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Research report

# Microinjections of a dopamine D1 receptor antagonist into the ventral tegmental area block the expression of cocaine conditioned place preference in rats

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## HIGHLIGHTS

- The selective D1 antagonist, SCH 23390, injected into the VTA blocks cocaine conditioned place preference.
- Dendritically released dopamine in the VTA plays a role in cocaine conditioned place preference.
- Cocaine-seeking related behaviors depend on VTA dopamine D1 receptor stimulation.

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## ABSTRACT

Stimulation of dopamine (DA) D1 receptors in the ventral tegmental area (VTA) is involved in primary rewards. In the current study we investigated whether VTA D1 receptor stimulation likewise plays a role in mediating the rewarding effects of cocaine-associated stimuli, using the cocaine conditioned place preference (CPP) paradigm. Rats were prepared with cannulae so as to allow microinjections in the VTA and later conditioned to a cocaine-associated environment using the CPP paradigm. Prior to each conditioning session rats were injected with either saline or cocaine (10 mg/kg, intraperitoneally) and then placed in one of the two sides of the CPP apparatus. Sessions lasted 30 min a day over a period of eight days, such that rats alternated daily between consistently experiencing cocaine in one side and saline in the other. On the test day, which was conducted one day after conditioning, rats were given bilateral microinjections of one of four doses of the D1 antagonist, SCH 23390, (0, 2, 4 or 8 μg/0.5 μl) directly into the VTA and allowed free access to both sides of the apparatus. Preference for either side was measured as time spent in each side and compared to the same measures taken before conditioning. The D1 antagonist produced a dose-related, significant reduction in the preference for the cocaine-paired side compared to vehicle. These data suggest that the expression of cocaine conditioned place preference requires stimulation of VTA D1 receptors and, as such, are the first to suggest a role for VTA dendritically released DA in cocaine-, or other reward-, related learning.



Dopamine (DA) is strongly implicated in motivation and reward-related learning [1–7]. The rewarding effects of natural stimuli (e.g. food, water, sexual contact) and drugs of abuse are mediated by the mesocorticolimbic DA system [8–11], the DA neurons that originate in the ventral tegmental area (VTA) and project primarily to the nucleus accumbens and prefrontal cortex [12–14]. The VTA is reciprocally connected with various forebrain structures. The major excitatory projection into the VTA is the glutamatergic input that synapses onto GABA neurons and directly onto the cell bodies and dendrites of DA neurons [6,15–17]. As part of a feedback loop in response to DA axonal release the VTA also receives inhibitory GABA afferents from the nucleus accumbens and ventral pallidum [18–21]. In addition, DA neurons are enervated by GABAergic interneurons. On the terminals of these glutamate and GABA afferents are DA D1 receptors [22–24] whose role is to regulate the local release of both glutamate and GABA. These neurotransmitters stimulate receptors found on DA cells and consequently modulate the activity of DA neurons through excitation or inhibition [25–28]. Dopamine produced in the VTA is

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released not only axonally in terminal regions but also dendritically in the VTA [29]. Stimulation of D1 receptors facilitates the release of VTA glutamate, which excites DA cells and non-DAergic neurons [30–33]. Stimulation of D1 receptors also facilitates the release of VTA GABA that, through inhibitory action on DA cells, can inhibit dendritic [25,34,35] and axonal release of DA [36–38].

Stimulation of D1 receptors in the VTA plays a role in food and cocaine reward. Animals treated with bilateral injections of the D1 receptor antagonist, SCH 23390, directly into the VTA respond less for food reward under a progressive ratio (PR) schedule of reinforcement [39]. This reduction in break point is interpreted as a reduction in motivation for food, suggesting a reduction in food reward. Likewise, bilateral microinjections of SCH 23390 into the VTA alter the intravenous self-administration of cocaine under both fixed ratio (FR) and PR schedules of reinforcement; under a FR schedule of reinforcement rats treated with intra-VTA SCH 23390 respond at higher rates whereas under a PR schedule of reinforcement they respond less [40]. These results suggest that stimulation of D1 receptors in the VTA is involved in cocaine reward.

