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Role of hypocretin/orexin receptor blockade on drug-taking and ultrasonic vocalizations (USVs) associated with low-effort self-administration of cathinone-derived 3,4-methylenedioxypyrovalerone (MDPV) in rats

Steven J. Simmons*, Rose Martorana, Helene Philogene-Khalid, Fionya H. Tran, Taylor A. Gentile, Xu Xinyan, Su Shu, Scott M. Rawls, PhD, and John W. Muschamp, PhD

Center for Substance Abuse Research (CSAR); Lewis Katz School of Medicine at Temple University; Philadelphia, PA, 19140

Abstract

Rationale—Synthetic psychostimulant abuse, including cathinone-derived 3,4-methylenedioxypyrovalerone (MDPV), continues to increase in many countries. Similar to cocaine but with greater potency, MDPV elicits a transient sympathomimetic response by blocking cellular uptake of dopamine (DA) and norepinephrine (NE)—administration in some users is reported as euphoria-inducing much like cocaine and amphetamine. Pharmacological agents that disrupt excitatory transmission onto midbrain DA-producing neurons, including hypothalamic hypocretin/orexin (hcrt/ox) receptor antagonists, present attractive targets to aide abstinence maintenance by reducing psychostimulant-associated reward and reinforcement.

Objective—The present study sought to assess the degree to which suvorexant, a dual hcrt/ox receptor antagonist, influences drug-taking as well as ultrasonic vocalizations (USVs) associated with MDPV self-administration.

Methods—Rats were trained to self-administer MDPV (~0.03 mg/kg/inf, 3-s) for 14 days under a fixed-ratio 1 schedule of reinforcement, and effects of suvorexant (0, 3, 10, 30 mg/kg, i.p.) on drug-taking was assessed. USVs were recorded during a 30-minute pre-lever period as well as during 2-hours of MDPV self-administration.

Results—We observed that suvorexant modestly suppressed the number of MDPV infusions earned. Notably, we observed that suvorexant reduced 50-kHz USVs associated with pre- and post-lever time-points but did not noticeably alter call type profiles. Upon comparison of the two measures, we observed trending positive associations between suvorexant-induced changes in drug-taking and 50-kHz USVs.

Conclusions—Results from this exploratory study provide support for: (1) studying how suvorexant may provide benefit to humans with stimulant use disorders, (2) identifying a potential

*Correspondence addressed to: Steven J Simmons, Center for Substance Abuse Research, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, 19140, Phone: (856) 571-3894, steven.simmons@temple.edu, O RCIID: 0000-0002-6982-4740.

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role for orexin transmission in cathinone abuse, and (3) further interrogating the potential utility of rat USVs to predict drug consumption in preclinical models of substance use disorders.

Keywords

3, 4-methylenedioxypyrovalerone (MDPV); hypocretin/orexin; self-administration; suvorexant; synthetic cathinone; ultrasonic vocalizations

Introduction

Abuse of stimulant drugs including cocaine and amphetamine remains untreated pharmacologically, and the recent surge in development of novel psychoactive substances has worsened an ongoing public health problem. While listed as Schedule I by the U.S. Drug Enforcement Agency in 2012, synthetic cathinone drugs including mephedrone, methylene and 3,4-methylenedioxypyrovalerone (MDPV) gained popularity from their previously uncontrolled status. Their low cost and availability from online cryptomarkets continues to drive their use (for review, Glennon 2014). Synthetic cathinone drugs elevate heart rate/respiration, decrease fatigue, and can induce euphoria, delirium and hallucination (Penders and Gestring 2011; Spiller et al. 2011). These drugs are used for their positive, euphoric effects, but acute overdose or repeated frequent use can be life-threatening or result in both cardiovascular and psychiatric adverse events that require emergency medical treatment.

