

# Brain Disorders: Involvement of D1/D2 dopamine receptors within the nucleus accumbens and ventral tegmental area in the development of sensitization to antinociceptive effect of morphine- Leila Zarepour-Iran University of Medical Sciences

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## Abstract

The nucleus accumbens (NAc) and therefore the ventral tegmental area (VTA) are two major areas for the mesolimbic dopaminergic system which are strongly involved within the development of behavioral sensitization. In the present study, we investigated the role of D1/D2 dopaminergic receptors within the NAc or VTA in response to sensitization to morphine by the tail-flick test as a model of acute pain. Sensitization was induced by subcutaneous (SC) injection of morphine (5 mg/kg), once daily for 3 days followed by five days freed from drug. After the sensitization period, antinociceptive responses induced by an ineffective dose of morphine (1 mg/kg; SC) were obtained by the tail-flick test, and represented as maximal possible effect (%MPE). In experimental groups, D1 and D2 receptor antagonists, SCH-23390 and sulpiride (0.25, 1 and 4 µg/rat), were separately microinjected into the NAc or VTA, 10 min before morphine administration during the sensitization period, respectively. Results showed that injection of morphine during the sensitization period (development of sensitization) increased %MPE of the ineffective dose of morphine from  $2.43 \pm 1.4\%$  in naive to  $47.75 \pm 4.01\%$  in sensitized animals. Unilateral microinjections of various doses of the D1/D2 receptor antagonists, SCH-23390 and sulpiride, into the NAc dose-dependently decreased %MPEs in morphine-sensitized animals. Nonetheless, %MPEs were only suffering from intra-VTA administration of SCH-23390 in morphine-sensitized animals. Our findings suggest that both the D1/D2 dopamine receptors in the NAc and the D1 receptors in the VTA may be of more important in the development of sensitization to in rats.

## Introduction:

While the precise role for dopamine has been debated, dopamine is thought to be a key ingredient in both the development and expression of behavioral sensitization to repeated drug administration (Lodge and Grace, 2008; Pierce

and Kalivas, 1997; Robinson and Berridge, 1993). It was found earlier that long-term opioid treatment leads to antinociceptive tolerance and causes a paradoxical sensitization (opioid-induced hyperalgesia) toward mildly painful (hyperalgesia) and normally innocuous (allodynia) stimuli. Prolonged morphine administration was also found to up-regulate pain neurotransmitter (such as calcitonin gene-related peptide; CGRP) levels in primary sensory neurons (Tumati et al., 2011; Zarrindast et al., 2007). Anatomical and pharmacological evidence indicates that the nucleus accumbens (NAc) is involved in opioid sensitization (Azizi et al., 2009; Kalivas and Duffy, 1995; Robinson and Kolb, 2004). The common circuitry in behavioral sensitization includes dopamine projections from the ventral tegmental area (VTA) to the NAc and glutamate projections from the medial prefrontal cortex (mPFC) to the NAc (Pierce and Kalivas, 1997). The NAc is a complex forebrain structure (Jongen-Rêlo et al., 1994), which receives massive dopaminergic input from the VTA and glutamatergic input from structures such as the hippocampus, amygdala and mPFC (Heyman et al., 1989). In rats, dopamine-mediated antinociception has been reported in many studies (Altier and Stewart, 1998; Morgan and Franklin, 1991). The binding of dopamine to its receptors causes a change in the release of neurotransmitters which plays a key role in behavioral sensitization. For example, enhanced excitability of the VTA dopaminergic neurons that occurs with repeated cocaine is associated with a decrease in dopamine D2 auto receptor sensitivity (White and Wang, 1984). Moreover, repeated intra-VTA injections of low doses of the D2 receptor antagonist, eticlopride, which is presumably an auto receptor-selective, enhanced subsequent stimulant response to amphetamine (sensitization). Blockade of the dopamine D1 receptors in the VTA during the initiation phase prevents the development of amphetamine, but not cocaine sensitization (Vezina, 1996). Morphine and amphetamine-induced analgesia are involved in increasing dopamine levels in the NAc. In a study by Altier and Stewart (1998), dopamine receptor antagonists injected into the NAc blocked the