In the current study we investigated whether stimulation of DA D1 receptors in the VTA plays a role in the rewarding effects of cocaine-related cues, specifically in cocaine conditioned place preference (CPP). In a standard CPP paradigm animals spend more time in the compartment that contains cues associated with the drug, suggesting that the drug-paired compartment, through learning processes, produces rewarding effects [41–43]. Cocaine can be used to establish a conditioned place preference [42,44–46]. This preference is associated with DA release in terminal regions of the mesocorticolimbic DA system [47] and is dependent on this DA release [48–53]. As reviewed above, stimulation of VTA DA D1 receptors plays a role in the rewarding effects of cocaine itself. But whether or not it also is involved in the learned rewarding effects of cocaine, as are manifested in the expression of cocaine CPP, is not known. Given the importance of learned rewarding effects of cocaine-associated stimuli in aspects of cocaine addiction (e.g., cue-induced cocaine craving [54–56]) it is important to know if stimulation of VTA DA D1 receptors also plays a role in this aspect of cocaine-related behavior. We hypothesized that it does. We tested this hypothesis by investigating the effects of SCH 23390 injected into the VTA on the expression of cocaine CPP in rats.

## 1. Methods

This study was carried out in accordance with the guidelines established by the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and was approved by the Queens College Institutional Animal Care and Use Committee.

### 1.1. Subjects

The subjects consisted of fifty-four male Long Evans rats taken from our in-house colony bred from males and females obtained from Charles River Laboratories (Kingston, NY). The rats were housed individually on a reversed 12 h light:12 h dark cycle (lights on at 10 pm). The rats had free access to food (LabDiet chow) and water at all times except when in the testing chambers. All experimental procedures were conducted during the dark cycle.

### 1.2. Surgery

The animals were injected with 0.54 μg of atropine sulfate concentrated in 0.1 ml of distilled water (to decrease bronchial secretion) and 5 minutes later were deeply anesthetized with sodium pentobarbital (65 mg/kg). A small incision was made on the midline of the scalp to expose the skull and two holes were drilled to allow cannula implantation. Stainless steel guide cannulae (outer diameter 0.028 mm, inner diameter 0.016 mm) were bilaterally lowered to a depth that allowed for microinjections into the VTA using the following coordinates from Paxinos and Watson [57]: −5.6 mm caudal to bregma, ±2.0 mm from the midline at a 10° angle toward the midline and −8.7 mm below the surface of the skull. For the anatomical control groups cannulae were placed using the same coordinates except for the dorsal-ventral coordinate, which was −7.2 mm below the surface of the skull. The cannulae were permanently fixed to the skull using dental acrylic anchored to four stainless steel screws screwed into the skull. Obturators, extending 1 mm beyond the cannulae, were inserted into the cannulae to prevent blockage and remained there at all times, except during microinjections.

### 1.3. Apparatus

Testing was conducted using six conditioned place preference chambers, each placed in a sound-attenuating ventilated box. Each conditioning chamber, measuring 43 cm × 43 cm × 30 cm, was equipped with 32 photo-beam emitters spaced 2.5 cm apart along two adjacent walls (16 per wall) and 6 cm above the floor (with photo-detectors directly opposite the emitters). These photobeams detected the position of the rats in one compartment or the other. Chambers consisted of two compartments that had distinct combinations of wall patterns (striped or non-striped) and floor texture (grid or rods) and were separated by a black plastic wall with an opening that could be closed with a guillotine door.

### 1.4. Drugs

Cocaine (a gift from the National Institute on Drug Abuse, Bethesda, MD) was dissolved in 0.9% saline to achieve a dose of 10 mg/kg and injected in volumes of 1 ml/kg.

The D1 receptor antagonist, SCH 23390 (Sigma–Aldrich, St. Louis, MO), was dissolved in artificial cerebrospinal fluid to achieve doses of 0, 2, 4 and 8 μg and was delivered in a volume of 0.5 μl.

### 1.5. Procedure

#### 1.5.1. Conditioned place preference

Four groups of rats were tested for the effects of intra-VTA SCH 23390 on the expression of cocaine CPP. One day prior to placement in the testing chambers, all rats were injected with saline intraperitoneally (IP) and immediately placed back in their home cages. These home-cage injections were done in order to habituate the rats to injections. The CPP procedure began with a pre-exposure session where all animals were injected with saline (IP) and placed directly in the open doorway of the CPP apparatus. During this session the rats could freely explore both compartments for 15 min. The time spent in each compartment was measured. This pre-exposure session was followed by a conditioning phase consisting of 8 consecutive, daily sessions. For all of these sessions the doorway between compartments was closed. Cocaine (10 mg/kg) was administered IP immediately prior to a 30 min exposure to one compartment. This conditioning procedure was repeated once every other day for a total of four cocaine conditioning days. On alternating days, rats were injected with IP saline and placed in the other compartment. The order of cocaine conditioning was counterbalanced across rats. Also, for half of the rats cocaine injections were paired with the preferred compartment and for the other half with the non-preferred compartment. The preferred compartment was defined as the compartment in which a rat spent the most time during the pre-exposure session.