BINGE-LIKE CONSUMPTION OF ETHANOL AND OTHER SALIENT REINFORCERS IS BLOCKED BY OREXIN-1 RECEPTOR INHIBITION

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ABSTRACT

Jeffrey Jon Olney: Binge-like consumption of ethanol and other salient reinforcers is blocked by orexin-1 receptor inhibition
(Under the direction of Todd E. Thiele)

Orexin (OX) neurons originating in the lateral hypothalamus (LH) are ideally positioned to modulate reward processing as they form connections with several key brain regions known to be involved in the reward pathway. Consistent with these findings, a growing number of studies have implicated the OX system in modulating the rewarding properties of several drugs of abuse, including ethanol. However, the role of the OX system in excessive binge-like ethanol intake remains relatively unexplored. Here we assessed the participation of the OX-1 receptor (OX1R) in binge-like ethanol consumption using the drinking-in-the-dark (DID) paradigm to model binge-like ethanol drinking in male C57BL/6J mice. Binge-like ethanol and saccharin drinking following peripheral injections of 0.0, 5.0, or 10.0 mg/kg SB-334867 (SB), a selective OX1R antagonist was examined. Finally, mice were given peripheral injections of SB and open-field locomotor activity was measured. Results indicated that, inhibition of the OX1R via SB blunted ethanol and saccharin drinking, but did not alter open-field locomotor activity. Together, these data suggest that the OX system participates in the consumption of salient reinforcers regardless of calories without affecting general locomotor activity.

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I would like to thank Dr. Todd E. Thiele for his guidance and mentorship with this project. I would also like to thank Dr. Montse Navarro and Dr. Jennifer Rinker for their technical expertise and guidance, as well as Rhiannon D. Thomas, Benjamin R. Cox, Dr. Simon Alex Marshall, Nathan Burnham, and Timothy Gilliam for their technical assistance.

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LIST OF ABBREVIATIONS

BEC	Blood ethanol concentration
C57	C57BL/6J
DID	Drinking-in-the-dark
i.p.	Intraperitoneal
LH	Lateral hypothalamus
NAc	Nucleus Accumbens
OX	Orexin
OX1R	Orexin 1 Receptor
OX2R	Orexin 2 Receptor
SB	SB-334867
VTA	Ventral tegmental area

Chapter 1: Introduction

In 1998, two independent research groups simultaneously discovered the existence of a novel peptide. de Lecea and colleagues (1998) noted that this new peptide possessed structural similarities to the gut-peptide, secretin, but displayed an expression pattern limited to the hypothalamus; thus, these researchers deemed this new peptide hypocretin (hypothalamus + secretin = hypocretin). Alternatively, Sakurai and colleagues (1998) shrewdly observed that the peptide was located in the hypothalamus, a brain region long-known for its involvement in feeding behavior (Hetherington & Ranson 1940). After demonstrating that this peptide possessed highly robust orexigenic properties, these researchers named this peptide orexin (OX).

Cloning studies have revealed that the OX system is comprised of two peptides, orexin-A and orexin-B, which are derived from the precursor, prepro-orexin, and act on two G-protein coupled receptors, OX1R and OX2R (de Lecea et al. 1998; Sakurai et al. 1998). These peptides interact with equal affinity at the OX2R, but orexin-A has been found to have a greater affinity for OX1R with orexin-B exerting minimal actions on OX1R (Sakurai et al. 1998). These receptors are generally excitatory and are involved in a myriad of neurobiological functions, such as feeding (Sakurai et al. 1998), reward-related behavior (Harris et al. 2005), stress (Sakamoto et al. 2004; Winsky-Sommerer et al. 2004), and arousal (Chemelli et al. 1999).

Although central OX originates solely within the hypothalamus (Sakurai et al. 1998), its afferents form connections with regions across the brain (Yoshida et al. 2005). Consistent with these findings, OX projections originating from the lateral hypothalamus (LH) facilitate the activity of several brain regions in the reward circuit, such as the ventral tegmental area

(VTA; Korotkova et al. 2003; Borgland et al. 2006; Moorman & Aston-Jones 2010) and shell of the nucleus accumbens (NAc; Patyal et al. 2012).

In agreement with the widespread connections of hypothalamic OX neurons to various regions in the reward circuit, OX has been found to be involved in the neurobiological responses to ethanol. Lawrence and colleagues (2006) first observed this relationship by systemically injecting the selective orexin-1 receptor (OX1R) antagonist, SB-334867 (SB), to reduce operant self-administration of ethanol. Furthermore, SB was found to selectively reduce ethanol consumption in high-, but not low-, ethanol preferring rats (Moorman & Aston-Jones 2009). Further investigations suggest that modulation of OX signaling via SB is specific to ethanol as Jupp and colleagues (2011) found that SB significantly attenuated the motivational effects of ethanol but not sucrose. Although these data suggest an essential role for OX1R signaling in ethanol drinking, recent evidence suggests the OX2R antagonists are also capable of suppressing responses to ethanol (Shoblock et al. 2011; Anderson et al. 2014; Barson et al. 2014). Moreover, more comprehensive investigations of the role of OX in ethanol drinking have revealed that signaling within the reward-related pathway is responsible for modulating this behavior. Indeed, direct infusions of orexin-A to the LH (Schneider et al. 2007) and a non-selective OXR antagonist into the VTA (Srinivasan et al. 2012) significantly increased and decreased ethanol drinking, respectively. As a whole, these findings indicate ethanol drinking directly parallels OX signaling in the reward pathway.

