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Sleep deprivation enhances conditioned place preference in an orexin receptor modulated manner

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Abstract: Drug addiction and withdrawal are characterized by sleep disruption, but the effects of sleep disruption on these states are not well characterized. Sleep deprivation (SD) immediately prior to the cocaine conditioning trials enhanced cocaine conditioned place preference (CPP) in a dose-dependent manner (3, 8 mg/kg but not 15 mg/kg) in mice. SD immediately prior to the post-conditioning test, also enhanced cocaine CPP preference in a dose dependent manner (8 mg/kg, but not 3, 15 mg/kg). Exposure to orexin-receptor antagonism (1 mg/kg SB334867, an orexin 1 receptor antagonist) just prior to cocaine-conditioning trials or the post-conditioning test, attenuated SD-enhanced preference. This suggests a potential therapeutic role for the manipulation of the orexin system to mitigate drug seeking, especially in the context of sleep loss prior to drug exposure.

Significance statement: Drugs of abuse, including cocaine, disturb sleep and sleep disturbance has been implicated in probability of relapse; however, there have been few direct tests of sleep disturbance on drug seeking behavior. Here, we show that acute (4h) sleep deprivation enhances the rewarding properties of cocaine, a drug with high abuse potential. Furthermore, antagonism of the orexin system, a neuromodulator involved in motivation-based arousal, reduces this SD-induced enhancement of cocaine preference.

Introduction:

Cocaine, a psychostimulant with high abuse potential due to its strong reinforcing properties (Johanson et al., 1976; Bozarth and Wise, 1985), blocks monoaminergic transporters. Of these, the dopamine transporter blockade is predominately responsible for reinforcement (Ritz et al., 1987). Cocaine is readily self-administered in non-humans to the point that unlimited access is often fatal (Johanson et al., 1976; Bozarth and Wise, 1985). These appetitive properties can be measured using conditioned place preference (CPP; Bardo and Bevins, 2000); an associative learning task in which rewarding properties of stimuli are inferred based on time spent in a context associated with a specific drug/stimuli (such as cocaine) relative to a neutral stimuli (such as saline). As expected for a drug that produces reinforcement, animals show preference for environments in which they have previously received cocaine (Mucha et al., 1982; Spyraki et al., 1982).

Acute cocaine exposure potentiates arousal by increasing sleep latency and increasing the amount of time in waking in a dose dependent manner (Dugovic et al., 1992; Knapp et al., 2007; Bjorness and Greene, 2018). Increases in subsequent sleep compensate for the sleep loss to the extent that there is no overall change in the amount of sleep/waking over the 24h period in response to either acute (Bjorness and Greene, 2018) or to several days of repeated (Dugovic et al., 1992) cocaine exposure. Non-compensated reductions in sleep are observed following withdrawal from cocaine self-administration, with decreases in Non-Rapid Eye Movement Sleep emerging one week into withdrawal, decreases in Rapid Eye Movement Sleep emerging one day into withdrawal, and decreases in both persisting through three weeks of withdrawal (Chen et al., 2015). Thus, chronic sleep disturbance emerges after more extensive exposure to cocaine, while limited cocaine induces a sleep deprivation plus recovery response.

SD can influence drug use, as suggested by evidence that subjective sleep quality is a robust predictor of relapse to alcohol consumption (Brower et al., 2001) and that lack of SWS time recovery across abstinence is associated with relapse to cocaine use (Angarita et al., 2014). Furthermore, subjective sleep disturbance is associated with cocaine relapse following treatment in a large cohort study (Dolsen and Harvey, 2017). In rodents, chronic sleep restriction can increase motivation for cocaine (i.e. the amount of work an animal will do to obtain a cocaine reward) in a subset of animals (Puhl et al., 2013). SD also influences reward in that SD increases preference for the stimulant methylphenidate in humans (Roehrs et al., 1999) and induces preference to a low dose of amphetamine in mice (Berro et al., 2018).