Psychoactive stimulant drugs including MDPV and second generation analogues (e.g., α -pyrrolidinopentiophenone [PVP]) are reinforcing in animal models and can elevate brain catecholamine levels through multiple mechanisms. Specifically, MDPV blocks cellular uptake of dopamine (DA) and norepinephrine (NE) by binding to presynaptic transporters (Baumann et al. 2013). Preclinical studies find that MDPV elevates locomotor activity, primes reward threshold during intracranial self-stimulation, induces place preference, and is readily self-administered intravenously (Bonano et al. 2014; Nguyen et al. 2016; Schindler et al. 2016; Simmons et al. 2016; Watterson et al. 2014). At high doses, “bizarre behaviors” can be observed in rats following acute injection of synthetic cathinone drugs including sporadic jumping while facing away from chamber wall, retropulsion and flattened body posture (Marusich et al. 2014). In the first study of its kind, Hicks and colleagues demonstrated therapeutic utility of restoring glutamate clearance following MDPV administration—an effect critical for reinstating cocaine-seeking (Cornish and Kalivas 2000)—by pharmacologically inhibiting degradation of an endogenous agonist of metabotropic glutamate receptors (mGluR) 2/3 (Hicks et al. 2017). At present, no treatments have been found to block reinforcement associated with synthetic cathinone self-administration. However, an array of transmitter and peptide systems remain viable targets in efforts to normalize behaviors that propagate synthetic cathinone abuse.

Within the hypothalamus, hypocretin/orexin (hcrt/ox) peptides are synthesized and transported to numerous structures across the central nervous system (de Lecea et al. 1998; Sakurai et al. 1998). Hcrt/ox signals through two excitatory G-protein coupled receptors (OX₁R, OX₂R) and enhances neuronal excitability in catecholamine-producing cell types (Blasiak et al. 2015; Borgland et al. 2006; Brown et al. 2001; Eriksson et al. 2001; Hagan et

al. 1999). Notably, fiber immunoreactivity and receptor mRNA analyses revealed dense innervation across midbrain regions including ventral tegmental area (VTA), and hcrt/ox transmission was initially found to underlie arousal and appetite (Fadel and Deutch 2002; Marcus et al. 2001; Peyron et al. 1998). Soon thereafter, it was found that narcoleptic patients have depleted circulating levels of hcrt/ox (e.g., Nishino et al. 2000). In 2005, Aston-Jones and colleagues provided evidence that neurons producing hcrt/ox express the neuronal activity marker c-Fos after exposure to a chamber paired with palatable food or morphine, and that intra-VTA hcrt/ox receptor blockade reduces reinstatement of place preference for a morphine-paired context (Harris et al. 2005). Research teams have since demonstrated that blocking OX₁R transmission by systemic or intra-VTA injection reduces motivated responding and reinstated reward-seeking behavior for palatable reinforcers including self-administered cocaine (Bentzley and Aston-Jones 2015; Borgland et al. 2009; Boutrel et al. 2005; James et al. 2011; Muschamp et al. 2014; Smith et al. 2010). Reward suppression following hcrt/ox receptor antagonism may be driven in part by reductions in psychostimulant-evoked dopamine efflux within ventral striatum as has been supported using *in vivo* fast-scan cyclic voltammetry (España et al. 2011; España et al. 2010).

Suvorexant, a first-in-class hcrt/ox receptor antagonist, recently received approval by the U.S. Food and Drug Administration to treat insomnia. In clinical trials, suvorexant aided sleep onset and permitted more sustained sleep relative to placebo-treated control subjects (Herring et al. 2012). In rodent models of addiction, we found that suvorexant attenuates responding for cocaine in self-administering rats (Gentile et al. 2017). Additionally, we showed that suvorexant suppresses cocaine-elicited 50-kHz ultrasonic vocalizations (USVs)—a measure that may reflect a suppression of positive subjective response to cocaine—relative to vehicle pre-treatment levels. 50-kHz USVs are readily and robustly observed following cocaine self-administration as well as during exposure to a cocaine-paired context (Barker et al. 2014; Maier et al. 2010; Simmons *et al.* 2016) but can also be observed from aggressed male rats (Thomas et al. 1983) and following electrical footshock (Taylor et al. 2017).

In the present report, we expand these findings in an exploratory study by testing the ability of suvorexant to attenuate operant responding for MDPV and 50-kHz USVs associated with self-administration of MDPV. Results show a non-significant dose-related effect of suvorexant on MDPV infusions earned as well as on 50-kHz USVs during pre- and post-lever time-points. We also show that the effects of suvorexant on drug-taking trends towards positive correlation with reduction of 50-kHz USVs during MDPV self-administration—a finding that supports potential utility of USVs as a predictive measure for subsequent drug-taking behavior. Combined, our results contribute to an expanding literature positioning hcrt/ox as potential adjunctive pharmacotherapy target for treating substance use disorders.

