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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 (DORA), recently received approval from the U.S. Food and Drug Administration (FDA) to treat insomnia. In Experiment 1, rats self-administered cocaine under a progressive-ratio (PR) schedule of reinforcement and effects of suvorexant on motivation to self-administer cocaine were measured. In Experiment 2, effects of suvorexant on cocaine reward were assessed using a place conditioning paradigm, and 50-kHz ultrasonic vocalizations (USVs) 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, 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 PR 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 towards a growing literature supporting therapeutic roles of orexin receptor antagonists in treating substance use disorders.

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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.

Tables.

Keywords

Addiction; Affect; Dopamine; Orexin; Self-Administration; Ultrasonic Vocalizations

Introduction

Illicit drug use remains a major problem in the United States costing approximately \$181 billion annually and leading to deteriorated lifestyles 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 DA (Heal *et al* 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—afferents within the ventral tegmental area (VTA) transmit DA to post-synaptic 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 coeruleus, and dorsal raphe nucleus (Darwinkel *et al* 2014; Peyron *et al* 1998). Orexins exert excitatory effects by signaling through two G-protein coupled receptors (OX₁R, OX₂R). Accordingly, orexin transmission participates in various behavioral states including arousal, sleep/wakefulness, and motivation to retrieve natural and drug rewards (Borgland *et al* 2009; de Lecea *et al* 1998; Mahler *et al* 2012; Sakurai *et al* 1998; Smith *et al* 2009). Systemically-administered OX₁R antagonists reduce morphine-conditioned place preference (Harris *et al* 2005), block cued and stress-induced reinstatement of cocaine-seeking (Boutrel *et al* 2005; Smith *et al* 2009) and reduce self-administered cocaine (Brodnik *et al* 2015; Muschamp *et al* 2014). Dual orexin receptor antagonists 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 preclinical models.

Converging anatomical and functional reports highlight the significance of orexin transmission within the mesolimbic reward circuit for addiction-related psychiatric disorders (Calipari and España 2012). Orexins provide both direct and feed-forward excitatory input to dopaminergic neurons of the VTA (Fadel and 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* 2010). 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.

The goal of the present study was to evaluate the therapeutic potential of suvorexant, a clinically-available dual orexin receptor antagonist, 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 effects of suvorexant on conditioned cocaine reward and on hedonic processing of cocaine using conditioned place preference (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 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 one 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 9:00 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 Institutional Animal Care and Use Committees of Temple University and Drexel University.

Drugs

For all Experiments, suvorexant (Selleckchem; Munich, Germany) was dissolved in 100% dimethyl sulfoxide (DMSO) through vortexing and ultrasonication and was administered at a fixed volume (100 μ L, i.p.). For all Experiments, cocaine hydrochloride (Sigma; St. Louis, MO, USA) was dissolved in 0.9% 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% induction, 2–3% 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 μ L experimenter-administered infusion of heparinized saline to aide 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 \sim 0.36mg/kg/infusion for all infusions. For fixed-ratio (FR) reinforcement, session duration was 2-hours or 60 infusions, and the intertrial-interval (ITI) 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 ITI was 30-seconds. Rats began cocaine self-administration training on a FR-1 schedule of reinforcement, and criterion for advancement was 20 infusions for 3 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, 15...), and criterion for advancement was 3 consecutive sessions where the number of infusions was maintained within \pm 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 pretreatment, and (3) PR with suvorexant pretreatment was used. A summary timeline of events can be found in Figure 1A.

Experiment 2: Conditioned Place Preference (CPP), 50-kHz Ultrasonic Vocalizations (USVs), and Locomotor Activity

—For CPP, a two-chamber apparatus with visually- and tactilely-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; 4 trials) or Context B (preferred; 4 trials), respectively. Lastly, rats were given a post-test for 30 minutes and time spent on each Context was recorded. Pre- 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 Context A and Context B of CPP (described above; $n=6-8$ /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) on an IBM laptop. Recorded “.wav” files were analyzed offline 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* 2014) 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 pretreatment (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 activity 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 stereotaxic 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 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 μ M increments until a 0.5-s, 60-Hz monophasic (4 ms; 250 μ A) stimulation train elicited a robust DA response. Stimulation trains were delivered every 5 mins for at least 30 mins until DA release reached stability (three consecutive collections within 10%). Once stability was achieved, mice were injected with suvorexant (30mg/kg, i.p.) and subsequent changes in DA release recorded for 30 mins. 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 mins.