The mechanism/s by which sleep loss could influence reward seeking have yet to be determined; however, the peptide neuromodulator orexin [also known as hypocretin] shows differential activity across sleep/waking states with increased activity during extended waking (i.e. SD) compared to typical waking (Estabrooke et al., 2001; Yoshida et al., 2001), is modulated by cocaine and other drugs of abuse (Thannickal et al., 2018; James et al., 2019), and influences reward seeking (Hollander et al., 2012; Gentile et al., 2018) making it an attractive candidate system. Furthermore, since orexin is heavily implicated in both maintenance of arousal (Sakurai et al., 2010) and motivated behavior (James et al., 2017), it has been hypothesized to integrate arousal and motivation (Tyree et al., 2018).

In the present study we tested the hypothesis that acute SD enhances cocaine CPP and that the orexin system has an important role in this modulation.

Materials and Methods:

Animals: Adult male C57BL/6 mice were obtained from Charles River Laboratories. Mice were assigned into groups (described below) and placed into cages atop a treadmill apparatus with food and water available *ad libitum* in rooms with an ambient temperature of 22.0 +/− 1 °C and a 12:12 h light/dark cycle. All experiments were approved by the VA North Texas Health Care System IACUC and were in accordance with recommendations in the Guide for Care and Use of Laboratory Animals (U.S. National Research Council).

Cocaine conditioned place preference:

An unbiased design was used with three chambered CPP boxes (Med Associates). These boxes were unbiased in that there was no overall preference for either of the side chambers which feature different wall and flooring patterns to make them easily distinguishable. First, mice were given a pre-conditioning test in which they were placed into the center chamber (doors open) and allowed to explore for 20 min. Mice were excluded if they showed an innate preference for either side (as defined by >20% difference in percent time spent between sides) or if they spent more time in the center chamber than either side chamber. A subset of “excluded” mice underwent a second pre-conditioning test using a different CPP box (featuring different floor and wall patterns). These double pre-tested animals were divided evenly across groups. Next, mice underwent four conditioning trials (doors closed) in which they received cocaine (3, 8, or 15 mg/kg) or saline (vehicle control) with one, 30 min trial per day. Finally, mice were given a 20 min post-conditioning test (doors open). Testing and conditioning trials

occurred between ZT4-ZT7 and were conducted under low light level to encourage exploration. Time in each chamber was determined by IR beam break (automated) or video (manual). Based on previous reports, preference is expected for the 8 mg/kg dose (Campbell et al., 2000) and for the 15 mg/kg dose (Nomikos and Spyraki, 1988), but not for the 3 mg/kg dose (Zachariou et al., 2001). While conditioning protocols vary, two trials are expected to be sufficient to support the development of cocaine preference (McClung et al., 2005; Graham et al., 2009).

Cocaine CPP Study 1 (Figure 1A): For each cocaine dose (3, 8, 15 mg/kg), two groups of animals were compared (group names are designated based on the sleep parameters [SD or undisturbed {noSD}] before each set of conditioning trials [cocaine {Coc} or saline {Sal}]. Both groups received cocaine and saline on alternating days. The experimental group of mice underwent SD for 4 h immediately prior to cocaine conditioning trials and, on alternate days, were undisturbed prior to saline conditioning trials (**SD Coc, noSD Sal**). The control group was sleep deprived for 4 h immediately prior to the saline conditioning trials (**noSD Coc, SD Sal**) but was undisturbed prior to the cocaine conditioning trials. An additional experiment was used to test whether SD is sufficient to induce preference. Subjects received saline on both side chambers with (**SD Sal, noSD Sal**) or without SD (**noSD Sal, noSD Sal**) prior to saline-conditioning trials on one side of the box. Animals were weighed prior to each conditioning trial. There was no difference in pre-conditioning relative time values (side A – side B) between groups for any of the doses (0 mg/kg, *p=0.96*; 3 mg/kg, *p=0.98;* 8 mg/kg, *p=0.99;* 15 mg/kg, *p=0.71*).