Despite this growing body of literature implicating the OX system in ethanol consumption, relatively less is known of its role in binge drinking behavior. Considering recent evidence that indicates different neurocircuitry may be engaged during binge-like versus moderate levels of ethanol drinking (Lowery et al. 2010; Lowery-Gionta et al. 2012; Sparta et al. 2008), the goal of the present study was to further characterize the role of the central OX system in binge-like drinking behavior. To this end, we peripherally administered the selective OX1R antagonist, SB, to characterize the participation of the OX1R in binge-like ethanol

consumption, and we assessed the specificity of SB in modulating ethanol drinking by examining its effects on saccharin, a salient but non-caloric reinforcer. Additionally, we also examined the effects of this pharmacological inhibition on more general behavioral responses by assessing locomotor activity following treatment with SB.

Chapter 2: Materials and Methods

Animals

A total of forty male C57BL/6J (C57) adult mice (aged 7-9 weeks; Jackson Laboratories, Bar Harbor, ME) were used in the present study. Relative to other strains of mice, C57s have demonstrated superior drinking in the DID paradigm (Rhodes et al. 2005; Rhodes et al. 2007). Mice were individually housed in plastic cages located in a vivarium with an ambient temperature of approximately 22ºC and a reverse light/dark cycle with lights off at 7:00 am. Food and water were available *ad libitum* except during testing (see below). All procedures used were in accordance with the National Institute of Health guidelines and were approved by the University of North Carolina Institutional Animal Care and Use Committee.

General Procedure

Drugs

SB-334867 (SB; 0.0, 5.0, or 10.0 mg/kg; Tocris Bioscience, Minneapolis, MN) was dissolved using 0.01% Tween® 80 (Sigma-Aldrich, St. Louis, MO) in saline as described previously (Anderson et al. 2014). All doses were administered in a 10.0 ml/kg injection volume.

Drinking-in-the-dark

A 4-day drinking-in-the-dark (DID) procedure was used to model binge-like drinking (Rhodes et al. 2005). The DID procedure is a commonly used animal model of binge-like ethanol drinking that promotes high levels of consumption and generates physiologically relevant blood ethanol concentrations (BECs) of 80.0 mg/dl or greater

(Rhodes et al. 2005; Rhodes et al. 2007). On days 1-3, standard water bottles were removed three hours into the dark cycle and the animals were given access to test bottles containing either ethanol (20% v/v) or saccharin (0.15% w/v) solutions for two hours. Using the standard DID protocol, binge-like consumption is assessed on the fourth day when access is extended to four hours. However, SB has been reported to have a relatively short half-life of less than 30 min (Porter et al. 2001). In agreement with this report, our initial findings indicated that the effects of SB were rather short-lived. Due to the hyper-transient nature of the drug, a shortened, modified DID procedure was used in the current experiments to better capture the transient effects of the drug. Therefore, binge-like consumption was assessed on the fourth day when the animals were given two-hour access to the test bottles.

Experiment 1: Binge-like Ethanol Drinking Following Treatment with SB

In order to examine the contribution of the OX1R in binge-like ethanol drinking, animals were given intraperitoneal (i.p.) injections of 0.0, 5.0, or 10.0 mg/kg SB 30 min prior to ethanol access. Immediately after testing, tail-blood samples were collected from each animal and BECs were measured using the Analox blood ethanol analyzer (Analox Instruments, Luneburg, MA). In order to increase power during statistical analysis, each animal received all three doses of the drug over repeated trials to allow for within-subjects comparison of ethanol drinking behavior. Mice were given three days rest between subsequent 4-day DID sessions in order to avoid carryover effects of the drug.

Experiment 2: Binge-like Saccharin Drinking Following Treatment with SB

A separate cohort of 20 C57 mice was used to assess binge-like consumption of saccharin following treatment with SB. The procedures used here were identical to those described above with the exception that these mice were given access to saccharin (0.15% w/v) during the modified, two-hour DID procedure and tail-blood samples were not taken from these animals. All other experimental parameters remained unchanged. A Latin square design was used such that each animal received all three doses of the drug over repeated trials. Mice were

given three days of rest between subsequent 4-day DID sessions in order to avoid carryover effects of the drug.