For data acquisition, the electrode potential was linearly scanned (-0.4 to 1.2 V and back to -0.4 V vs Ag/AgCl) and cyclic voltammograms were recorded at the carbon fiber electrode every 100 ms with a scan rate of 400 V/s using a voltammeter/ampereometer (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 (*tau*) using Demon Voltammetry and Analysis software written in Labview language (National Instruments; Austin, TX, USA; Yorgason *et al* 2011).

Statistical Analyses

For self-administration data, Infusions and Correct Responses were expressed as % Baseline relative to prior-day performance. One-way repeated-measures ANOVAs were used with Drug Treatment as the within-subjects factor (0, 3, 10, 30 mg/kg of suvorexant). Bonferonni-corrected contrast analyses proceeded for all suvorexant-pretreated groups (3, 10, 30 mg/kg) against vehicle-pretreated (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 Bonferonni-corrected contrasts were conducted against the Veh-Sal control group.

For USV analyses, standardized change scores (“ 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 using the formula [(B-A)/(A+B)]. For between-groups

analysis, independent samples 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-groups one-way ANOVA was used to analyze total activity during minutes 0 to 120 (post-cocaine). Tukey’s HSD post-hoc tests were used for pairwise comparisons, and an additional series of independent samples 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 dopamine release from VTA stimulation (recorded every 5 minutes for 30 minutes prior to drug pretreatment). 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 (α) within each family of comparisons was maintained at 0.05.

Results

Experiment 1: Suvorexant Decreases Cocaine-Seeking (Figure 1)

Repeated-measures ANOVAs examining Infusions [$F(3, 8) = 2.286$, n.s.] or Correct Responses [$F(3, 8) = 1.679$, n.s.] 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 [$t(11) = 2.583$, $p = 0.075$].

Experiment 2: Suvorexant Prevents Conditioned Cocaine Reward and Attenuates Initial Hedonic Reactivity to Cocaine (Figures 2 and 3, Supplemental Figure 1)

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 [$t(14) = 1.662$, n.s.].

One-sample t-tests examining USV Score found that positive affective reactivity to cocaine was significant for the vehicle-pretreated group [$t(7) = 5.461$, $p < 0.001$] but not for the suvorexant-pretreated group [$t(5) = 0.408$, n.s.] 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 USV Score did not find a significant interaction between Treatment Group and Time but did find a significant main effect of Time [$F(5, 60) = 11.415$, $p < 0.001$] 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 ($M_{\text{Veh-Coc}} = 57.5$ ms, $M_{\text{Suvo-Coc}} = 59.4$ ms) or

bandwidth ($M_{\text{Veh-Coc}} = 45.0$ kHz, $M_{\text{Suvo-Coc}} = 34.5$ kHz) following systemic cocaine injection.

A one-way ANOVA examining activity counts during minutes 0 to 120 revealed a significant main effect of Treatment Group [$F(2, 72) = 11.640$, $p < 0.001$]. Post-hoc tests revealed significantly greater activity from minutes 0 to 120 in cocaine-treated groups irrespective of pretreatment compared to the vehicle-pretreated, acute saline-treated group [Veh-Coc: $p < 0.001$; Suvo-Coc: $p < 0.001$]. T-tests within each 5-minute bin revealed significantly lower activity at minutes -20, -15 and -10 [all $p < 0.05$] in the Suvo-Coc animals relative to Veh-Sal control animals.

