Suvorexant, an orexin/hypocretin receptor antagonist, attenuates motivational and hedonic properties of cocaine

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ABSTRACT

Orexins (‘hypocretins’) are peptides produced by neurons of the hypothalamus that project to structures implicated in reward and emotion processing. Converging evidence demonstrates functional roles of orexin signaling in arousal, sleep/wakefulness and motivated behaviors for natural and drug rewards. Suvorexant, a dual orexin receptor antagonist, recently received approval from the US Food and Drug Administration to treat insomnia. In Experiment 1, rats self-administered cocaine under a progressive-ratio schedule of reinforcement and the effects of suvorexant on motivation to self-administer cocaine were measured. In Experiment 2, the effects of suvorexant on cocaine reward were assessed by using a place conditioning paradigm, and 50-kHz ultrasonic vocalizations were also recorded to track changes in hedonic reactivity to cocaine. To rule out potentially confounding effects of suvorexant-induced somnolence, locomotor activity was also measured. In Experiment 3, the effects of suvorexant on cocaine-evoked elevations in ventral striatal dopamine were examined. Data reveal that suvorexant (i) reduced the number of cocaine infusions earned during progressive-ratio self-administration; (ii) attenuated initial positive hedonic reactivity to cocaine and prevented cocaine place preference; (iii) did not affect cocaine-induced hyperlocomotion and (iv) reduced cocaine-induced elevations in extracellular ventral striatal dopamine. The present study examined the therapeutic potential of suvorexant in rodent models of cocaine use disorder. These results contribute toward a growing literature supporting therapeutic roles of orexin receptor antagonists in treating substance use disorders.

Keywords Addiction, affect, dopamine, orexin, self-administration, ultrasonic vocalizations.

INTRODUCTION

Illicit drug use remains a major problem in the USA costing approximately $181 billion annually and leading to deteriorated life-styles for the drug user as well as friends and families of drug users. Psychostimulants such as cocaine cause rapid and long-lasting changes in brain reward circuitry principally by elevating synaptic levels of dopamine (DA) (Heal, Gosden, & Smith 2014). Cocaine administration transiently causes euphoria and subjective positive affect. It is generally believed that the positive affective state following cocaine administration functions as a reward signal, which is conducted in part through mesolimbic DA transmission—aferents within the ventral tegmental area (VTA) transmit DA to postsynaptic targets within ventral striatum. Pharmacological interventions that work to reduce positive affect following cocaine use may prove useful by reducing the rewarding value of the drug.

Orexins (‘hypocretins’) are peptides produced within the hypothalamus that innervate monoaminergic nuclei of the brainstem, including VTA, locus coruleus and dorsal raphe nucleus (Peyron et al. 1998; Darwinkel et al. 2014). Orexins exert excitatory effects by signaling

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through two G-protein coupled receptors (OX₁R and OX₂R). Accordingly, orexin transmission participates in various behavioral states including arousal, sleep/wakefulness and motivation to retrieve natural and drug rewards (de Lecea et al. 1998; Sakurai et al. 1998; Borgland et al. 2009; Smith, See, & Aston-Jones 2009; Mahler et al. 2012). Systemically administered OX₁R antagonists reduce morphine-conditioned place preference (CPP) (Harris, Wimmer, & Aston-Jones 2005), block cued and stress-induced reinstatement of cocaine-seeking (Boutrel et al. 2005; Smith et al. 2009) and reduce self-administered cocaine (Muschamp et al. 2014; Brodnik et al. 2015). Dual orexin receptor antagonists (DORA) also reduce cocaine-seeking behavior in part through augmenting cocaine-evoked elevations in ventral striatal DA (Prince et al. 2015). Moreover, signaling via OX₁Rs appears to selectively modulate motivation for high-incentive rewards, such as cocaine and high-fat food but not normal chow (Borgland et al. 2009). Recently, selective OX₂R antagonism was shown to reduce escalation in self-administered heroin under extended-access conditions (Schmeichel et al. 2015). Orexin transmission thus appears to mediate aspects of the rewarding and reinforcing properties of abused drugs through both receptor subtypes, and antagonists reliably reduce the rewarding properties of cocaine in pre-clinical models.