Experiment 3: Locomotor Activity Following Treatment with SB

The same 20 mice from the previous saccharin experiment were used to assess locomotor activity following treatment with SB. One hour before locomotor testing, animals were brought into a dark room adjacent to the room that housed the locomotor chambers. Thirty minutes before locomotor testing, animals were given i.p. injections of 0.0 or 10.0 g/kg SB as described above. The decision to use only the 10.0 mg/kg dose of SB was based on the findings from our previous experiment that only the higher dose produced significant reductions in ethanol consumption. Three hours into the dark cycle, animals were placed in a 16.5 x 16.5 in2 open-field locomotor chamber (Accuscan Instruments, Columbus, OH) and locomotor activity was recorded in five minute bins for two hours using VersaMax software (Omnitech Electronics, Columbus, OH). After the two-hour test period, mice were placed back into their homecages and returned to the vivarium. Unlike our previous pharmacological experiments, a Latin square was not used in order to avoid previous experience with the locomotor chamber confounding the animals' locomotor activity.

Data Analysis

For experiment 1, a repeated-measures ANOVA was used to assess hourly ethanol consumption with both time (hour 1 and hour 2) and SB dose (0.0, 5.0, or, 10.0 mg/kg) being within-subject variables. Additionally, total ethanol consumption across the two-hour test period was assessed using a repeated-measures ANOVA with SB dose (0.0, 5.0, or, 10.0 mg/kg) as the within-subject variable. Since the first round of DID in the within-subjects design followed the standard, four-hour DID model, the tail-blood samples from the first round were excluded from the analysis. Due to the exclusion of these data points, a univariate ANOVA was used to assess BECs with dose of SB (0.0, 5.0, or 10.0 mg/kg) as the independent variable.

Additionally, drug order was included as a between-subjects variable in these analyses to ensure the order in which the animals were presented the drug did not have any confounding effects on ethanol consumption. Tukey's HSD post-hoc tests and Bonferroni corrections were used when applicable.

For experiment 2, a repeated-measures ANOVA was used to assess hourly saccharin consumption with both time (hour 1 and hour 2) and dose of SB (0.0, 5.0, or, 10.0 mg/kg) being within-subject variables. Total saccharin consumption was also assessed using separate repeated-measures ANOVAs with SB dose (0.0, 5.0, or, 10.0 mg/kg) as the within-subject variable. Drug order was also included as a between-subjects variable in these analyses. Tukey's HSD post-hoc tests and Bonferroni corrections were used when applicable.

For experiment 3, separate t-tests were used to assess the effects of SB on locomotor activity. In addition to measuring the effects of SB across the entire two-hour test period, the immediate effects of the drug during the first 15 min of testing were also assessed.

Chapter 3: Results

Experiment 1

Our first experiment sought to determine whether manipulating OX signaling via the selective OX1R antagonist, SB, could modulate binge-like ethanol drinking. As shown in Fig. 1A, treatment with SB significantly reduced binge-like ethanol consumption (main effect of dose: $F_{(2,38)} = 6.083$, $p = 0.005$) but the animals' drinking behavior did not change over time (main effect of time: $F_{(1,19)} = 0.372$, $p = 0.549$). Analysis also showed that there was a significant drug by time interaction effect ($F_{(2,38)} = 8.196$, $p = 0.001$). Further probing revealed that both the 5.0 and 10.0 mg/kg dose of SB significantly reduced binge-like ethanol drinking during the first hour relative to water ($p = 0.016$, $p < 0.001$, respectively), though the higher dose of SB significantly reduced binge-like ethanol drinking to a level beyond the lower dose ($p = 0.015$). Importantly, the effect of drug order was not significant ($F_{(2,17)} = 0.862$, $p = 0.481$) indicating that the order in which the animals received the SB treatment did not affect hourly ethanol drinking behavior. Additionally, SB significantly reduced ethanol drinking over the two-hour test period ($F_{(2,38)} = 9.870$, $p < 0.001$). Interestingly, the effect of the lower dose of SB appeared to be short-lived as only the higher dose significantly blunted ethanol drinking over the full two hours ($p < 0.001$). This effect of the higher dose across the two-hour test period appears to be driven by the effect at the first hour as no differences were observed during the second hour of testing. Similar to the hourly consumption, drug order did not impact total ethanol drinking behavior ($F_{(2,17)} = 0.862$, $p = 0.481$).

In accordance with the drinking data, treatment with SB significantly reduced measured BECs as well (Fig. 1B; $F_{(2,59)} = 4.312$, $p = 0.018$). Further probing of this effect indicated animals treated with the higher dose of SB exhibited significantly lower BECs relative to vehicle treated animals ($p = 0.013$). Finally, order of drug presentation did not affect measured BECs ($F_{(1,56)} = 0.729$, $p = 0.539$).

