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Environmental novelty modulates the induction and expression of single injection-induced behavioral sensitization to morphine

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ABSTRACT

Opioid addiction is a growing public health problem, being currently considered an epidemic in the United States. Investigating the behavioral effects of opioids and the factors influencing their development becomes of major importance. In animals, the effects of drugs of abuse can be assessed using the behavioral sensitization model, which shares similar neuronal substrates with drug craving in humans. Importantly, novelty plays a critical role on the development of behavioral sensitization. The aim of the present study was to investigate the influence of a new environment on both the induction and expression phases of morphine (Mor)-induced behavioral sensitization in the two-injection protocol. Mice were initially treated with saline, 15 or 30 mg/kg Mor (induction phase), and subsequently challenged 7 days later with 15 mg/Kg Mor (expression phase). Locomotor frequency was evaluated during behavioral sessions, performed as follow: induction session on a novel environment and expression on a familiar open-filed apparatus; induction session on animals' home-cage (familiar environment) and expression session on an unknown open-filed apparatus; both sessions on novel environments; and both sessions on familiar contexts. Mor-induced behavioral sensitization was only observed when animals were exclusively exposed to novelty during the induction phase, not being observed when both the induction and expression sessions were performed on similar (novel or familiar) environments. Our results suggest that the development of behavioral sensitization to Mor depends on the exposure to novelty during the induction phase and absence of novelty during the expression phase, indicating a complex relationship between novelty and Mor-induced behavioral effects.

1. Introduction

Drug addiction is a chronic disease that currently represents a public health problem. In particular, opioid abuse is a growing concern, being currently considered an epidemic in the United States (Skolnick, 2017). Opioids, such as morphine and heroine, have been increasingly used for non-therapeutic purposes, with the United Nations Office on Drugs and Crime (UNODC) having estimated that the use of opiates (opium, morphine and heroin) affected nearly 17 million people according to the most recent World Drug Report (UNODC, 2016). Research in the field has emphasized that a multifaceted approach is needed to reduce the burden of opioid abuse (Randolph, 2017), and understanding the factors influencing the development and expression of opioid-induced behavioral effects becomes of major importance.

In animals, the behavioral effects of drugs of abuse can be investigated using the behavioral sensitization model, which shares neuroadaptations with drug craving in humans (Robinson and Berridge, 1993). Behavioral sensitization in rodents is defined as a progressive increase in the behavioral responses to a drug after repeated administration (Robinson and Becker, 1986). Importantly, behavioral sensitization is directly affected by the drug administration regimen. For instance, intermittent and repeated administration of intermediate doses

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is usually more effective in inducing behavioral sensitization than the continued exposure to higher or escalating doses (Robinson and Becker, 1986; Stewart and Badiani, 1993; Vanderschuren et al., 1997). Importantly, it has been shown that a single administration of morphine is sufficient to induce both behavioral sensitization and neuroadaptations in rodents upon a subsequent drug challenge (two-injection protocol) (Vanderschuren et al., 2001; Marinho et al., 2015). In fact, Handal et al. (2008) have shown that morphine-induced behavioral sensitization in a two-injection protocol could be observed when the 2nd drug challenge was administered 6, 12 or 18 days after the induction treatment in rats.

Several studies have implicated the mesolimbic dopaminergic system in the behavioral and reinforcing effects of drugs of abuse (Kalivas, 2002). Specifically, increased dopamine levels in the nucleus accumbens is a common effect of almost all drugs with abuse potential (Haber and Knutson, 2010; Kalivas, 2002; McLellan et al., 2000; Stacy and Wiers, 2010). In particular, morphine stimulates dopamine systems indirectly by inhibiting GABAergic interneurons that inhibit dopaminergic neurons (Johnson and North, 1992), and morphine-induced behavioral sensitization has been proposed to be mediated by mesolimbic dopaminergic signaling (Sun et al., 2014).

Importantly, new environmental stimuli also induce an increase on dopamine release in the nucleus accumbens of rodents (Feenstra et al., 2000; Legault and Wise, 2001), which has prompted researchers to investigate the importance of novelty in modulating behavioral sensitization. For instance, Badiani et al. (1995a, 1995b, 1995c, 1998) have shown that behavioral sensitization to amphetamine and cocaine is potentiated when animals receive the drug treatment in a novel environment rather than in their home-cage. These data suggest that novelty and some behavioral effects of drugs of abuse may share similar neuronal substrates, and that novelty could possibly mimic the effects of drugs of abuse, therefore playing an important role in the development and expression of behavioral sensitization. However, no study to date has investigated the role of novelty on morphine-induced locomotor sensitization. The aim of the present study was to investigate if environmental novelty can facilitate the development of behavioral sensitization to morphine. While the induction phase of behavioral sensitization has been associated with the activation of dopaminergic neurons in the ventral tegmental area (VTA), the expression phase is thought to be mediated by neuroadaptations observed mainly in the nucleus accumbens after the initial (induction) treatment (Paulson and Robinson, 1991; Pierce and Kalivas, 1997; Brebner et al., 2005). Thus, this study also sought to investigate whether the exposure to novelty in the induction vs the expression phases could have distinct effects on morphine-induced behavioral sensitization using a single injection protocol.