Cocaine CPP Study 2 (Figure 2A): For each cocaine dose (3, 8, 15 mg/kg), two groups of animals were compared (group names are designated based on the sleep parameters, [SD], or undisturbed [noSD], before the post-conditioning test). Both groups received cocaine and saline on alternating days. In this study the experimental group of mice was sleep deprived on only one occasion, i.e. 4 h immediately prior to the post-test (**SD**), while a control group was undisturbed (**noSD**). There was no difference in pre-conditioning relative time values between groups for any of the doses (3 mg/kg, *p=0.99;* 8 mg/kg, *p*=0.73; 15 mg/kg, *p=0.68*).

Orexin-receptor antagonism during conditioning and SD-enhanced Cocaine CPP Study 3 (Figure 3A): Subsets of mice receiving 3 or 8 mg/kg cocaine were injected with the orexin 1 receptor antagonist (OX1R) SB 334867 (1 mg/kg [SB]) or vehicle [Veh] 15 min prior to the conditioning trials (group names are designated based on sleep parameters and OX1R-antagonism status prior to each conditioning test). The experimental group was sleep deprived for 4 h and given SB prior to cocaine conditioning trials (**SD SB Coc, noSD Veh Sal**), while a control subset of mice was sleep deprived and given SB prior to the saline paired trials (**noSD Veh Coc, SD SB Sal**). For the 8 mg/kg dose, a third subset of mice was injected with SB prior to the cocaine paired trials but was not sleep deprived, serving as an OX1R antagonist-only control (**noSD SB Coc, noSD Veh Sal**). There was no difference in pre-conditioning relative time values between groups at either dose (3 mg/kg, *p=0.9;* 8mg/kg, *p=0.99*). SB 334867 was chosen on the basis of its common use in addiction-related studies, while the cocaine doses chosen were based on the doses in which there were significant group differences in Study 1 (3, 8 mg/kg).

OX1R antagonism after conditioning and SD-enhanced Cocaine CPP Study 4 (Figure 4A): Subsets of mice receiving 8 mg/kg cocaine were injected with SB 15 min prior to the post-test. The experimental group was sleep deprived for 4 h prior to receiving SB (**SD SB**), while the control group was undisturbed prior to receiving SB (**noSD SB**). There was no difference in pre-conditioning relative time values between groups (*p=0.98)*. The cocaine dose chosen was based on the dose in which there was a significant group difference in Study 2 (8 mg/kg).

Sleep deprivation: Mice were sleep deprived using the treadmill method (Bjorness et al., 2009) in which waking is enforced through slow walking; the belt speed was ~3cm/s (for comparison belt speeds of ~20 cm/s are used for exercise, Um et al., 2011). SD began early in the light phase (ZT0-2) and concluded immediately before CPP conditioning or testing for a total of 4 h. Food and water were available throughout the SD period. Four hours of sleep deprivation was used since this duration reliably induces a homeostatic response as measured by an increase in slow wave activity (SWA, 0.5-4.5 Hz) during slow wave sleep (Bjorness et al., 2018b). Furthermore, this duration does not increase expression of glucocorticoid-related genes as determined by transcriptome analysis of cortical tissue (Bjorness et al., 2020).

Drugs: Cocaine hydrochloride (Sigma Aldrich) was dissolved in sterile saline and injected in doses of 3, 8, or 15 mg/kg with a volume of ≤0.1ml. Sterile saline was used as the vehicle control. SB 334867 (Sigma Aldrich) was dissolved in DMSO then diluted in sterile water (10% DMSO). DMSO diluted with sterile water was used as the vehicle control.

Outcome measures: The main outcome measure for cocaine CPP was preference score which was calculated as Preference (sec) = Post-conditioning test [side A_{time}– side B_{time}] – Pre-conditioning test [side A_{time} – side B_{time}] with A side conditioning in trials 1, 3 and B side in trials 2, 4. For Study 1 and 2, control and experimental groups were compared using one tailed unpaired T tests (3, 8, or 15 mg/kg cocaine) or two tailed unpaired T test (0 mg/kg cocaine). For Study 3 and 4, control and experimental groups were compared using two tailed unpaired T test (3 mg/kg Study 3, 8 mg/kg Study 4) or one way ANOVA with Sidak correction for multiple comparisons (8 mg/kg Study 4). One tailed T tests were used for comparisons in which there is literature support for an effect of SD on preference outcomes, while two tailed T tests were used for comparisons lacking direct literature support for an effect of SD on preference outcomes. Additionally, pre-conditioning relative time values (time in side to be paired with cocaine – time in side to be paired with saline) were also compared for each experiment to ensure equal balancing (with respect to pre-conditioning preference time) of groups prior to cocaine exposure. For comparison to a theoretical mean of 0, a two-tailed one sample t test was used; positive values significantly different than 0 indicate preference. All statistical analyses were performed using GraphPad prism. Values are given as average +/- standard error of the mean and significance is set at $p<0.05$. For all studies, pre and post-conditioning relative values are shown (Figure 1B, 2B, 3B, 4B) for the 8 mg/kg dose in order to illustrate variability between animals within each group alongside the general pattern of increased time in the cocaine-paired side.