Experiment 2

The previous experiment demonstrated that pharmacological inhibition of the OX1R is capable of reducing excessive ethanol consumption in a DID paradigm. Interestingly, a recent study by Anderson and colleagues (2014) observed that systemic treatment with SB reduces binge-like sucrose consumption, which suggests the OX system modulates binge-like consumption of general, salient reinforcers beyond ethanol. However, as sucrose and ethanol are both reinforcing substances that have calories, it remains to be seen whether or not these effects are due alterations in caloric need. Thus, the next experiment sought to characterize the effects of SB on saccharin, a salient, non-caloric reinforcer.

Analysis of hourly intake revealed that SB significantly reduced saccharin consumption (Fig. 2; main effect of dose: $F_{(2,38)} = 4.231$, $p = 0.022$). Further probing of this main effect showed that only the higher dose (10.0 mg/kg) of SB significantly attenuated saccharin intake after Bonferroni's correction ($p = 0.011$). A significant main effect of time indicated that all animals drank more saccharin during the first hour than during the second (main effect of time: $F_{(1,38)} = 11.756$, $p = 0.003$). The dose by time interaction effect did not reach significance ($F_{(2,38)} = 1.089$, $p = 0.347$). Additionally, SB significantly reduced total saccharin intake across the full two-hour test period ($F_{(2,38)} = 4.231$, $p = 0.022$). Similar to the hourly consumption, follow-up tests revealed that only the higher dose of SB significantly attenuated saccharin intake after Bonferroni's correction ($p = 0.011$). As with the previous experiment, drug order did not affect hourly ($F_{(2,17)} = 0.024$, $p = 0.977$) or total ($F_{(2,17)} = 0.018$, $p = 0.982$) saccharin drinking behavior. Together, findings from this experiment indicate that the effect of reduced binge-like drinking following pharmacological inhibition of the OX1R via SB is not specific to ethanol but appears to blunt consumption of salient caloric (ethanol) and non-caloric (saccharin) reinforcers.

Experiment 3

Perhaps one of the most widely studied functions of the OX system is its role in sleep and arousal (see de Lecea 2012 for review). To rule out the possibility that our observed effects

of SB on ethanol or saccharin consumption could be secondary to the effects of SB on general motor behavior, the next experiment sought to test the effects of SB on open-field locomotor activity. Simple t-tests showed that, relative to vehicle, treatment with the 10.0 mg/kg dose of SB had no discernable effect on immediate locomotor activity during the first 15 min of testing ($t_{(18)}$ = 0.2129, p = 0.834) nor across the entire two-hour test period ($t_{(18)}$ = 1.630, p = 0.1204; Fig. 3). Together, these data demonstrate that our observed effects of SB on the consumption of salient reinforcers are not a consequence of impaired locomotor activity.

Chapter 4: Discussion

The goal of the present experiments was to examine the contribution of the OX system in binge-like ethanol drinking. Through our series of experiments, we demonstrated that blocking the OX1R signaling via SB protects against excessive ethanol consumption. Interestingly, this effect is not specific to ethanol as treatment with SB also reduced binge-like consumption of saccharin; however, no other nonspecific effects were observed as SB did not significantly alter locomotor activity.

We observed that selective inhibition of the OX1R protected against excessive ethanol consumption using the DID procedure. This observation confirms several others that have reported that prior treatment with SB significantly reduces responding to ethanol in a variety of paradigms (Moorman & Aston-Jones 2009; Jupp et al. 2011; Anderson et al. 2014; Voorhees & Cunningham 2011). Notably, our mice exhibited robust ethanol intake yet the BEC levels were slightly below binge criteria, which may stem from the shortened 2 hour test. What is more, our findings indicate the protective effect of SB is not specific to ethanol as we also observed that SB reduced excessive intake of saccharin, a non-caloric reinforcer. In fact, it has recently been reported that systemic SB protects against binge-like ethanol and sucrose intake (Anderson et al. 2014; Alcaraz-Iborra et al. 2014). Together with our observation that peripherally administered SB similarly blunts ethanol and saccharin drinking, these findings suggest the OX1R modulates the consumption of salient reinforcers regardless of caloric content. Although previous findings have demonstrated that the protective effect of peripherally administered SB is specific to ethanol and not sucrose (Jupp et al. 2011), this difference could be attributed to

procedural differences. That is, the prior study used operant responding to ethanol and sucrose in alcohol-preferring rats while the present study used the DID procedure in C57 mice.

Moreover, the recent report by Anderson and colleagues (2014) described similar success in using SB to significantly reduce binge-like ethanol drinking in C57s using the standard DID model. Interestingly, that same report found that only a high dose of SB (30.0 mg/kg) reduced binge-like ethanol drinking while lower doses were ineffective. Though the effective dose of SB between this previous report and our current study are at variance with one another, there are methodological differences that may be able to explain this disparity. First, a great deal of variability in SB has been documented between different vendors and even different batches of the same vendor (Mahler et al. 2012; Jupp et al. 2011). The previous study used SB that was synthesized by one of the researchers at Lilly Research Laboratories while we ordered ours from a private vendor. This may be of importance as the manner in which the compound is synthesized may alter its effectiveness (McElhinny et al. 2012). Therefore, the possibility that the different effective dose observed between our studies is due to some inherent difference between the two SB compounds cannot be ruled out.