Converging anatomical and functional reports highlight the significance of orexin transmission within the mesolimbic reward circuit for addiction-related psychiatric disorders (Calipari & España 2012). Orexins provide both direct and feed-forward excitatory input to dopaminergic neurons of the VTA (Fadel & Deutch 2002; Korotkova et al. 2003; Muschamp et al. 2014). Intra-VTA application of the orexin-A peptide increases cocaine-seeking and enhances cocaine-evoked increases in DA transmission to ventral striatal targets (España et al. 2011). Blockade of orexin transmission appears to diminish the reinforcing effects of cocaine by attenuating cocaine-induced mesolimbic DA transmission at its origin in the VTA (e.g., España et al. 2010).

The goal of the present study was to evaluate the therapeutic potential of suvorexant, a clinically available DORA, in rodent models of cocaine use disorder. Specifically, we used a self-administration model to study the effects of suvorexant on cocaine-seeking. We further assessed the effects of suvorexant on conditioned cocaine reward and on hedonic processing of cocaine by using CPP and through recording positively valenced 50-kHz ultrasonic vocalizations (USVs), respectively. Next, we measured the effects of suvorexant on cocaine-induced locomotor activity. Finally, we performed in vivo fast-scan cyclic voltammetry to assess the effects of suvorexant on cocaine-evoked elevations of ventral striatal DA.

MATERIALS AND METHODS

Animals

Adult male Sprague–Dawley rats (Charles River; Horsham, PA, USA), arrived at Temple University’s vivarium, were pair-housed and given food and water ad libitum. Rats acclimated to the vivarium for at least 1 week before beginning experiments. For Experiment 1, rats were singly housed following jugular vein catheterization surgery and were placed on a reverse 12-hour : 12-hour light:dark cycle with lights turning off at 09:00 AM. For Experiment 2, rats were pair-housed throughout the experiment. For Experiment 3, mice (Charles River; Horsham, PA, USA) were used and were housed in cages of 2–5 mice per cage until surgery and were provided food and water ad libitum. All experimental procedures were approved by the Institutional Animal Care and Use Committees of Temple University and Drexel University.

Drugs

For all Experiments, suvorexant (Selleckchem; Munich, Germany) was dissolved in 100 percent dimethyl sulfoxide through vortexing and ultrasonication and was administered at a fixed volume (100 μl, i.p.). For all experiments, cocaine hydrochloride (Sigma; St. Louis, MO, USA) was dissolved in 0.9 percent physiological saline.

Experimental procedures

Experimental designs can be found in Table 1.

Experiment 1: Cocaine self-administration

For jugular vein catheterization surgery to permit cocaine self-administration in Experiment 1, rats were anesthetized with isoflurane gas (5 percent induction, 2–3 percent maintenance) and an aseptic environment was maintained throughout. Once under surgical anesthesia, a permanent indwelling catheter (Camcaths; Cambridgeshire, United Kingdom) was implanted in the right jugular vein of the rat and was connected to a stainless steel exit port of the rat’s mid-scapular region. Incisions were closed with 9-mm wound clips and treated post-operatively with antibiotic ointment. Rats recovered from surgery for 5–7 days before beginning cocaine self-administration training.

