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# Hypocretin receptor 1 blockade preferentially reduces high effort responding for cocaine without promoting sleep

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## HIGHLIGHTS

- Blockade of HCRTr1 reduces cocaine self-administration under high effort conditions.
- Blockade of HCRTr1 does not alter cocaine self-administration under low effort conditions.
- HCRTr1 antagonism does not promote sleep at levels that alter cocaine self-administration.

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## ABSTRACT

Recent evidence suggests that blockade of the hypocretin receptor 1 may act as a useful pharmacotherapy for cocaine abuse. Here we investigated the extent to which various doses of a hypocretin receptor 1 antagonist, SB-334867, affect cocaine self-administration at varying doses of cocaine and across a range of effort requirements, and tested if these SB-334867 doses produce sedative effects. First, we trained animals to self-administer one of three doses of cocaine on a progressive ratio schedule, and then tested the effects of three doses of SB-334867. Responding for cocaine was then analyzed to segregate features of relatively high and low effort requirements across the progressive ratio session. In another set of experiments, we tested potential sleep-promoting effects of the same doses of SB-334867. Our data indicate that blockade of hypocretin receptor 1 preferentially reduces high effort responding for cocaine at levels that do not promote sedation.



## 1. Introduction

Hypocretins (also known as orexins) are excitatory neuropeptides synthesized by a confined group of neurons located in the lateral hypothalamus and perifornical regions. These neurons project widely throughout the brain and interact with two G-protein coupled receptors, the hypocretin receptor 1 (HCRTr1), and hypocretin receptor 2 (HCRTr2) [1,2]. Early investigation into the function of this system established its role in the regulation of arousal and arousal-related processes (for a review see; [3]). Later, a series of studies indicated that the hypocretin system may impact motivational processes via projections to the ventral tegmental area (VTA) [4–6]. Consistent with the anatomy, hypocretin peptides increase firing frequency of VTA dopamine (DA) neurons directly [7] and through enhancement of glutamatergic inputs to DA neurons [8,9]. Moreover, hypocretin peptides enhance DA signaling in VTA target regions including the prefrontal cortex and the nucleus accumbens (NAc) [10–12], and increase the effects of cocaine on DA tone and stimulated DA release in the NAc core [12]. In accordance with these observations, blockade of HCRTr1 reduces DA neuron firing [13], however, the impact of HCRTr1 blockade on synaptic output of DA is more complex. While systemic blockade of HCRTr1 has no effect on DA tone in the NAc core [11,14] or shell [15] as measured by in vivo microdialysis, it does reduce phasic release of DA in the NAc core as measured by in vivo fast scan cyclic voltammetry [14]. Moreover, blockade of HCRTr1 reduces the effects of cocaine on DA tone and stimulated DA release in the NAc core [14]. These reports provide strong evidence that the hypocretin system participates in the regulation of reward and reinforcement processes dependent on DA signaling.

*Abbreviations:* DA, dopamine; EEG, electroencephalogram; EMG, electromyogram; FR, fixed ratio; I.P., intraperitoneal; I.V., intravenous; HRCTr1, hypocretin receptor 1; HRCTr2, hypocretin receptor 2; NAc, nucleus accumbens; PR, progressive ratio; VTA, ventral tegmental area.

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In addition to influencing DA signaling, the hypocretin system regulates aspects of cocaine self-administration behavior. Specifically, it has been suggested that hypocretin preferentially regulates appetitive behaviors that require high effort, but has little effect on consummatory behaviors associated with low effort [12,14]. Classically, the modulation of appetitive behaviors has been tested using a progressive ratio (PR) schedule that increases response requirements, and therefore effort requirements, across a given session [16]. Under a PR schedule, hypocretin-1 peptide promotes responding for cocaine while blockade of HCRTr1 produces the opposite effects [9,14]. In contrast, modulation of consummatory behaviors has classically been tested with fixed ratio (FR) schedules where lever press requirements, and therefore effort requirements, remain low and constant across a session. Under these conditions, hypocretin manipulations leave consummatory behaviors intact [12,14,17].

It should be noted, however, that the degree of effort an animal is required to expend within any self-administration paradigm is a function of both the number of responses required to obtain drug as well as the dose of drug provided [18], and the ability of pharmacological pretreatments to reduce cocaine self-administration appears to depend on this relationship [19]. The hypocretin antagonist studies outlined above used similar doses of cocaine (0.50–0.75 mg/kg) and analyzed responding solely at low or high response requirements. Therefore, it remains unclear as to whether the effects of hypocretin manipulations on cocaine self-administration will vary as a function of dose provided and response requirement.