Results:

Cocaine CPP; Study 1: To examine the effects of SD on cocaine CPP we compared CPP in experimental and control groups of mice. Both groups were alternately (every other day) conditioned to cocaine and saline (Coc, Sal), however, the experimental group's cocaine conditioning was preceded by 4 hours of SD (SD Coc, noSD Sal) whereas the control group's saline conditioning was preceded by SD (noSD Coc, SD Sal) as illustrated in figure 1A. To control for the potential effects of SD in the absence of cocaine conditioning, an additional two cohorts of mice received saline every day either with (SD Sal, noSD Sal) or without SD (noSD Sal, noSD Sal).

As expected, most animals (8/12 noSD Coc, SD Sal, 12/12 SD Coc, noSD Sal) that received the 8 mg/kg dose of cocaine showed an increase in the time spent in the cocaine-paired side, tested after conditioning (Figure 1B); however, SD immediately prior to cocaine conditioning trials resulted in an increase in time spent on the cocaine-paired side over animals experiencing SD preceding saline (Figure 1C, Table 1). In the absence of SD, animals did not show any preference for the 3mg/kg cocaine-conditioned side. In contrast, SD immediately prior to cocaine conditioning trials induced preference to a 3 mg/kg dose of cocaine (Figure 1C; Table 1). SD did not influence preference to a 15 mg/kg dose of cocaine, a possible ceiling-like effect (Figure 1C; Table 1). Controls (noSD Coc, SD Sal) showed preference at 15 mg/kg but not at 8 mg/kg, possibly due to high variability driven by one animal (not identified as an outlier when using the ROUT method [GraphPad Prism]). SD in the absence of cocaine did not induce preference (0 mg/kg, Figure 1C; Table 1).

Cocaine CPP; Study 2: To examine the effects of SD on cocaine CPP after conditioning has been established, cocaine-conditioned animals underwent SD for 4 hours immediately prior to the post-conditioning test. The control group was similarly conditioned but remained undisturbed prior to testing for CPP (Figure 2A).

As expected, most animals (n=9/13 noSD; 10/11 SD) that received the 8mg/kg dose of cocaine showed an increase in the time spent in the cocaine-paired side from the pre-conditioning to post-conditioning tests (Figure 2B). SD immediately prior to the post-conditioning test induced a non-significant trend towards preference to a 3 mg/kg dose of cocaine (Figure 2C; Table 2) and significantly increased preference to an 8 mg/kg dose of cocaine (Figure 2C; Table 2). There was no difference in preference between groups to a 15 mg/kg dose of cocaine (Figure 2C; Table 2), but the sleep deprived group showed a non-significant trend towards preference for the cocaine-paired side.

OX1R antagonism during conditioning and SD-enhanced Cocaine CPP; Study 3: A previous observation indicates that SB 334867 during conditioning attenuates cocaine CPP (Rao et al., 2013). To examine the effects of OX1R antagonism on SD-induced enhancement of cocaine CPP, we compared CPP in an experimental and control group as in Study 1, except that immediately following SD, but prior to each training session, animals received the OX1R antagonist SB 334867 (SB) or vehicle (Veh) on alternating days (Figure 3A). To test for OX1R antagonism effects in the absence of SD, SB 334867 was also given to cohort that did not undergo SD.