Methods

Animals

Adult male Sprague-Dawley rats, aged 7–8 weeks at start of experiment, were used for the present report. Rats arrived from Harlan Laboratories (Indianapolis, IN, USA), were pair-housed and provided food chow (LabDiet 5012; St. Louis, MO, USA) and water *ad libitum*

until surgery. Rats were kept on a reverse 12-h: 12-h light cycle (lights off at 9:00 AM). All surgical and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Temple University.

Drugs

3,4-methylenedioxypyrovalerone (MDPV) was synthesized locally by Dr. Allen Reitz (Fox Chase Chemical Diversity Center, Inc.; Philadelphia, PA, USA) who verified purity using high-performance liquid chromatography. MDPV was dissolved in 0.9% saline by vortexing, was filtered through 0.45 μ m cellulose acetate and was stored at 4° C until distributed in syringes for intravenous self-administration—average bodyweight of cohort was used to adjust MDPV concentration once every 5–7 days to ensure infusion dose consistency across experiment. Suvorexant (AstaTech; Bristol, PA, USA) was dissolved in 0.1 mL dimethyl sulfoxide (100%) by vortexing and ultrasonication for 15–20 min (Branson 1800). Suvorexant solution was prepared immediately prior to pre-treatment injections as described below.

Surgery

For intravenous drug self-administration, rats were implanted with polyethylene catheter tubing (PE-20; SAI Infusion Technologies [RJVR-10]) in the right jugular vein. Rats were induced (5%) and thereafter maintained (2–3%) under isoflurane gas anesthesia mixed with oxygen (1.0 – 1.5 L/min) throughout surgery and were given injectable analgesia (meloxicam 2.0 mg/kg, s.c.) pre-operatively. Mid-scapular and neck regions were shaved and treated with betadine and alcohol three times each. On the dorsal surface, a mid-scapular midline incision was made and connective tissues cleared to allow for placement of backmount mesh secured to a 22-gauge stainless steel exit port (PlasticsOne; Roanoke, VA, USA). On the ventral surface, small sharp scissors were used to make an incision, and jugular vein was found and isolated. Catheter tubing (SAI Infusion Technologies; Lake Villa, IL, USA) ran from backmount to neck, terminated in the right jugular vein and was secured with absorbable suture thread (Coated Vicryl [polyglactin 910]; Ethicon). Incision sites were closed using 9 mm stainless steel wound clips (MikRon Precision Inc), were wiped with alcohol and treated topically with antibiotic ointment (bacitracin zinc, neomycin sulfate, polymyxin B sulfate) and lidocaine hydrochloride jelly USP (2%; Akorn). Rats were allowed 7 days to recover before beginning intravenous drug self-administration.

Self-administration: apparatus and session

Plexiglas chambers (12.0" [L] $\times$ 9.5" [W] $\times$ 11.5" [H]; Med Associates; St. Albans, VT, USA) used for self-administration were encased within wooden, ventilated, sound-attenuating cabinets. Rats were allowed to self-administer MDPV (~0.03 mg/kg/inf; ~50 μ L per 3-s inf) for 14 consecutive 2-h sessions (or 80 maximum infusions) under fixed-ratio (FR) 1 access conditions with programmed 20-s intertrial interval "timeout" periods following each infusion. To begin sessions, rats were connected to polyethylene tubing that itself was encased within a metal spring tether (PlasticsOne; Roanoke, VA, USA). Tubing connected to a plastic fluid swivel (375/22PS; Instech Laboratories, Inc; Plymouth Meeting, PA, USA), attached to a fulcrum arm, and automated syringe pump thereafter located outside of the Plexiglas chamber. Two levers extended into the chamber upon session start.