Another difference between these two reports is the time intervals at which consumption data were measured. The previous study reported that only the 30.0 mg/kg dose of SB was effective in significantly reducing ethanol drinking over a four-hour test period. However, the rather short half-life of the SB compound (Porter et al. 2001) prompted us to shorten the test period as well as take hourly measures in order to assess drinking over time. Using this design, we were able to discover that lower doses are indeed capable of reducing excessive ethanol consumption- though the effect is short-lived.

Although this report demonstrates that inhibition of the OX system protects against binge-like ethanol consumption, the systemic nature of our treatment procedure is unable to determine the locus of this effect. Future studies will attempt to identify the OX circuits that convey these effects via site-specific delivery of OX agents directly to candidate regions. The

LH is known to be involved in reward-related behavior (Wise 1996; DiLeone et al. 2003) and OX neurons originated from the LH have been reported to project to key brain regions in the reward circuit (Marcus et al. 2001; Yoshida et al. 2005). Both the NAc shell and VTA are densely innervated by OX neurons and are known to be involved in reward-related behavior. Indeed, application of OX peptide within the shell of the NAc *in vitro* causes a robust depolarization (Mukai et al. 2009). However, direct infusion of orexin-A into the NAc shell failed to alter ethanol consumption (Schneider et al. 2007) suggesting that OX signaling in this brain region may not modulate ethanol drinking behavior. On the other hand, it has been previously demonstrated that application of orexin-A alone initiates plastic changes in the VTA that facilitate activity in this region (Borgland et al. 2006). Moreover, blocking OXRs in the VTA has been shown to protect against ethanol self-administration (Srinivasan et al. 2012). Taken together, these findings suggest the OX neurons originating in the LH that project to the VTA may be a key circuit that modulates ethanol drinking.

Finally, since the OX system is known to be critically involved in sleep and arousal (Chemelli et al. 1999), it was important to rule out the possibility that our observed effects of SB on responding to salient reinforcers was not due to an induction of a hypoactive state. Given that treatment with SB did not alter locomotor activity at any time point measured, it is unlikely that inhibiting the OX1R could have produced any effects on arousal that could have confounded our effects on binge-like consummatory behavior.

Findings from the present report indicate that pharmacological inhibition of signaling onto the OX1R served to protect against excessive ethanol and saccharin consumption without altering locomotor activity. Together, these data provide evidence for a role of the OX system in binge-like ethanol consumption and warrant further investigation. Future studies will investigate the specific brain regions exerting these effects via site-specific infusions of SB. Moreover, though this study outlines a critical role for the OX1R in these behaviors, future investigations will characterize the role of the OX2R as well. Additionally, the ability of OX1R inhibition to reduce

binge-like ethanol drinking suggests that the OX system is perturbed during this behavior; thus, further studies using immunohistochemistry will examine changes in the OX system following multiple episodes of DID. Importantly, these findings provide promise that targeting the OX system through OXR antagonists may serve as an effective treatment for alcohol use disorders.