Experiment 3: Suvorexant Reduces Cocaine-Induced Elevations in Ventral Striatal Dopamine (Figure 4, Supplemental Figure 2)

For time bins prior to acute cocaine injection (-30 to 0 minutes), a two-way ANOVA examining % Dopamine revealed a non-significant interaction [$F(5, 48) = 0.598$, n.s.], a non-significant main effect of Time [$F(5, 48) = 0.242$, n.s.] and a trend towards 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 [$F(11, 96) = 0.167$, n.s.] nor a main effect of Time [$F(11, 96) = 0.597$, n.s.]. There was, however, a significant main effect of Treatment Group indicating greater % Dopamine in the Veh-Coc group compared to Suvo-Coc group [$F(1, 96) = 20.072$, $p < 0.001$]. No significant effects of suvorexant on cocaine's propensity to block the dopamine transporter (tau) were observed [main effect of Treatment Group during the post-cocaine phase: $F(1, 96) = 0.902$, n.s.].

Discussion

Results from the present study suggest that suvorexant, a clinically-available dual orexin receptor antagonist (DORA), reduces cocaine-seeking in a preclinical cocaine self-administration model (Figure 1). These results corroborate a recent report showing that either single or dual orexin receptor antagonism reduces cocaine-seeking using progressive-ratio 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 orexinergic transmission to and signaling within the VTA is 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-salient rewards (Borgland *et al* 2009; Martin-Fardon and Weiss 2014). Furthermore, significant effects of orexin receptor blockade on drug infusions are usually observed when rodents are placed on a progressive-ratio schedule of reinforcement and not under fixed-ratio access conditions (e.g. España *et al* 2010; Hutcheson *et al* 2011; Mahler *et al* 2013; Smith *et al* 2009). A separate line of evidence reveals a critical role for signaling via OX₂Rs in mediating escalation of drug-taking in self-administering rats using an extended-access design (Schmeichel *et al* 2015). OX₂Rs additionally potentiate VTA neuronal activity by enhancing

pre-synaptic glutamate transmission (Borgland *et al* 2008). Orexins normally excite VTA DA neurons through somatodendritic OX₁Rs (Korotkova *et al* 2003) as well as by heteroceptors on glutamatergic terminals in VTA (Borgland *et al* 2006). Mechanistically, our data support that suvorexant, a clinically-available DORA, may occlude cocaine-evoked striatal DA release by reducing excitability of VTA DA neurons. Notably, OX₁Rs in NAcc positively regulate phasic DA release when tested *ex vivo*, but our study revealed a selective effect of systemic suvorexant on cocaine-evoked DA changes and not on NAcc DA dynamics before cocaine injection (Patyal *et al* 2012). Results from the present study add to a growing literature detailing receptor-specific aspects of targeting OX₁Rs and OX₂Rs alone or in combination (Khoo and Brown 2014).

Results from the present study further suggest that suvorexant modestly attenuates cocaine CPP (Figure 2A). An earlier report demonstrated that systemic OX₁R antagonism significantly attenuated morphine CPP (Harris *et al* 2005), while other studies find that systemic OX₁R antagonism attenuates expression of amphetamine CPP and reduces motivation for conditioned cocaine reinforcement (Hutcheson *et al* 2011; Shaw *et al* 2016). CPP assesses reward following passive contextual conditioning, and thus orexin transmission may only be modestly recruited for development of conditioned reward. Additionally, orexin peptide levels in plasma and cerebrospinal fluid are highest during active hours (i.e. when in dark lighting) and lowest during inactive hours (Blouin *et al* 2013; Kiyashchenko *et al* 2002; Lee *et al* 2005; Mileykovskiy *et al* 2005). As CPP training and testing took place during the rats' inactive hours, effects of orexin transmission blockade may have been relatively weak compared to transmission blockade during active hours.