The hypocretins also participate in the regulation of arousal, and in particular sleep/wake behavior. This has raised concerns that some of the behavioral effects of HCRTr1 blockade may be mediated through gross deficits in arousal rather than more direct disruption of circuits implicated in motivation. Several studies have begun to disentangle the roles of hypocretin in the regulation of sleep/wake cycle and motivational processes, and it appears that the reward and reinforcement influences of hypocretin may be mediated primarily through the HCRTr1 receptor [12,14,17,20], without associated changes in sleep/wake activity [21–23]. Nevertheless, there remains some debate over the sleep promoting/sedative effects of HCRTr1 blockade [24][see 24], and no study as of yet has monitored the effects of HCRTr1 blockade on motivation and sleep using identical hypocretin agents and dosing.

The current studies examined the extent to which the HCRTr1 antagonist SB-334867 affects responding for three unit doses of cocaine with varying reinforcing efficacies (0.375, 0.75, and 1.5 mg/kg), including the maximally reinforcing dose of cocaine for this schedule (1.5 mg/kg) [16]. Rats were trained to take cocaine on a PR schedule of reinforcement and were then tested with systemic treatment of 7.5, 15, or 30 mg/kg SB-334867, a HCRTr1 antagonist with 50 fold affinity for HCRTr1 over HCRTr2 [25]. Data were then analyzed in accordance to a two phase model that addresses features of relatively low effort and high effort behavior within the PR session. Finally, we tested the effects of 7.5, 15, and 30 mg/kg SB-334867 on sleep with electroencephalographic (EEG) and electromyographic (EMG) recordings in order to rule out sleep associated confounds.

## 2. Materials and methods

### 2.1. Animals

Male Sprague–Dawley rats (340–440 g, Harlan, Frederick, MD) were given ad libitum access to food and water and kept on a reverse 12:12 h light:dark cycle (lights on at 15:00 h). All protocols and animal care procedures were maintained in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals: Eighth Edition (The National Academies Press, Washington, DC, 2011) and approved by the Institutional Animal Care and Use Committee at Drexel University College of Medicine.

### 2.2. Chemicals and dosing

SB-334867 is considered to be a relatively selective HCRTr1 antagonist with some off target interactions, but at least 30–100 fold higher selectivity for the HCRTr1 over HCRTr2 and other potential targets [25,26]. SB-334867, was obtained as a free base (Tocris R&D, Minneapolis, MN), and was stored desiccated for no more than 3 months in a light impermeable bottle to minimize decomposition [27]. Drug was prepared fresh daily as a suspension in 10% β-cyclodextran + 4% dimethyl sulfoxide in distilled $H_2O$, and was administered 30 min prior to behavioral testing as a single 2 ml intraperitoneal (i.p.) dose. Selected doses were based on previous studies indicating changes in drug associated behavior and DA signaling [14,17,28–30].

### 2.3. Self administration

#### 2.3.1. Surgery

Rats used for self-administration experiments were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg), and implanted with an intravenous (i.v.) silastic catheter placed into the right jugular vein. Rats received post-surgical antibiotic (Neo-Predef, Pharmacia & Upjohn Company, New York, NY) and analgesic (5 mg/kg; Ketoprofen, Patterson Veterinary, Devens, MA) and recovered for 3 days prior to training.

#### 2.3.2. Training and testing

Rats were trained to self-administer cocaine on a FR schedule in which single lever presses result in single injections of cocaine. i.v. catheters were connected through a stainless steel spring to a counterbalanced swivel (Instech Laboratories, Plymouth Meeting, PA, USA). Lever responses resulted in delivery of 0.75 mg/kg cocaine (in saline; National Institute on Drug Abuse) over an approximate 5 s period followed by a 20 s inter-trial interval. FR training sessions were terminated after 20 injections. Once stable patterns of cocaine self-administration were reached (~2–4 days) rats were separated into one of three groups, and switched to 0.375, 0.75, or 1.5 mg/kg cocaine dose on a PR schedule for additional training and SB-334867 testing.

Throughout the PR schedule rats were given access to a response lever at 10:00 h, and single cocaine injections were contingent upon an increasing number of responses: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, and 603 [16]. When the required number of responses was made, a single 0.375, 0.75, or 1.5 mg/kg cocaine injection was delivered. The self-administration session was terminated after 6 h. Following 3 days of stable baseline responding (less than 20% variance with no ascending or descending trends), rats were treated with vehicle or varying doses of SB-334867. Rats received i.p. vehicle or SB-334867 in the middle of their dark phase, which corresponded to 30 min before onset of the self-administration session (09:30 h). Rats were treated with vehicle and each dose of SB-334867 based on a Latin-square design, with a minimum of 3 days between treatments. Individual rats were trained on the PR schedule at one dose of cocaine, and tested at each dose of SB-334867. All rats were tested during the dark/activity phase of the light/dark cycle.

#### 2.3.3. Data analysis

To examine the effect of SB-334867 on patterns of responding we averaged the cumulative number of injections across animals into 5 min time bins across the duration of the PR session. Initial