For all self-administration sessions, rats were moved to sound-attenuating, ventilated behavioral chambers (MED Associates; St. Albans, VT, USA) after receiving a 100-μl experimenter-administered infusion of heparinized saline to aid in maintaining catheter patency. Rats were weighed daily and connected to polyurethane tubing via
the stainless steel exit port, which was enclosed within a metal spring leash, attached to a fluid swivel and connected to a syringe pump for drug delivery. Cocaine infusion duration was adjusted each session based on rat bodyweight, averaging around 3 seconds, and dose was maintained at ~0.36 mg/kg/infusion for all infusions. For fixed-ratio (FR) reinforcement, session duration was 2 hours or 60 infusions, and the intertrial interval was 20 seconds. For progressive-ratio (PR) reinforcement, session duration was 4 hours, 60 infusions or an absence of responding for 90 minutes, and the intertrial interval was 30 seconds. Rats began cocaine self-administration training on a FR-1 schedule of reinforcement, and criterion for advancement was ≥20 infusions for three consecutive sessions. Rats were then moved to a PR schedule of reinforcement, where infusions were rewarded upon performing an incrementing number of lever-press operant responses for subsequent infusions (e.g. 1, 1, 2, 4, 6, 9, 12, and 15), and criterion for advancement was three consecutive sessions where the number of infusions was maintained within ±1 infusion relative to prior-day performance. Rats were then moved to the treatment phase of self-administration where a 3-day repeating block of sessions consisting of (1) FR-1; (2) PR without suvorexant pre-treatment and (3) PR with suvorexant pre-treatment was used. A summary timeline of events can be found in Fig. 1a.

Experiment 2: Conditioned place preference, 50-kHz ultrasonic vocalizations and locomotor activity

For CPP, a two-chamber apparatus with visually and tactically distinguished contexts, separated by a removable partition, was used following a biased, forced-choice design. Rats (n = 8/group) were first allowed to freely shuttle between the two contexts during a 30-minute pre-test to assess natural preference, and time on each context was recorded. Eight daily, 30-minute conditioning trials proceeded. Rats were pre-treated with either suvorexant (30 mg/kg, i.p.) or vehicle followed by injections of either cocaine (10 mg/kg, i.p.) or saline vehicle and placed in Context A (non-preferred; four trials) or Context B (preferred; four trials), respectively. Lastly, rats were given a post-test for 30 minutes, and time spent on each context was recorded. Pre-test and post-test times were used to calculate CPP Score.

For USV recording, a condenser microphone (CM16/CMPA; Avisoft Bioacoustics; Berlin, Germany) was suspended above each of two distinguished contexts and recorded 50-kHz USVs during first conditioning trials in Contexts A and B of CPP (described earlier; n = 6–8/group). Audio was sampled at 192 kHz (16 bits) from an amplification unit (UltraSoundGate 116H; Avisoft Bioacoustics; Berlin, Germany) and was processed by recording software (Avisoft Bioacoustics; Berlin, Germany) and was processed by recording software (Avisoft Bioacoustics; Berlin, Germany) on an IBM laptop. Recorded ‘.wav’ files were analyzed offline by using either RAVENPRO software (Cornell Lab of Ornithology, Bioacoustics Research Program; Ithaca, NY, USA) or from an XBAT/MATLAB-based auto-detection program (Barker et al. 2014a) for generating 50-kHz USV count data.

For the locomotor activity assay, rats were placed individually into activity chambers and allowed to acclimate for 30 minutes. Activity was measured as beam breaks collected in 5-minute time bins for 180 minutes. Baseline activity was recorded for 30 minutes, followed by suvorexant pre-treatment.
(30.0 mg/kg, i.p.) or vehicle (0.1 ml, i.p.) and a subsequent activity recording for 30 minutes. An acute cocaine (10.0 mg/kg, i.p.) or saline (1.0 ml/kg, i.p.) injection was administered, and the activity were recorded for the following 120 minutes. Digiscan DMicro system (Accuscan, Inc.; Columbus, OH, USA) measured ambulatory activity as consecutive beam breaks resulting from horizontal movement and non-ambulatory activity as repetitive-beam breaks.

Experiment 3: In vivo fast-scan cyclic voltammetry in ventral striatum

Mice for in vivo voltammetry (n = 5/group) were anesthetized with isoflurane, placed into a stereotoxic apparatus and subsequently implanted with a carbon fiber microelectrode aimed at the ventral striatum (AP: +1.3, ML: +1.3, DV: −4.5), and a Ag/AgCl reference electrode were placed in contralateral cortex. A bipolar stimulating electrode (Plastics One; Roanoke, VA, USA) aimed at the VTA (AP: −3.0, ML: +1.1, DV: −4.0) was lowered in 100 μM increments until a 0.5-s, 60-Hz monophasic (4 ms; 250 μA) stimulation train elicited a robust DA response. Stimulation trains were delivered every 5 minutes for at least 30 minutes until DA release reached stability (three consecutive collections within 10 percent). Once stability was achieved, mice were injected with suvorexant (30 mg/kg, i.p.) and subsequent changes in DA release were recorded for 30 minutes. Following the last collection, mice were injected with 10 mg/kg cocaine i.p. (6 mg/ml) and the change in DA release was recorded for at least 60 minutes.

