects of the PAG on the production of this state, since this structure is well-known to be involved in defensive behaviour elicited by anxiety-evoking stimuli. Different groups of animals were submitted to 10 days of i.p. morphine injections, challenged 2 h after with an i.p. injection of naloxone (0.1 mg/kg), and submitted to the plus-maze, open-field and light–dark transition tests. The effects of morphine withdrawal on anxiety-induced Fos immunolabelling were evaluated in four animals that passed by the light–dark transition test randomly chosen for Fos-protein analysis. Besides the PAG, Fos neural expression was conducted in other brain regions involved in the expression of anxiety-related behaviours. Our results showed that morphine withdrawn rats presented enhanced anxiety accompanied of few somatic symptoms. Increased Fos immunolabelling was noted in brain regions well-known to modulate these states as the prelimbic cortex, nucleus accumbens, amygdala and paraventricular hypothalamus. Increased Fos labelling was also observed in the ventral and dorsal aspects of the PAG, a region involved in anxiety-related processes suggesting that this region could be a common neural substrate enlisted during anxiety evoked by dangerous stimuli as well as those elicited by opiate withdrawal.

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1. Introduction

Early studies on drug dependence focused upon the physical consequences of withdrawal as the main component of drug abuse. Later, it was suggested that the positive reinforcing properties of the addictive drugs themselves would play a primary role in the development of drug dependence [1–3]. In the field of opiates, since physical dependence appears later than the positive motivational effects during chronic opioid exposure, great attention has been paid to both positive reinforcing properties of opiates promoting substance abuse and the negative reinforcing effects eliciting craving and inducing relapse during withdrawal [1]. In this context, several experimental protocols have been used to promote the physical and emotional alterations verified after abrupt interruption from chronic morphine exposure. For example, in rodents, the administration of a single dose of a morphine antagonist in morphine-pretreated animals elicits a pattern of somatic and affective withdrawal symptoms that is highly dependent on the doses of the antagonist used: low doses promoting mainly alterations on emotionality and higher doses being effective in eliciting full somatic withdrawal symptoms [4,5]. The study of Higgins and Sellers [5] demonstrated that doses of naloxone as low as 0.05 mg/kg failed to consistently produce physical withdrawal signs. They were however effective in promoting alterations on emotionality as revealed by the place and taste aversion conditioning and suppression of operant responding. Other studies showed a quite similar pattern of results, with decreases on spontaneous locomotor activity, suppression for operant responding for food, elevation of intracranial self-stimulation threshold, significant conditioned place aversion and decrease of the time spent in the open arms of the elevated plus-maze, all highly sensitive indexes of aversive motivational states produced by morphine withdrawal, obtained with doses of naloxone, as low as 0.01 mg/kg [4,6,7]. In addition, immunohistochemical studies on opioid dependence have revealed that two different neural systems seem to underlie the physical and affective aspects of opioid withdrawal. In general, it is assumed that low doses of opiate antagonist, known to induce the negative-affective component of opiate withdrawal, promote the activation mainly of prosencephalic regions, such as the amygdala, through its central nucleus, shell region of the nucleus accumbens,

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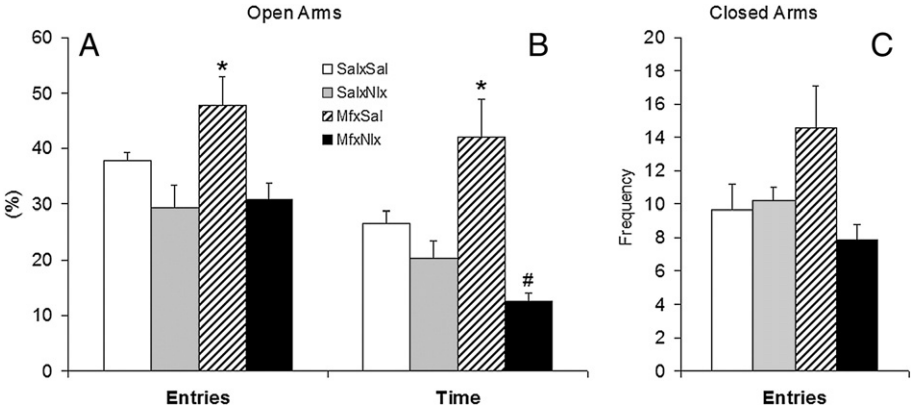

Fig. 1. Percentage of entries (A), time spent in the open arms (B) and number of enclosed arms entries (C) of the EPM of rats chronically treated (i.p.) with saline or morphine. Two hours after the last injection the animals were challenged with an i.p. injection of saline or naloxone and tested 5 min later. The groups depicted are saline–saline (Sal × Sal), saline–naloxone (Sal × Nlx), morphine–saline (Mf × Sal) and morphine–naloxone (Mf × Nlx). *Significant difference between morphine treatment and its control group (Sal × Sal). #Significant difference between naloxone treatment and its control group (Sal × Nlx). Data are presented as mean ± SEM. Two-way ANOVA followed by post-hoc Newman–Keuls; $p \leq 0.05$.

lateral septal nucleus and the bed nucleus of stria terminalis [8,9]. On the other hand, the main components of the neural substrates mediating the somatic aspects of opiate dependence induced by high doses of antagonists appear to be localized in the diencephalon and mesencephalon being mainly represented by the paraventricular and lateral nuclei of the hypothalamus, ventral tegmental area, *locus coeruleus* and the periaqueductal grey (PAG) [9].