In response to 8 mg/kg cocaine, all sleep deprived animals showed an increase in the time spent on the cocaine-paired side; however, only a subset (6/11) of animals receiving OX1R antagonism in the absence of SD showed this relative increase (Figure 3B). In contrast to the observations of Study 1 in which SD induced cocaine CPP to a 3 mg/kg dose of cocaine, SB 334867 prevented this induction. Finally, SB 334867 prevented preference in the absence of SD and blocked SD-induced enhancement of preference to a 8 mg/kg dose of cocaine as determined by a lack of difference between groups; however SD animals treated with SB 334867 did show preference for the cocaine-paired side suggesting that SD-dependent enhancement is reduced but not entirely prevented (Figure 3C; Table 3).

OX1R antagonism after conditioning and SD-enhanced Cocaine CPP; Study 4: Previous observations indicate that SB after conditioning does not influence cocaine CPP (Sharf et al., 2010; Sartor and Aston-Jones, 2012). The effect of OX1R antagonism together with SD on cocaine CPP after establishment of conditioning was examined by comparing CPP in an experimental and control group of mice as in Study 2, except that all animals received the OX1R antagonist prior to the post-conditioning test (Figure 4A).

A similar majority of undisturbed or sleep deprived animals administered SB 334867 just prior to the post-conditioning test, showed an increase in the time spent on the cocaine-paired side (Figure 4B). The antagonism of OX1R after establishment of conditioning was sufficient to prevent the SD-induced enhancement of cocaine CPP to an 8 mg/kg dose of cocaine (Figure 4C; Table 4).

Sleep deprivation shifts the cocaine CPP dose-response curve leftward in an orexin-influenced manner: SD immediately prior to cocaine-conditioning trials shifts the preference dose-response curve leftward (Figure 5A) which is consistent with an increasing sensitivity to the rewarding properties of cocaine. However, OX1R antagonism immediately prior to the cocaine-conditioning trials reduces this shift (Figure 5B). An SD-related leftward shift in the dose response curve is also apparent when SD occurs immediately prior to the post-conditioning test (Figure 5C) and it is reduced by OX1R antagonism. Unexpectedly, OX1R antagonism in undisturbed animals prior to the post-conditioning test [Study 4; noSD SB] led to preference values similar to that of sleep deprived animals in the absence of OX1R antagonism [Study 2; SD], thereby reversing the polarity of the effect of SD as determined by dividing the group average preference score of the sleep deprived group by the group average preference score of the undisturbed group (i.e. [Study 2, SD/noSD]; [Study 4, SD SB/noSD SB]). A score above 1 indicates that SD results in a higher relative preference score compared to the undisturbed condition, while a score below 1 indicates that SD results in a lower relative preference score compared to the undisturbed condition. Statistical comparisons across studies were not performed because of data collection constraints (see discussion section) so these comparisons are observational in nature and should be interpreted with caution.

Discussion: SD enhanced cocaine CPP in a dose-dependent manner resulting in a leftward shift in the dose-response curve, indicating SD increased the rewarding properties of cocaine. This

shift was more pronounced when SD occurred immediately prior to cocaine exposure compared to SD after cocaine conditioning was already established, consistent with a greater SD-induced enhancement of acquisition of preference than of its expression. OX1R antagonism reduced the SD-induced enhancement of both acquisition and expression.

On the low end of the dose-response curve, SD induced preference to a subthreshold dose of cocaine, which is similar to a previously reported SD-dependent induction of preference to subthreshold amphetamine (Berro et al., 2018), suggesting SD may increase sensitivity to psychostimulants in general. On the high end of the dose-response curve, SD did not alter preference to a sensitizing dose of cocaine, possibly due to a ceiling effect and/or, an aversive effect elicited by higher doses of cocaine.