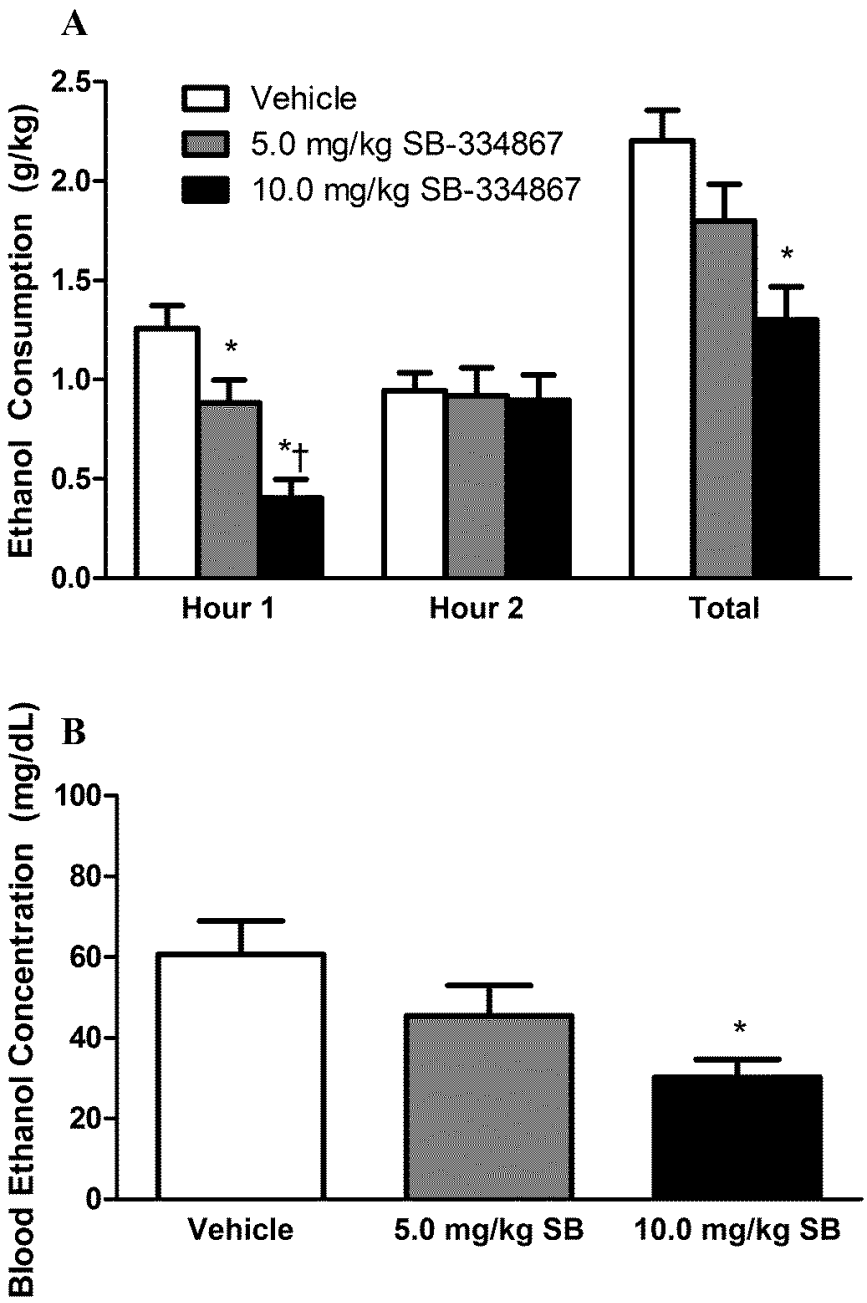

Figure 1. Pharmacological inhibition of the OX1R reduces excessive ethanol consumption and BEC. Both the 5.0 mg/kg and 10.0 mg/kg doses of SB protected against excessive ethanol consumption during the first hour of testing relative to vehicle treated controls; however, only the 10.0 mg/kg dose caused a significant reduction in ethanol drinking across the full two-hour test period **(A)**. Prior treatment with the 10.0 mg/kg dose of SB caused a significantly lower BEC relative to vehicle treated controls **(B)**. * denotes $p < 0.05$ relative to vehicle group at the same time point; † denotes $p < 0.05$ relative to 5.0 mg/kg group at the same time point. Data are presented as Mean ± SEM.

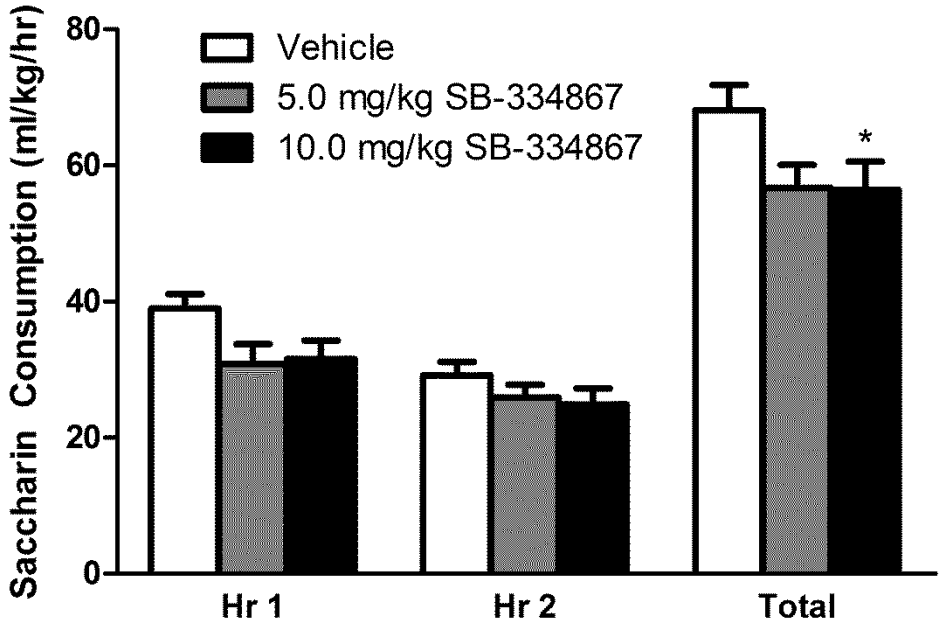

Figure 2. Pharmacological inhibition of the OX1R reduces excessive saccharin consumption. Both doses of SB significantly reduced excessive saccharin drinking over the two hours of DID testing. * denotes $p < 0.05$ relative to vehicle group at the same time point. Data are presented as Mean ± SEM.