Ultrasonic vocalizations (USVs) have been used in preclinical models of substance use disorders to characterize changes in affective processing upon drug administration as well as during withdrawal and relapse (Barker *et al* 2014; Barker *et al* 2015; Knutson *et al* 2002). Experimenter- and self-administered cocaine robustly evoke 50-kHz USVs in rats (Barker *et al* 2010; Maier *et al* 2012; Mu *et al* 2009; Williams and Undieh 2010). Further, while 50-kHz USVs elicited following cocaine injection belong to fixed-frequency, frequency-modulated and "trill" categories, 22-kHz USVs associated with negative affective states are typically monotonic and thus classified predominantly as fixed-frequency USVs. Results from the present study suggest that DORAs are capable of reducing affective reactivity to cocaine as indicated by an augmentation of USV Score (Figure 2B, Supplemental Figure 1). *In vivo* voltammetry data from previous investigations (Prince *et al* 2015) as well as the present study (Figure 4, Supplemental Figure 2) find that single or dual orexin receptor antagonists 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 nucleus accumbens (NAcc) reduces 50-kHz USVs elicited by psychostimulant injection (Thompson *et al* 2006; Williams and Undieh 2010; Wright *et al* 2013). Furthermore, systemic OX₁R antagonism decreases reward sensitivity in mice performing an operant task for brain stimulation reward in an intracranial self-stimulation paradigm (ICSS). 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 support 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 progressive-ratio cocaine self-administration, but data reveal that all but one suvorexant-pretreated rat did indeed self-administer cocaine (Supplemental Figure 3). Notably, systemic cocaine elicited comparable hyperlocomotor responses irrespective of drug pretreatment (Figure 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 preclinical models of addiction relapse and to determine circuits responsible for the effects of suvorexant on motivation versus affective processing.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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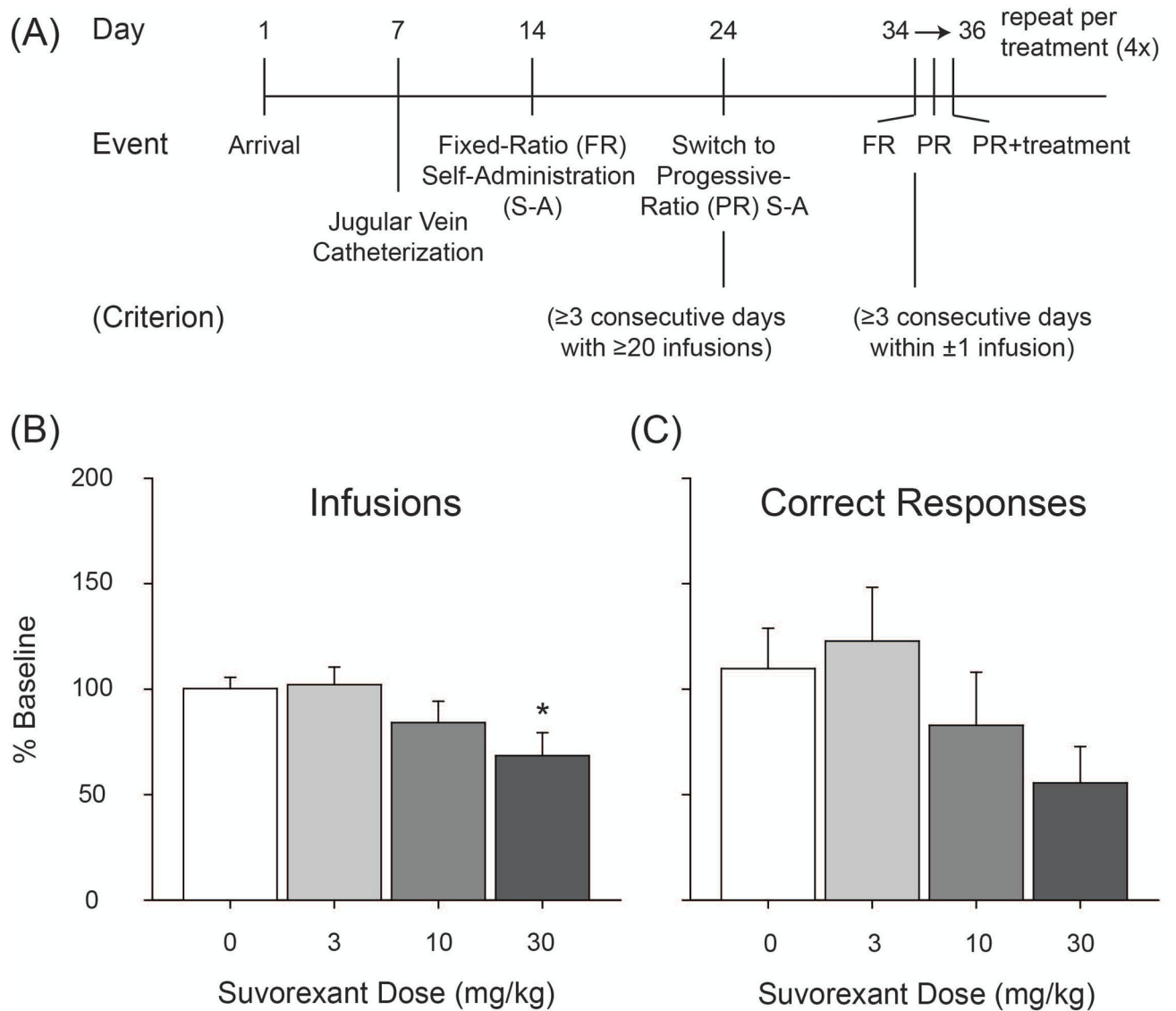