For data acquisition, the electrode potential was linearly scanned (−0.4 to 1.2 V and back to −0.4 V versus Ag/AgCl) and cyclic voltammograms were recorded at the carbon fiber electrode every 100 ms with a scan rate of 400 V/s by using a voltmeter/amperometer (Chem-Clamp, Dagan Corporation; Minneapolis, MN, USA). The magnitude of electrically evoked DA release was monitored. DA overflow curves were analyzed as previously described for DA release (peak concentrations of DA) and uptake (t/au) by using DEMON VOLTMETRY AND ANALYSIS SOFTWARE written in Labview language (National Instruments; Austin, TX, USA; Yorgason, España, & Jones 2011).

Statistical analyses

For self-administration data, infusions and correct responses were expressed as % Baseline relative to prior-day baseline performance. * p < 0.05 relative to vehicle-pre-treated control data. Data are presented as mean ± SEM, n = 12
day performance. One-way repeated-measures ANOVAs were used with drug treatment as the within-subjects factor (0, 3, 10, and 30 mg/kg of suvorexant). Bonferroni-corrected contrast analyses proceeded for all suvorexant-pre-treated groups (3, 10, and 30 mg/kg) against vehicle-pre-treated (0 mg/kg) control data.

For CPP data, CPP Score was expressed as % Baseline relative to pre-test time (s) in Context B. A one-way ANOVA was used with treatment group as the between-groups factor (Veh-Sal, Suvo-Sal, Veh-Coc, Suvo-Coc), and Bonferroni-corrected contrasts were conducted against the Veh-Sal control group. For USV analyses, standardized change scores (Δ USV Score) were used to normalize data for parametric assessment and to assess changes in 50-kHz USVs following initial exposure to cocaine or saline by using the formula [(B – A)/(A + B)]. For between-groups analysis, independent sample t-tests were conducted against ‘0’—the point of no-change. For within-session analyses, a two-way mixed ANOVA examining treatment group by time was conducted.

For locomotor activity, a between-group one-way ANOVA was used to analyze the total activity during minutes 0 to 120 (post-cocaine). Tukey’s honest significant difference post hoc tests were used for pairwise comparisons, and an additional series of independent sample Bonferroni-corrected t-tests were used to examine locomotor differences within each 5-minute time bin during the pre-drug phase (–30 to 0 minutes).

For voltammetry analyses, % Dopamine was expressed as % Baseline relative to evoked DA release from VTA stimulation (recorded every 5 minutes for 30 minutes prior to drug pre-treatment). Two-way ANOVAs were used examining % Dopamine with treatment group and time as factors in 5-minute bins for time prior to or following acute cocaine administration.

For contrasts on self-administration, CPP and USV data, Type I error rate (a) within each family of comparisons was maintained at 0.05.

RESULTS

Experiment 1: Suvorexant decreases cocaine-seeking

Repeated-measure ANOVAs examining infusions [F(3, 8) = 2.286, NS] or correct responses [F(3, 8) = 1.679, NS] by drug treatment were not significant although contrast analyses revealed that the high dose of suvorexant (30.0 mg/kg) caused a significant reduction in infusions earned relative to prior-day performance [|t(11)| = 2.892, p < 0.05] and had a minor effect at reducing the number of correct responses [|t(11)| = 2.583, p = 0.075] (Fig. 1).