The lever located on the right side when facing lever-containing wall yielded drug infusions during availability periods, and the other lever designated as the inactive lever whereupon pressing had no programmed outcome. Syringe pump activation, tone, house- and cue-lights were controlled by a local PC running MED-PC IV software (MED Associates; St. Albans, VT, USA). MDPV infusions were 3-s in length and were paired with concomitant presentation of tone (~8.5-kHz; Sonalert) and cue light illumination ~1-in above active lever. Following MDPV infusion, house lights were turned off for a 20-s timeout period and operant responding on active lever was tallied but did not yield infusion until initiation of subsequent availability period. Catheter lines were kept patent with injections of 150 μ L of 0.9% saline containing heparin (Sagent Pharmaceuticals, 100 USP units/mL) and gentamicin (Henry Schein, 10 mg/mL) to maintain catheter patency and reduce infection risk prior to and immediately following each session.

Ultrasonic vocalization (USV) recording and analysis

Condenser microphones (UltraMic 200; Dodotronic; Italy) suspending above Plexiglas chambers were used to record USVs. Audio was sampled at 192-kHz, inputted to a local PC and recorded to 10-min. wav files using recording software (RavenPro; Cornell Laboratory of Ornithology; Ithaca, NY, USA). Spectrographic analysis proceeded using RavenPro software, and each detected USV was boxed to export start time, end time, lower and upper frequencies. USVs lasting at least 15 ms in length were included in analyses. USVs with a mean frequency (average between lower and upper) between 38- and 70-kHz were included as “50-kHz” calls and between 18- and 28-kHz as “22-kHz” calls. For call type analysis, identifiable contiguous audio fluctuations of 3-kHz were used to classify putative 50-kHz USVs as fixed-frequency (FF; 0 fluctuations), frequency-modulated with one to two fluctuations (FM₁₋₂) and frequency-modulated with three or more fluctuations (FM₃₊) call types.

Experimental procedures and timeline (Figure 1)

After 7 d of acclimating to vivarium conditions, rats were operated on to implant jugular vein catheters. As is detailed above, rats then underwent 14 daily sessions of MDPV self-administration. Rats then underwent pre-treatment sessions using a within-subjects design at which point suvorexant (0, 3, 10, 30 mg/kg, i.p.) was administered 15 min before placement in self-administration chamber. Pre-treatment assignment order followed a Latin square design. During each pre-treatment session, USVs were recorded during a 30-min “Pre-Lever” period followed by 2-h of self-administration which co-terminated along with self-administration programs. An initial cohort of rats (n=7) received 0 and 10 mg/kg pre-treatment whereas a second cohort (n=7) received 0, 3, 10 and 30 mg/kg pre-treatment doses. Upon comparison of lever responses (via independent samples t-tests) and of total USV count data (via Mann-Whitney U-Tests) between the two cohorts, no significant differences were observed (lever presses, Veh: [$t(12)$] = 0.15, n.s.], 10 mg/kg: [$t(12)$] = 2.06, n.s.]; total USV count data, Veh: [$U_1 = 11$, $|Z| = 1.73$, n.s.], 10 mg/kg: [$U_1 = 12$, $|Z| = 0.893$, n.s.]) and thus data were pooled for these pre-treatment doses in final analyses. While USVs during anticipation and self-administration were classified as belonging to 22- or 50-kHz USV categories, call type analysis (FF, FM₁₋₂, FM₃₊) proceeded on the first 5 minutes of “Pre-Lever” and “Post-Lever” time-points.

Data analysis

Lever response data (infusions earned) was analyzed using a one-way ANOVA with Treatment Group as between-groups factor. 50-kHz USV data were analyzed using non-parametric Wilcoxon signed-rank tests for “Pre-Lever” and “Post-Lever” time-points as well as for Total 50-kHz USVs. For % change analyses, the ratio of total 50-kHz USVs from suvorexant pretreatment (3, 10, 30 mg/kg) to total 50-kHz USVs from vehicle pretreatment was computed and compared to “100”—the point of no-change—using Bonferroni-corrected one-sample t-tests. Due to hardware error and file loss, two sessions in the 10 mg/kg pretreatment group were not able to be included in analysis. Correlations between % change in infusions and % change in USVs were conducted using Bonferroni-adjusted Spearman correlations (all p -values displayed are corrected for multiple comparisons). Pie charts for call type analysis were calculated by determining proportions of FF, FM₁₋₂ and FM₃₊ per subject followed by averaging across subjects per drug pretreatment conditions. For correlations, one subject was omitted from analysis as no 50-kHz USVs were detected in either vehicle- or suvorexant-pretreatment session (thus % change values could not be calculated).