With regard to the present study, it is important to note that most of the behavioural models cited above have mainly been used in the studies on the neurobiological mechanisms implicated in the production of fear and anxiety elicited in animals exposed to dangerous situations or contextual cues signalling the delivery of aversive stimuli [10–12]. In addition, studies on chemical or electrical stimulation, lesions of brain structures and immunohistochemical techniques such as Fos-like immunoreactivity detection have also been reported [13,14]. In fact, Fos-protein detection technique has been employed in several studies as a way to access changes in transcription of immediate early genes in brain regions following exposure to several types of aversive stimuli. These include, for example, brain electrical stimulation [15], local injection of neurotransmitters [16], stress induced by immobilization [17], pain [18] and abstinence of several types of drugs of abuse such as psychostimulants [19], alcohol [20], benzodiazepines [21] and opiates [22]. The results obtained in these studies showed a similar type of neuronal activation as those observed following acute withdrawal from morphine. For instance, a simple exposure to the elevated plus-maze [23,24] produces high Fos labelling in limbic areas related to the production of the behavioural and autonomic responses associated with the expression of anxiety-like behaviours, many of them also activated during opiate withdrawal [22].

Withdrawal from morphine may function as an unconditioned stressor promoting unconditioned withdrawal responses and, as such, could activate a particular set of structures involved with the organization of anxiety-like states, particularly those belonging to the well-known brain aversion system, such as the amygdala, hypothalamus and PAG [14,25]. With regard to the PAG, overall, some of the studies on morphine withdrawal have linked this structure mainly to expression of the somatic effects promoted during withdrawal [26–28], and have not considered the anatomical constitution and the functional differences of its columns. In fact, as far as we are aware, only one study has been conducted to evaluate separately the differential pattern of neural activation of the PAG columns promoted during morphine withdrawal [29]. In that study, significant Fos immunolabelling was achieved in the lateral and ventral aspects of the PAG. Unfortunately, no anxiety-provoking stimuli was used since the Fos-labelling procedure was conducted in brain areas of anaesthetized and awake rats under naloxone-precipitated morphine withdrawal, who were kept in their cages and perfused 2 h after naloxone injections.

In spite of the role of the PAG on opiate withdrawal has been well demonstrated, its importance is mainly linked to the production of the physical aspects of opiate abstinence, particularly through the activation of its ventral subdivision [29]. In this context, the present study aimed to evaluate the role of its dorsal aspects, well-known to contribute for the expression of anxiety- and fear-related behaviours in animals facing dangerous situations, on the production of the negative-affective states observed after a chronic morphine treatment break. To achieve this goal, different groups of morphine-pretreated animals were submitted to a naloxone-induced withdrawal procedure in which few physical symptoms but clear affective-negative state

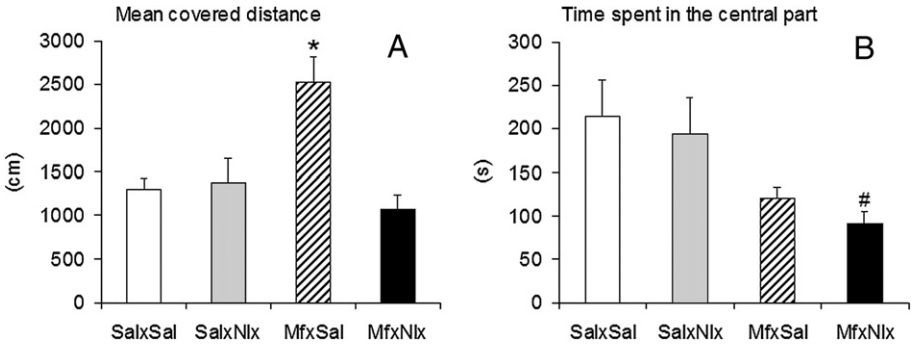

Fig. 2. Mean covered distance (A) and mean total time spent in the central part of the open-field (B) of rats treated i.p. with saline or morphine and challenged with saline or naloxone injections (i.p.). The groups are depicted as in Fig. 1. Independent groups of animals were tested 5 min after the saline or naloxone i.p. injections. *Significant difference between morphine treatment and its control group (Sal × Sal). #Significant difference between naloxone treatment and its control group (Sal × Nlx). Data are presented as mean ± SEM. Two-way ANOVA followed by post-hoc Newman–Keuls; $p \leq 0.05$.

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