Most groups showed preference for the 8 mg/kg dose of cocaine, a dose in which preference is expected (Campbell et al., 2000); however, animals sleep deprived prior to saline conditioning trials (noSD Coc, SD Sal, Study 1) did not reach statistical significance for preference despite the majority of animals showing an increase in time spent on the cocaine-paired side from the pre to post-conditioning tests (8/12). This lack of preference is likely attributable to high variability in preference scores relative to the group average (126.4 +/- 83.6) and is driven by a single animal as can be seen in Figure 1B, though this animal does not qualify as an outlier. As can be seen from the raw data plots with the 8 mg/kg dose across studies, most animals show an increase in relative time in the cocaine-paired side from pre to post-conditioning, though not all animals do so. We cannot explain the source of the individual differences, but these are consistent with individual differences seen with locomotor sensitization to cocaine (Hooks et al., 1991; Allen et al., 2007) and cocaine self-administration (Glick et al., 1994; Griffin et al., 2007).

The ability of OX1R antagonism to reduce SD-induced enhancement of cocaine CPP is consistent with the well-known role of orexin in motivated behavior (James et al., 2017) and maintenance of arousal (Sakurai et al., 2010). Orexin neuronal activity increases during SD (Estabrooke et al., 2001) as does orexin release (Yoshida et al., 2001). Furthermore, orexin agonists promote cocaine self-administration (Espana et al., 2011), while antagonism of orexin activity can reduce reward behavior (Sartor and Aston-Jones, 2012; Rao et al., 2013, Shaw et al., 2017).

The SD-induced enhancement of cocaine CPP is consistent with previous studies in which SD increases preference of methylphenidate in humans (Roehrs et al., 1999) and induces preference to a low dose of amphetamine in rodents (Berro et al., 2018). However, there are several additional studies that would be of interest in further delineating the ability of sleep loss to influence reward behavior. First, thus far all studies have used stimulants so the generalizability of the SD-induced enhancement of preference across drug class is unknown. Additionally, the time course of this enhancement preference is unclear. A long term enhancement of preference would likely be more relevant to the development of addiction than if the SD-induced enhancement is quickly lost. Finally, it is unknown if SD-induced enhancement of stimulant reward is sustained in drug experienced animals since existing studies have included drug naïve rodents or non-dependent humans.

These studies had several limitations. First, Study 4 lacked a vehicle control group; a control group of noSD SB was used for the experimental group SD SB in which the ability of OX1R-antagonism to counter SD-induced enhancement of cocaine CPP to an 8 mg/kg dose of cocaine (from Study 2) was tested. DMSO was diluted in order to reduce the concentration below that which behavioral effects are observed (Cavas et al., 2005). However, the effect of vehicle alone on CPP expression was not determined so the possibility that SD-induced enhancement of cocaine CPP was reduced by the vehicle cannot be excluded. Another limitation relates to the lack of direct statistical comparisons across related studies (Study 1&3, Study 2&4) due to the manner of data collection. Within each study, control and experimental animals were littermates and data were collected concurrently across groups with multiple sets of control and experimental animals collected for each study; however, there was a considerable time lag between data collection of related studies. A superior design would have included concurrent data collection for related studies so that these could be directly compared. Additionally, since activity measures are not available for conditionings performed with all of the CPP boxes, we cannot exclude a possibility that enhanced preference is associated with an increase in locomotor activity; however, we have previously shown that acute SD does not influence the magnitude of locomotor sensitization to cocaine (Bjorness and Greene, 2018b) so an SD-dependent increase in locomotor activity is not expected. Finally, the current experiments did not include female subjects so it is unknown whether gender influences SD-enhancement of cocaine CPP.

In conclusion, acute SD increases the rewarding properties of cocaine in a cocaine dose dependent manner as measured by the CPP task which suggests that sleep loss may facilitate the transition towards addiction. OX1R antagonism reduces this effect, suggesting a potential therapeutic avenue for careful consideration as an aid in abstinence maintenance. Recently, Suchting and colleagues provided preliminary proof-of-concept for use of orexin receptor antagonism in individuals with cocaine use disorder (Suchting et al., 2020). Although the study design precludes an assessment of the efficacy of a OX1R/OX2R antagonist, there is evidence for its having improved objective sleep (actigraphy) and self-reported craving measures (Cocaine Craving Questionnaire), suggesting the clinical relevance for our findings.