Results

Effect of suvorexant on self-administered MDPV (Figure 2)

Under a fixed-ratio 1 schedule of reinforcement, suvorexant did not significantly reduce MDPV infusions earned across doses tested [$F(3, 38) = 0.42$, n.s.] (Figure 2A). A qualitative analysis on latency to self-administer the first ten infusions of MDPV (referred to as “load-up” period during intravenous drug self-administration) supported the finding that suvorexant increased time taken to retrieve initial MDPV infusions (Figure 2B).

Effect of suvorexant on USVs associated with MDPV self-administration (Figure 3)

50-kHz USVs emitted during “Pre-Lever” and “Post-Lever” time-points by suvorexant pre-treatment dose are plotted in Figure 3A. A significant reduction in 50-kHz USVs was observed following moderate-dose suvorexant pre-treatment (10 mg/kg) during “Pre-Lever” ($W_s = 7.0$, $p < 0.05$) and “Post-Lever” ($W_s = 11.0$, $p < 0.05$) time-points as well as upon examination of total 50-kHz USVs ($W_s = 6.0$, $p < 0.05$). When comparing % total 50-kHz USVs relative to vehicle-pretreatment baseline levels, a significant reduction was observed following suvorexant pretreatment at 10 mg/kg [$|t(11)| = 3.14$, $p < 0.05$] (data not shown). No apparent patterns of USV call type changes were observed when examining 50-kHz USVs during “Pre-Lever” and “Post-Lever” time-points irrespective of suvorexant pre-treatment (dark grey shading in Figure 3B). From analyzed samples, the majority of USVs emitted during both “Pre-Lever” and “Post-Lever” time-points irrespective of pre-treatment belonged to FM₁₋₂ 50-kHz USV call type.

Association between drug-seeking and affect in MDPV self-administering rats (Figure 4)

Correlations associating suvorexant’s effects on drug-taking and 50-kHz USVs are displayed in Figure 4. The degree to which moderate-dose suvorexant (10 mg/kg) decreased MDPV infusions earned trended towards positive association with change in “Pre-Lever” [$r_s(9) =$

0.62, $p = 0.13$] and “Post-Lever” [$r_s(9) = 0.62$, $p = 0.13$] 50-kHz USVs (Figures 4A.ii and B.ii, respectively). Similar trends were observed following pretreatment with high-dose (30 mg/kg) suvorexant (Pre-Lever: [$r_s(5) = 0.82$, $p = 0.07$]; Post-Lever: [$r_s(5) = 0.79$, $p = 0.11$]) (Figures 4A.iii and B.iii, respectively).

Discussion

In this report, we provide evidence that suvorexant, a hcrt/ox receptor antagonist presently in clinical use, exerts modest efficacy in attenuating the reinforcing effects of MDPV in self-administering rats when tested under fixed-ratio 1 schedule of reinforcement. Synthetic cathinone drugs pose potential harm to users, and this public health problem is aggravated by the continuous development of novel cathinone analogs. Behavioral therapy remains the most practiced form of intervention for treatment-seeking stimulant users, however high incidence of relapse suggests adjunctive pharmacotherapies are needed.