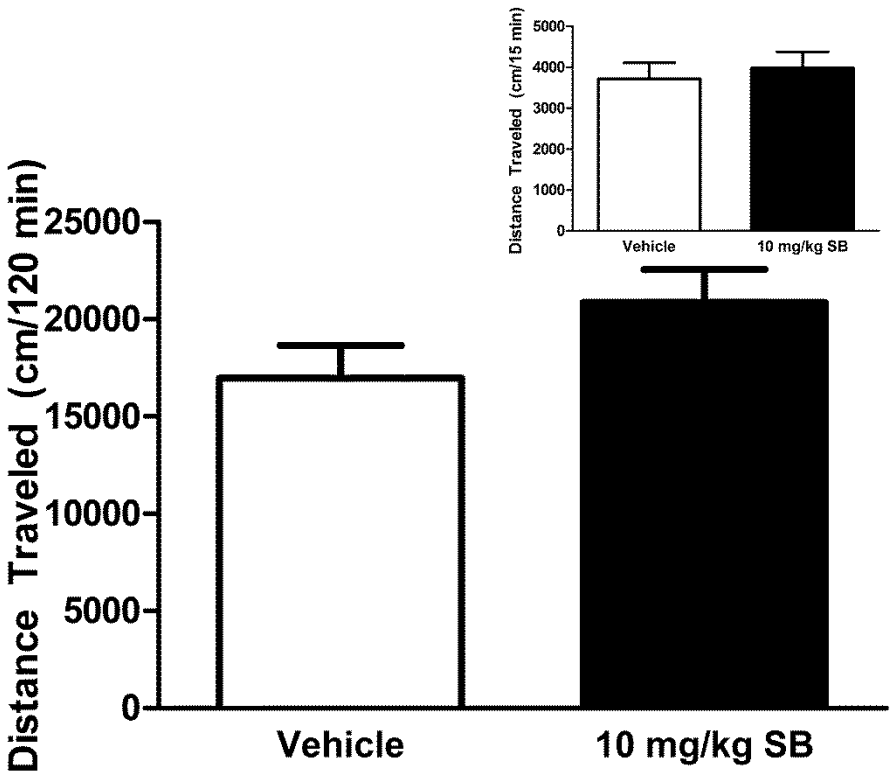

Figure 3. Treatment with SB does not affect locomotor activity. The 10.0 mg/kg dose of SB had no discernable effects on locomotor activity across the full two hours of testing nor did it have any immediate effects during the first 15 min of locomotor testing (inset). Data are presented as Mean ± SEM.

REFERENCES

Alcaraz-Iborra, M. et al., 2014. Binge-like consumption of caloric and non-caloric palatable substances in ad libitum-fed C57BL/6J mice: Pharmacological and molecular evidence of orexin involvement. *Behavioural Brain Research*, 272, pp.93–99.

Anderson, R.I. et al., 2014. Orexin-1 and orexin-2 receptor antagonists reduce ethanol self-administration in high-drinking rodent models. *Frontiers in Neuroscience*, 8.

Barson, J.R., Ho, H.T. & Leibowitz, S.F., 2014. Anterior thalamic paraventricular nucleus is involved in intermittent access ethanol drinking: role of orexin receptor 2. *Addiction Biology*.

Borgland, S. et al., 2006. Orexin A in the VTA is critical for the induction of synaptic plasticity and behavioral sensitization to cocaine. , 49, pp.589–601.

Chemelli, R.M. et al., 1999. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell*, 98(4), pp.437–451.

DiLeone, R.J., Georgescu, D. & Nestler, E.J., 2003. Lateral hypothalamic neuropeptides in reward and drug addiction. *Life Sciences*, 73(6), pp.759–768.

Harris, G.C., Wimmer, M. & Aston-Jones, G., 2005. A role for lateral hypothalamic orexin neurons in reward seeking. *Nature*, 437(7058), pp.556–559.

Hetherington, A.W. & Ranson, S.W., 1940. Hypothalamic lesions and adiposity in the rat. *The Anatomical Record*, 78, pp.149–172.

Jupp, B. et al., 2011. The orexin1 receptor antagonist SB-334867 dissociates the motivational properties of alcohol and sucrose in rats. *Brain research*, 1391, pp.54–59.

Korotkova, T.M. et al., 2003. Excitation of Ventral Tegmental Area Dopaminergic and Nondopaminergic Neurons by Orexins/Hypocretins. *The Journal of Neuroscience*, 23(1), pp.7–11.

Lawrence, A.J. et al., 2006. The orexin system regulates alcohol-seeking in rats. *British journal of pharmacology*, 148(6), pp.752–759.