Figure 1.

(A) Timeline of events to train rats in cocaine self-administration. Effects of suvorexant on the number of (B) infusions and (C) correct responses relative to prior-day baseline performance. * $p < 0.05$ relative to vehicle-pretreated control data. Data are presented as mean \pm *S.E.M.* $n=12$.

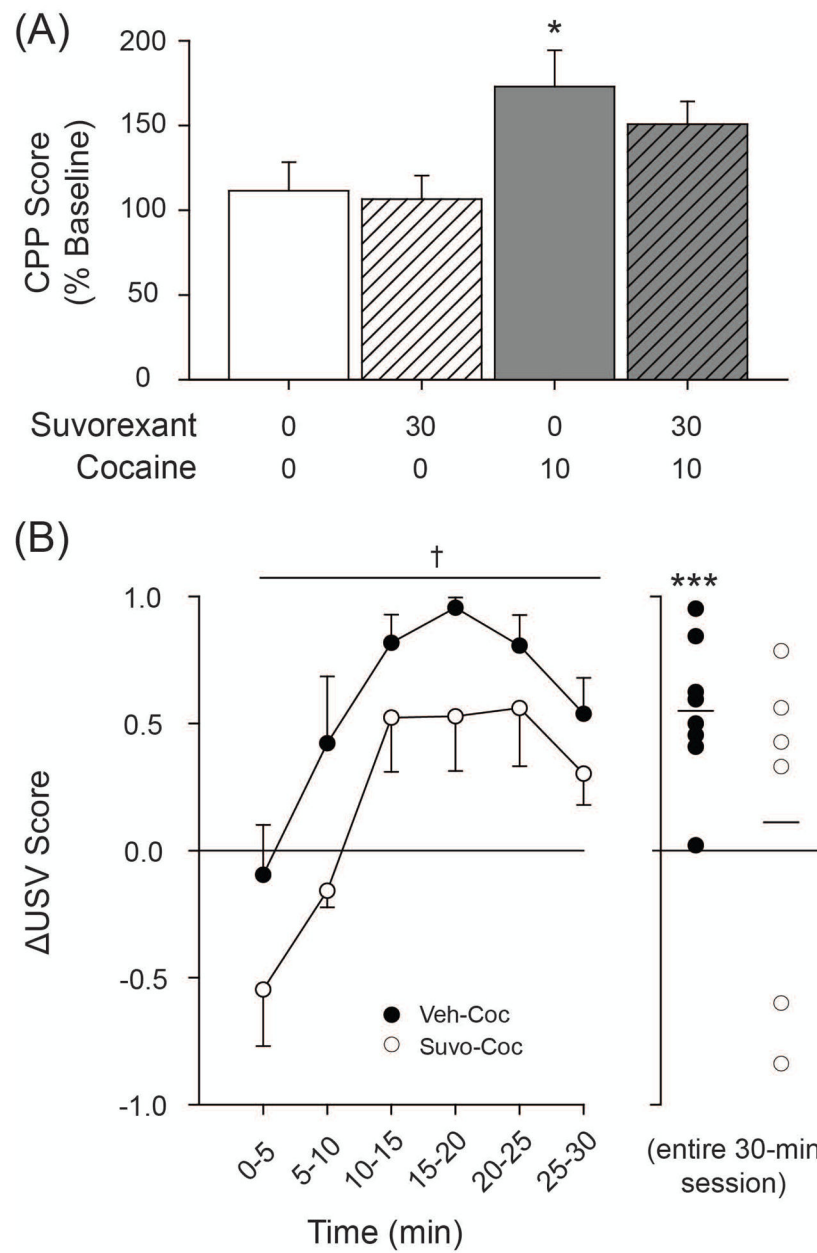


Figure 2. Effects of suvorexant on (A) CPP Score and (B) ΔUSV Score. † $p = 0.053$ main effect of Drug Type. *** $p < 0.001$ compared to “0” in (B). Data in (A) are presented as mean \pm *S.E.M.* $n = 6-8$ /group.

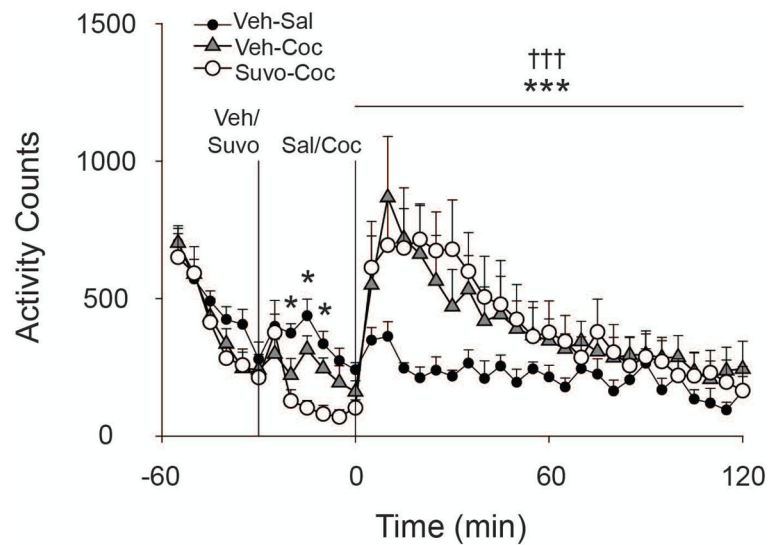


Figure 3. Effects of suvorexant on cocaine-induced locomotor activity. Solid vertical line indicates pretreatment with either suvorexant (30 mg/kg) or vehicle at -30 minutes. Solid vertical line indicates acute treatment with either cocaine (10 mg/kg) or vehicle at 0 minutes. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ indicates activity count difference between Veh-Sal and Suvo-Coc groups. † $p < 0.05$, †† $p < 0.01$, ††† $p < 0.001$ indicates activity count difference between Veh-Sal and Veh-Coc groups. Data are presented as mean \pm *S.E.M.* $n=8$ /group.