In a complementary study, we found that suvorexant decreased cocaine infusions earned from self-administering rats when taxed under a progressive-ratio schedule of reinforcement (Gentile *et al.* 2017). A series of prior research reports demonstrated that hcrt/ox functionally contributes to motivational effects of psychostimulant drugs. Hcrt/ox antagonists reduce drug-taking predominantly in tasks requiring more effort to receive intravenous drug infusions (e.g., Bentzley and Aston-Jones 2015; Brodnik *et al.* 2015). In the present report, suvorexant failed to significantly suppress drug-taking (Figure 2A). It remains possible that suvorexant may reduce drug-taking when employing effortful operant tasks as has been discussed in detail elsewhere (James *et al.* 2016). España and colleagues (2010) assessed the efficacy of systemically-injected SB-334867, a selective OX₁R antagonist, on cocaine self-administration under varying schedules of reinforcement. Results from España *et al.* (2010) revealed that SB-334867 reduced cocaine infusions earned on 6-h discrete-trials as well as progressive-ratio but not fixed-ratio 1 schedules of reinforcement. One mechanism found to underlie the ability of hcrt/ox receptor antagonists to reduce reinforcing and motivational efficacy of drugs of abuse is by attenuating stimulant-evoked elevations in ventral striatal DA efflux (e.g., Brodnik *et al.* 2015; Prince *et al.* 2015). In a prior study, we observed that suvorexant reduces cocaine-evoked elevations in ventral striatal DA (Gentile *et al.* 2017), and others have bridged functional contribution of mesolimbic DA transmission in the context of psychostimulant-evoked 50-kHz USVs. For example, intra-accumbal microinjections of amphetamine robustly evokes 50-kHz USVs (Burgdorf *et al.* 2001), and systemic blockade of D1- and/or D2-like receptors inhibits amphetamine-elicited 50-kHz USVs (Wright *et al.* 2013). The D1-like receptor antagonist SCH23390 dose-dependently attenuates 50-kHz USVs elicited following presentation of a cue predicting chocolate food availability (Buck *et al.* 2014). Notable other experiments reveal that both active as well as passive electrical stimulation of the medial forebrain bundle and optical stimulation of DA-releasing terminals in ventral striatum elicits a robust yet transient intensity-dependent bout of 50-kHz trill USVs (Scardochoio *et al.* 2015). It is possible that suvorexant suppressed MDPV-associated 50-kHz USVs in the current study by antagonizing DA transmission to ventral striatal targets.

Our prior report shows that self-administered MDPV—which potentially elevates extracellular DA levels in ventral striatum (Baumann *et al.* 2013)—evokes 50-kHz USVs only during initial drug infusions (Simmons *et al.* 2016). Here, we report a 30-min assessment of pre-lever USVs elicited by exposure to a context paired with MDPV self-administration. This observation expands on an original finding that rats emit “anticipatory” 50-kHz USVs when exposed to cocaine-predictive cues (Maier *et al.* 2010). We surmise that inclusion of a 30-min pre-lever time-period enables resolution between two distinctive bouts of 50-kHz USVs (second bout occurring after lever extension/access to self-administer MDPV; Figure 3C). We observed that suvorexant attenuated 50-kHz USVs during each of pre- and post-lever time-points. Our prior work shows that suvorexant pretreatment suppresses cocaine-evoked elevations in ventral striatal dopamine (Gentile *et al.* 2017) which supports the hypothesis that mesolimbic dopamine underlies the elicitation of 50-kHz USVs (for reviews, see Barker *et al.* 2015; Brudzynski 2015). From the three categories of call types analyzed in the present report, 50-kHz USV call type remained stable irrespective of suvorexant pre-treatment with majority of calls belonging to FM₁₋₂ call type which supports earlier work describing USVs associated with cocaine-paired cue presentation (Meyer *et al.* 2012). It should be noted that 50-kHz USVs are not exclusively emitted upon presentation of positively-valenced stimuli. In a recent report, Taylor and colleagues (2017) detected a transient emission of 50-kHz USVs without evident frequency modulation (termed “constant frequency” 50-kHz USVs) following initial electrical footshock (Taylor *et al.* 2017). Further, 50-kHz USVs have been observed from agitated adult male rats (Thomas *et al.* 1983). Thus, total 50-kHz USVs may reflect a state of general arousal but may not be necessarily limited to a positive subjective state. Differences in efficacy of suvorexant to reduce “Pre-Lever” 50-kHz USVs may be due to differences in relative value attributed to the context paired with MDPV self-administration. Rats dichotomized on this basis have been termed “sign”- and “goal”-trackers by research teams in prior studies (for review, see Flagel *et al.* 2009). Future studies could assess the hypothesis that rats assigning high value to drug-predictive cues/context (sign-trackers) may be more susceptible to reward attenuation following suvorexant relative to those assigning value strictly to reward receipt (goal-trackers).