Experiment 2: Suvorexant prevents conditioned cocaine reward and attenuates initial hedonic reactivity to cocaine

A between-groups one-way ANOVA examining CPP Score revealed a significant main effect of treatment group [F(3, 28) = 3.635, p < 0.05]. Contrast analyses revealed that CPP Score from the Veh-Coc group was significantly greater than the Veh-Sal control group [t(14) = 2.600, p < 0.05], but that CPP Score from the Suvo-Coc group was not different from the Veh-Sal control group [t(14) = 1.662, NS] (Figs 2 & 3 and Supporting Information Fig. S1).

One-sample t-tests examining Δ USV Score found that positive affective reactivity to cocaine was significant for the vehicle-pre-treated group [t(7) = 5.461, p < 0.001] but not for the suvorexant-pre-treated group [t(5) = 0.408, NS] versus ‘0’—the point of no-change—when examining USVs across the entire 30-minute session. For within-session analyses, a two-way mixed model ANOVA examining Δ USV Score did not find a significant interaction between treatment group and time but did find a significant main effect of time [F(5, 50)]
60) = 11.415, p < 0.001] and a marginally significant main effect of treatment group [F(1, 12) = 4.600, p = 0.053]. No differences between Veh-Coc and Suvo-Coc groups were found when examining 50-kHz USV duration (M_Veh-Coc = 57.5 ms, M_Suvo-Coc = 59.4 ms) or bandwidth (M_Veh-Coc = 45.0 kHz, M_Suvo-Coc = 34.5 kHz) following systemic cocaine injection.

Experiment 3: Suvorexant reduces cocaine-induced elevations in ventral striatal DA

For time bins prior to acute cocaine injection (−30 to 0 minutes), a two-way ANOVA examining % Dopamine revealed a non-significant interaction [F(5, 48) = 0.598, NS], a non-significant main effect of time [F(5, 48) = 0.242, NS] and a trend toward significance in main effect of treatment group [F(1, 48) = 3.726, p = 0.06]. No significant pairwise comparisons were found. Following acute cocaine injection (0 to 60 minutes), a two-way ANOVA examining % Dopamine revealed neither a significant interaction between treatment group and time [F(11, 96) = 0.167, NS] nor a main effect of time [F(11, 96) = 0.597, NS]. There was, however, a significant main effect of treatment group indicating greater % Dopamine in the Veh-Coc group compared with Suvo-Coc group [F(1, 96) = 20.072, p < 0.001]. No significant effects of suvorexant on cocaine’s propensity to block the DA transporter (tau) were observed [main effect of treatment group during the post-cocaine phase: F(1, 96) = 0.902, NS] (Fig. 4 and Supporting Information Fig. S2).