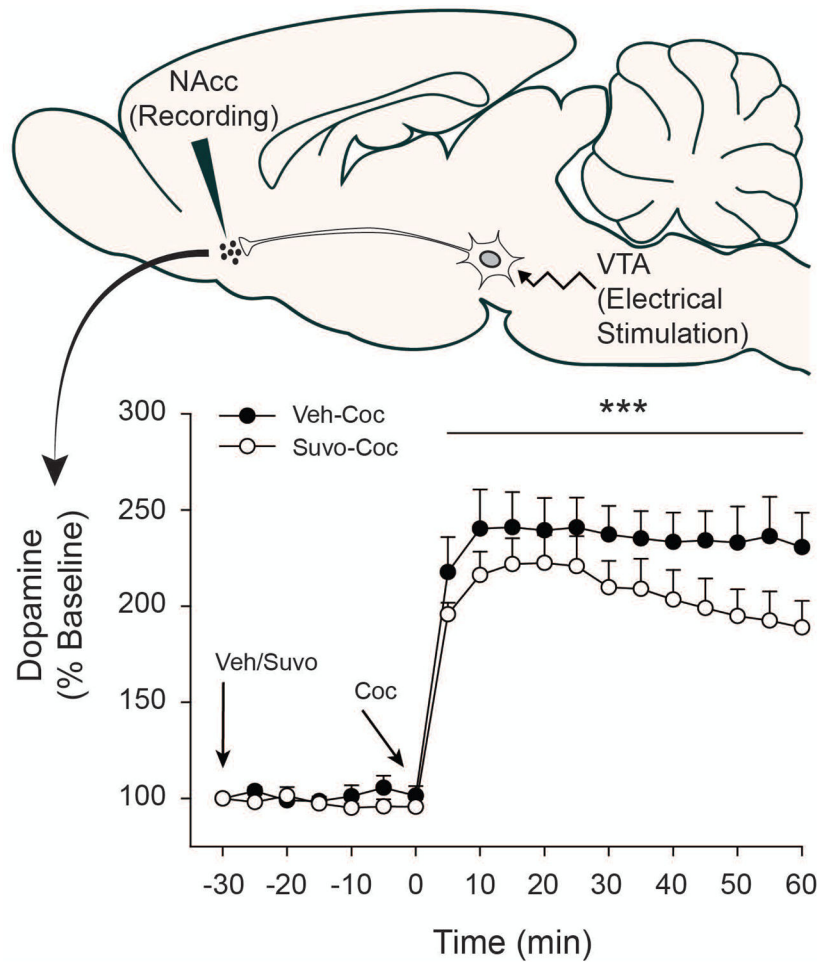


Figure 4. Dopamine levels (% Baseline) in ventral striatum between Veh-Coc and Suvo-Coc groups. Arrows indicate points of vehicle/suvorexant injection (-30 min; 30 mg/kg, i.p.) and cocaine injection (0 min; 10 mg/kg, i.p.). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ relative to Veh-Coc group. Data are presented as mean \pm *S.E.M.* $n=5$ /group.

Table 1

Experimental designs for cocaine self-administration (Experiment 1), CPP with 50-kHz USV recording and cocaine-induced locomotor activity (Experiment 2), and *in vivo* fast-scan cyclic voltammetry on cocaine-evoked dopamine levels in ventral striatum (Experiment 3).

Experiment	Assay/Paradigm	Group Design	Suvorexant Pretreatment	Cocaine Acute Treatment	Dependent Measures and Significance
Experiment 1	Self-Administration	Within	Veh	N/A	Infusions* Correct Responses
			3.0 mg/kg		
			10.0 mg/kg		
			30.0 mg/kg		
Experiment 2	Conditioned Place Preference (CPP)	Between	Veh	Sal	Preference [‡] USV Score [‡]
			30.0 mg/kg		
			Veh	10.0 mg/kg	
			30.0 mg/kg		
Experiment 3	Fast-Scan Cyclic Voltammetry	Between	Veh	Sal	Activity Count
			30.0 mg/kg	10.0 mg/kg	
			Veh	10.0 mg/kg	
			30.0 mg/kg		Dopamine [§]

* Designates significance versus “100” or “0”—the points of no-change—following suvorexant pretreatment.

‡ Designates significance versus vehicle-pretreated group.

§ Designates lack of significant cocaine-evoked effect following suvorexant pretreatment.