At moderate- and high-dose suvorexant, sporadic bouts of 22-kHz USVs (10 mg/kg: 88.7 ± 64.6 ; 30 mg/kg: 29.0 ± 13.5) were found throughout the 2.5-h recording relative to respective group’s vehicle pre-treatment session (7.7 ± 6.3). Acoustically, these 22-kHz USVs were typically long (> 1 s) and monotonic resembling “alarm” cries as described when rats are exposed to predatory stimuli or following direct injection of cholinergic agonist carbachol (Blanchard *et al.* 1991; Brudzynski 1994). It should be noted that only certain rats elicited high rates of 22-kHz USVs ($n=2/7$) which contributed to high variability between subjects whereas others emitted exclusively 50-kHz USVs during recorded time-points. Pioneering work shows that 24-h withdrawal from either orally or intravenously self-administered cocaine produces 22-kHz USVs (Barros and Miczek 1996; Mutschler and Miczek 1998). It is possible that the effect of globally blocking hcr/ox transmission potentiated a negative state and in turn promoted affective distress in some but not all rats. Administration of hcr/ox receptor antagonists can reduce sex drive in male rats (Muschamp *et al.* 2007) and can increase brain reward thresholds in electrically self-stimulating rodents

(Muschamp *et al.* 2014). Relationships between hcrt/ox transmission and MDPV-associated affective changes will need to be better disentangled in future studies.

Results from our exploratory study further develops upon recent work describing the potential therapeutic utility of hcrt/ox receptor antagonists for management of stimulant use disorders. We report here that, while suvorexant did not significantly block the reinforcing effects of cathinone-derived MDPV under a fixed-ratio 1 schedule of reinforcement, suvorexant altered the emission of pre- and post-lever 50-kHz USVs. As has been argued by other research teams, hcrt/ox likely modulates motivation underlying drug retrieval, and thus future studies employing effortful tasks would address this hypothesis. This report additionally resolves distinct behavioral signatures (pre- and post-lever USVs) during intravenous drug self-administration. Specifically, the trending positive associations between suvorexant's effects on drug self-administration and USVs suggests predictive utility of pre-drug USVs on subsequent drug-seeking—this will be worthwhile as an ancillary measure of abuse liability.

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Figure 1.

Timeline of experiment. (A) Following jugular vein catheterization and acquisition of MDPV self-administration, rats are given a counterbalanced series of sessions involving pre-treatment with suvorexant (0, 3, 10, 30 mg/kg, i.p.) using a within-subjects design. Arched lines depict expansion of detail for each drug pre-treatment session (i.e. days 28–34). (B) Following pre-treatment, rats stay in their homecage for 15 min followed by placement into self-administration chambers. After 30 min to assess anticipatory ultrasonic vocalizations (USVs), programs initiate to allow rats to self-administer MDPV for 2 h.

Figure 2.

Effect of suvorexant on MDPV self-administration. (A) Number of MDPV infusions earned by suvorexant pretreatment dose. (B) Latency to self-administer the first 10 infusions of MDPV. Data in (A–B) are means + *S.E.M.* *N*'s = 7–14.

Figure 3.

Effect of suvorexant on ultrasonic vocalizations (USVs) associated with MDPV self-administration. (A) Vertical bar charts depicting median 50-kHz USVs per drug pretreatment (Veh: $n=14$; 3 mg/kg: $n=7$; 10 mg/kg: $n=12$; 30 mg/kg: $n=7$) during Pre-Lever (upper-left panel) and Post-Lever (lower-left panel) time-points as well as total 50-kHz USVs (right panel). Dots correspond to individual data points corresponding to absolute 50-kHz USVs. (B) Pie charts showing USV type composition of 5-min samples during Pre-Lever and Post-Lever time-points following suvorexant pretreatment. (C) 50- and 22-kHz USVs plotted across session with black vertical line showing lever extension and access to MDPV. Data in (A) are medians + *I.Q.R.* * $p < 0.05$ relative to vehicle pre-treatment.

Figure 4.

Associating efficacy of suvorexant pre-treatment on MDPV infusions (x-axes) and on pre- or post-lever 50-kHz USVs (A. i–iii, B. i–iii, respectively). Dotted lines enclose “100”—the points of no-change of each measure relative to respective vehicle pre-treatment scores. *P*-values are two-tailed Bonferroni-adjusted Spearman correlations (listed if corrected $p < 0.20$). *N*'s = 7–11.