De Lecea, L., 2012. Hypocretins and the neurobiology of sleep–wake mechanisms. In *Progress in Brain Research*. Elsevier, pp. 15–24.

De Lecea, L. et al., 1998. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proceedings of the National Academy of Sciences*, 95(1), pp.322–327.

Lowery, E.G. et al., 2010. CRF-1 antagonist and CRF-2 agonist decrease binge-like ethanol drinking in C57BL/6J mice independent of the HPA axis. *Neuropsychopharmacology*, 35(6), pp.1241–1252.

Lowery-Gionta, E.G. et al., 2012. Corticotropin releasing factor signaling in the central amygdala is recruited during binge-like ethanol consumption in C57BL/6J mice. *The Journal of neuroscience*, 32(10), pp.3405–3413.

Mahler, S.V. et al., 2012. Multiple roles for orexin/hypocretin in addiction. *Progress in brain research*, 198, p.79.

Marcus, J.N. et al., 2001. Differential expression of orexin receptors 1 and 2 in the rat brain. *The Journal of comparative neurology*, 435(1), pp.6–25.

McElhinny, C.J. et al., 2012. Hydrolytic instability of the important orexin 1 receptor antagonist SB-334867: possible confounding effects on in vivo and in vitro studies. *Bioorganic & Medicinal Chemistry Letters*, 22(21), pp.6661–6664.

Moorman, D.E. & Aston-Jones, G., 2009. Orexin-1 receptor antagonism decreases ethanol consumption and preference selectively in high-ethanol–preferring Sprague–Dawley rats. *Alcohol*, 43(5), pp.379–386.

Moorman, D.E. & Aston-Jones, G., 2010. Orexin/Hypocretin Modulates Response of Ventral Tegmental Dopamine Neurons to Prefrontal Activation: Diurnal Influences. *The Journal of Neuroscience*, 30(46), pp.15585–15599.

Mukai, K. et al., 2009. Electrophysiological effects of orexin/hypocretin on nucleus accumbens shell neurons in rats: an in vitro study. *Peptides*, 30(8), pp.1487–1496.

Patyal, R., Woo, E.Y. & Borgland, S.L., 2012. Local hypocretin-1 modulates terminal dopamine concentration in the nucleus accumbens shell. *Frontiers in behavioral neuroscience*, 6, p.82.

Porter, R.A. et al., 2001. 1,3-Biarylureas as selective non-peptide antagonists of the orexin-1 receptor. *Bioorganic & Medicinal Chemistry Letters*, 11(14), pp.1907–1910.

Rhodes, J.S. et al., 2005. Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. *Physiology & behavior*, 84(1), pp.53–63.

Rhodes, J.S. et al., 2007. Mouse inbred strain differences in ethanol drinking to intoxication. *Genes, brain, and behavior*, 6(1), pp.1–18.

Sakamoto, F., Yamada, S. & Ueta, Y., 2004. Centrally administered orexin-A activates corticotropin-releasing factor-containing neurons in the hypothalamic paraventricular nucleus and central amygdaloid nucleus of rats: possible involvement of central orexins on stress-activated central CRF neurons. *Regulatory peptides*, 118(3), pp.183–191.

Sakurai, T. et al., 1998. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell*, 92(4), pp.573–585.

Schneider, E.R. et al., 2007. Orexigenic peptides and alcohol intake: differential effects of orexin, galanin, and ghrelin. *Alcoholism: Clinical and Experimental Research*, 31(11), pp.1858–1865.

Shoblock, J.R. et al., 2011. Selective blockade of the orexin-2 receptor attenuates ethanol self-administration, place preference, and reinstatement. *Psychopharmacology*, 215(1), pp.191–203.

Sparta, D.R. et al., 2008. Blockade of the corticotropin releasing factor type 1 receptor attenuates elevated ethanol drinking associated with drinking in the dark procedures. *Alcoholism, clinical and experimental research*, 32(2), pp.259–265.

Srinivasan, S. et al., 2012. The Dual Orexin/Hypocretin Receptor Antagonist, Almorexant, in the Ventral Tegmental Area Attenuates Ethanol Self-Administration. *PLOS ONE*, 7(9), p.e44726.

Voorhees, C.M. & Cunningham, C.L., 2011. Involvement of the orexin/hypocretin system in ethanol conditioned place preference. *Psychopharmacology*, 214(4), pp.805–818.

Winsky-Sommerer, R. et al., 2004. Interaction between the corticotropin-releasing factor system and hypocretins (orexins): a novel circuit mediating stress response. *The Journal of neuroscience: the official journal of the Society for Neuroscience*, 24(50), pp.11439–11448.

Wise, R.A., 1996. Addictive drugs and brain stimulation reward. *Annual Review of Neuroscience*, 19, pp.319–340.

Yoshida, K. et al., 2005. Afferents to the orexin neurons of the rat brain. *The Journal of comparative neurology*, 494(5), pp.845–861.