analgesic effects of intra-NAc or -VTA of substance P, morphine and amphetamine. This study suggests that tonic pain is inhibited, at least in part, by enhanced dopamine released from terminals of mesolimbic neurons. Human and animal imaging data also suggest that the NAc is an important neural substrate of pain modulation, and intra-accumbal injection of D2 receptor agonist inhibits persistent on-going nociception in the formalin test (Magnusson and Fisher, 2000; Taylor et al., 2003). Considering the above-mentioned findings and the interaction of opiate-mediated pain modulation and sensitization, in the present study, we tried to find out the role of dopamine D1 and D2 receptors within the NAc and VTA in the sensitization to morphine by the tail-flick test as a model of acute pain in rats.

## 2. Materials & methods

**2.1. Animals:** One hundred and thirty eight adult male albino Wistar rats (Pasteur Institute, Tehran, Iran) weighing 200–220 g were used in these experiments. Animals were housed in groups of three per cage a 12/12 h light/dark cycle (light on between 7:00 a.m. and 7:00 p.m.) with free access to chow and tap water. The animals were randomly allocated to different experimental groups. Each animal was used only once. Rats were habituated to their new environment and handled for one week before the experimental procedure started. All experiments were executed in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

**2.2. Drugs:** In the present study, the following drugs were used: morphine sulphate (Temad, Tehran Iran) and SCH-23390 (Tocris Bioscience, Bristol, UK), a D1 receptor antagonist, which were dissolved in sterile saline (0.9%). Sulpiride (Tocris Bioscience, Bristol, UK), a D2 receptor antagonist, was dissolved in 10% dimethyl sulfoxide (DMSO). In separate groups, control animals received either saline or 10% DMSO as a vehicle into the NAc or VTA.

**2.3. Stereotaxic surgery:** Rats were anesthetized by intraperitoneal injection of xylazine (10 mg/kg) and ketamine (100 mg/kg), and placed into a stereotaxic device (Stoelting, USA). An incision was made along the midline, the scalp was retracted, and the area surrounding bregma was cleaned and dried. In addition, lidocaine with epinephrine (0.2 ml) was injected in several locations around the incision. In separate groups of animals, a stainless steel 23-gauge guide cannula was unilaterally implanted 1 mm above the intended site (NAc or VTA) of drug injections according to the rat brain atlas (Paxinos and Watson, 2007). Stereotaxic coordinates for the NAc were 1–1.2 mm anterior to the bregma,  $\pm 0.8$ –1 mm

lateral to the sagittal suture and 6.8–7.8 mm ventral to the skull surface; and those for the VTA were 4.7–5 mm posterior to the bregma,  $\pm 0.8$ –0.9 mm lateral to sagittal suture and 8.2–8.4 mm from the skull surface. The guide cannula was secured in place using two stainless steel screws anchored to the skull and dental acrylic cement. After the cement completely dried and hardened, one stainless steel stylet was used to occlude the guide cannula during recovery period. Penicillin-G200000 IU/ml (0.2–0.3 ml/rat, single dose, intramuscular) was administered immediately after surgery. Animals were individually housed and allowed to recover for 5–7 days before experiments.

**2.4. Drug administration:** Microinjections were performed by lowering a stainless steel injector cannula (30-gauge needle) with a length of 1 mm longer than the guide cannula into the NAc or VTA. The injector cannula was connected to a 1- $\mu$ l Hamilton syringe by polyethylene tubing (PE-10). Drug solution or vehicle was infused over 60 s and left in place for 60 s to facilitate drug diffusion, which was followed by replacement of the obturator. Different doses of SCH-23390 and sulpiride were slowly administered in a total volume of 0.5 or 0.3  $\mu$ l into the NAc or VTA, respectively. The microinjection time for 0.1  $\mu$ l volume of drugs was 10 s to prevent lesions in these areas. All drug solutions were freshly prepared on the test day, and all microinjections were unilaterally administered into the NAc or VTA.

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