**DISCUSSION**

Results from the present study suggest that suvorexant, a clinically available DORA, reduces cocaine-seeking in a pre-clinical cocaine self-administration model (Fig. 1). These results corroborate a recent report showing that either single or dual orexin receptor antagonism reduces cocaine-seeking by using PR schedule of reinforcement (Brodnik et al. 2015; Prince et al. 2015). Moreover, intra-VTA OX1R antagonism reduces cocaine infusions earned during self-administration in rats (España et al. 2010; Muschamp et al. 2014), suggesting that orexigenic transmission to and signaling within the VTA are critical for the reinforcing properties of cocaine. While studies have shown that orexins can induce food consummatory behavior (Sakurai et al. 1998), signaling via OX1Rs preferentially modulates motivation to consume highly palatable sweet foods (Smith et al. 2015).
salient rewards (Borgland et al. 2009; Martin-Fardon & Weiss 2014). Furthermore, significant effects of orexin receptor blockade on drug infusions are usually observed when rodents are placed on a PR schedule of reinforcement and not under PR access conditions (e.g. Smith et al. 2009; España et al. 2010; Hutcheson et al. 2011; Mahler, Smith, & Aston-Jones 2013). A separate line of evidence reveals a critical role for signaling via OX2Rs in mediating OX1R antagonism attenuates expression of amphetamine when rodents are placed on a PR schedule of reinforcement receptor blockade on drug infusions are usually observed (Harris terminals in VTA (Borgland 2010)). Orexins normally excite VTA DA neurons through somatodendritic OX1Rs (Korotkova et al. 2008). Orexins normally excite VTA DA neurons through somatodendritic OX1Rs (Korotkova et al. 2003) as well as by heteroreceptors on glutamatergic terminals in VTA (Borgland et al. 2006). Mechanistically, our data support that suvorexant, a clinically available DORAs reduce cocaine-evoked elevations in ventral striatal DA. A separate line of evidence finds that 50-kHz USVs following cocaine injection are modulated in part by mesolimbic DA transmission—systemic or direct blockade of DA signaling in the NAcc reduces 50-kHz USVs elicited by psychostimulant injection (Thompson, Leonard, & Brudzyński 2006; Williams & Undieh 2010; Wright, Dobosiewicz, & Clarke 2013). Furthermore, systemic OX1R antagonism decreases reward sensitivity in mice performing an operant task for brain stimulation reward in an intracranial self-stimulation paradigm. This may indicate that blocking orexin signaling pharmacologically alters hedonic state (Muschamp et al. 2014). Results from the present study support the idea that dopaminergic signaling along the mesolimbic pathway is critical for positive affect associated with cocaine administration and that blockade of orexin transmission decreases activity of this pathway and subjective experience following cocaine. Suvorexant contributes to somnolence and typically decreases locomotor activity in animals (Winrow et al. 2011). Results from the present study suggest that suvorexant reduces activity but likely does not interfere with behavioral task performance when an operandum is employed (i.e. during cocaine self-administration). It is possible that suvorexant-associated sedation contributed to the observed reduction in infusions earned during PR cocaine self-administration, but data reveal that all but one suvorexant-pre-treated rat did indeed self-administer cocaine (Supporting Information Fig. S3). Notably, systemic cocaine elicited comparable hyperlocomotor responses irrespective of drug pre-treatment (Fig. 3). Taken together, it is unlikely that the reported decrease in motivation to self-administer cocaine was due to suvorexant-induced hypolocomotor effects.
Together, our results support the possibility that DORAs may promote abstinence from drug taking in human cocaine users. We demonstrate that suvorexant attenuates both the hedonic as well as the motivational properties associated with cocaine use. These effects are likely due to a reduction in cocaine-evoked forebrain DA transmission by suvorexant. It should be noted that suvorexant is currently used as a sleep aid and thus, if investigated clinically for treating substance use disorders, may elicit sedation that could interfere with normal waking hours. Future studies are needed to more thoroughly characterize the effectiveness of DORAs in pre-clinical models of addiction relapse and to determine circuits responsible for the effects of suvorexant on motivation versus affective processing.

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Authors Contribution
TAG and SJS designed experiments, collected/analyzed data and wrote the manuscript under advisory of JWM. DJB analyzed ultrasonic vocalization data sets. JKS and RAE conducted in vivo voltammetry and collected/analyzed corresponding data sets.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

Figure S1. 50-kHz ultrasonic vocalizations (USVs) across elicited by rats during initial 30-minute place conditioning sessions. ‘V’ subjects (n = 8) constitute rats that received a saline injection prior to placement in Context A and a combined vehicle-pre-treatment (dimethyl sulfoxide; 0.1 ml, i.p.) + acute cocaine (10 mg/kg, i.p.) before placement in Context B. ‘S’ subjects (n = 6) constitute rats that received a saline injection prior to placement in Context A, and a combined suvorexant-pre-treatment (30 mg/kg, i.p.) + acute cocaine before placement in Context B.

Figure S2. % change in tau in ventral striatum between Veh-Coc and Suvo-Coc groups. The arrows indicate points of vehicle/suvorexant injection (~30 minute; 30 mg/kg, i.p.) and cocaine injection (0 minute; 10 mg/kg, i.p.). No significant differences are detected between treatment groups. Data are presented as mean ± SEM, n = 6–8/group.

Figure S3. Infusions earned per subject during progressive-ratio cocaine self-administration following 30-minute suvorexant pre-treatment (Veh, 3, 10, 30 mg/kg) as well as prior-day performances—indicated as ‘(Pre)’. ‘−’ indicates missing data. n = 10–12/dose, within-subject design.