2. Material and methods

2.1. Subjects

Three-month-old male Swiss mice (40–45 g, outbred, raised, and maintained in the Centre for Development of Experimental Models in Medicine and Biology of Universidade Federal de São Paulo) were used. Animals were housed under controlled temperature (22–23 °C) and lighting (12 h light, 12 h dark; lights on at 6:45 a.m.). Food and water were available throughout the experiments. Animals used in this study were maintained in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications N° 8023, revised 2011). The experimental procedures were approved by the Institutional Animal Care and Use Committee under the protocol #01630/06.

2.2. Drug

Morphine sulfate (Mor; Sigma®) was freshly diluted in 0.9% saline solution (Sal) and administered subcutaneously (s.c.) at a volume of 10 ml/kg body weight. Control groups received an equivalent volume of Sal. The doses of Mor used were 15 and 30 mg/kg. The doses were chosen based on previous studies performed by our group (Patti et al., 2005, 2006; Marinho et al., 2015) and others (Zarrindast et al., 2003, 2005).

2.3. Quantification of locomotor activity – Open-field

The open-field apparatus consisted of a circular wooden box (40 cm in diameter and 50 cm high) with an open top and a floor divided into 19 floor units of similar size (~66 cm^2) by 3 concentric circles of different radii (8, 14 and 20 cm) intersected by radial line segments, as previously described (Chinen et al., 2006). All behavioral sessions were conducted in a room separate from the colony, maintained at controlled temperature (22–23 °C) and lighting (1600 lumens) mimicking the conditions in the vivarium. All animals were placed in the test room at least 1h prior to the beginning of behavioral manipulations in order to minimize any stress associated with transporting the animals from the vivarium into the test room. Animals were tested in sets of 5 animals (5 open-field apparatus), and, within the same experiment, animals from different groups were ran concomitantly in order to avoid any bias associated with the order and/or time of the behavioral session. All sessions were conducted during the same period of the day (between 14h00 and 16h00).

During a behavioral session, animals were individually placed in the center of the apparatus for direct quantification of motor activity. Hand-operated counters were used to score locomotion frequency (number of floor units entered, with one locomotion unit being scored when the animal entered into a floor unit with all four limbs) and stopwatches were used to record immobility (total seconds of lack of movements) and grooming (total seconds of mouth or paws on the body and on the head) durations in a 5-min session. A 5-min period was chosen based on previous studies from our group showing that a 5-min observation in the open-field shows the peak morphine effects when animals are exposed to the open-field apparatus 20min after the drug injection (Marinho et al., 2015; Hollais et al., 2014; Procópio-Souza et al., 2011; Patti et al., 2006). The apparatus was cleaned with alcohol–water (5%) solution before each behavioral test to eliminate possible bias due to odors left by previous mice.

Observers were unaware of the experimental design. Observers were trained using a standard procedures manual and completed at least 10 hrs of scoring prior to participating in the study. Percent agreement scores were used to determine inter-observer reliability, with a criterion of ≥90% required. Inter-observer reliability was redetermined every 3 months. Within a given experiment, a single individual conducted all injections. Because each experiment only involved a single behavioral observation, changes in observers only occurred between experiments, and not within experiments.

2.4. Experimental design

2.4.1. Experiment 1A – Effects of novelty during the induction phase of morphine-induced behavioral sensitization in a single injection protocol

Thirty-nine mice were habituated to the open-field during 3 consecutive days. During the habituation period, mice received a Sal injection 20 min prior to being exposed to the open-field apparatus for 5 min. On day 4 (induction phase), animals were randomly allocated to 4 groups (n = 9–10 per group). Nineteen animals (2 groups) were treated with Sal, and the 2 other groups received a single injection of either 15 or 30 mg/kg Mor. Twenty min after injections, animals were exposed to a wire mesh metallic cage (16 × 30 × 18 cm, novel environment), with no visual contact with other animals, during 5 min. Following the induction session, mice were left undisturbed in their home-cages for 7 days. On day 12 (expression phase), 1 group (n = 9) previously treated with Sal received a second Sal injection, and all other animals were challenged with an injection of 15 mg/kg Mor, forming