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Figure 1: A) Experimental timeline of cocaine CPP Study 1. B) Following conditioning to 8 mg/kg cocaine, most animals undisturbed prior to cocaine conditioning trials spent more time in the cocaine-paired side following conditioning as expected as compared to their pre-conditioning test times (top), which shifted to all animals when SD occurred immediately prior to cocaine conditioning trials (bottom). C) SD immediately prior to cocaine conditioning trials induced preference to 3 mg/kg cocaine and enhanced preference to 8 mg/kg cocaine without altering preference to 15 mg/kg cocaine. SD in the absence of cocaine (0 mg/kg) did not induce preference. Asterisks above columns indicate preference (as determined by a significant difference from 0), the carrot between columns indicates a significant difference between groups, and n.s. indicates a lack of significant difference between groups.

Figure 2: A) Experimental timeline for the cocaine CPP Study 2. B) Following conditioning to 8 mg/kg cocaine, most animals undisturbed prior to the post-conditioning test spent more time in the cocaine-paired side as compared to their pre-conditioning test times (top), which shifted to a higher proportion of animals when SD occurred immediately prior to the post-conditioning test (bottom). C) SD immediately prior to post-conditioning test induced a non-significant trend towards preference to 3 mg/kg cocaine and enhanced preference to 8 mg/kg cocaine, while reducing preference to a non-significant trend to 15 mg/kg cocaine. Asterisks signs above columns indicate preference (as determined by a significant difference from 0), the carrot between columns indicates a significant difference between groups, and n.s. indicates a lack of significant difference between groups.

Figure 3: A) Experimental timeline for the cocaine CPP Study 3. B) All animals spent more time in the cocaine-paired side following conditioning as compared to their pre-conditioning test values when Veh was administered prior to the cocaine trials (top) or when SB 334867 was administered immediately following SD (bottom); however, only a subset of animals spent more time in the cocaine-paired side when SB 334867 was administered in the absence of SD (right side). C) OX1R antagonism prior to cocaine conditioning trials blocked the SD-induced preference to 3 mg/kg cocaine and the SD-induced enhanced preference to 8 mg/kg cocaine, while OX1R antagonism in the absence of SD prevented the acquisition of preference to 8 mg/kg cocaine. Asterisks above columns indicate preference (as determined by a significant difference from 0) and n.s. indicates a lack of significant difference between groups.

Figure 4: A) Experimental timeline for cocaine CPP Study 4. B) Most animals spent more time in the cocaine-paired side following conditioning as compared to their pre-conditioning test values both when undisturbed animals were administered SB 334687 prior to the post-conditioning test (top) and when sleep deprived animals were administered SB 334867 prior to the post-conditioning test (bottom). C) OX1R antagonism prevents the SD-induced increase in preference to 8 mg/kg cocaine, though both groups show preference for the cocaine-paired side. Asterisks above columns indicate preference (as determined by a significant difference from 0) and n.s. indicates a lack of significant difference between groups.

Figure 5: A) Dose response plot of preference from cocaine CPP Study 1 in which SD shifts the curve leftward (data replotted from Figure 1C). B) OX1R antagonism mitigates the SD-induced shift in the dose response curve (data replotted from Figures 1C & 3C). C) Dose response plot of preference from cocaine CPP Study 2 in which SD shifts the curve leftward, though to a lesser degree than under cocaine CPP Study 1 (data replotted from Figure 2C). D) The SD enhancement of relative preference, as determined by the ratio of average preference in SD and noSD groups (from Study 2) and indicated by >1 value (left bar), is blocked by OX1R antagonism (SD SB/noSD SB from Study 4; right bar). Notably, the relative preference of <1 under OX1R antagonism indicates that OX1R antagonism in the presence of SD reduces relative preference, while OX1R antagonism in the absence of SD increases relative preference.

Table 1: Cocaine CPP, Study 1

Study 1	0 mg/kg		3 mg/kg	
	noSD Sal, no SD Sal	SD Sal, noSD Sal	noSD Coc, SD Sal	SD Coc, noSD Sal
N	12	12	10	11
Mean+/-SEM	97.81+/-52.49	100.6+/-63.38	41.83+/-90.53	180.4+/-62.75
95% CI	-17.73 to 213.4	-49.92 to 251.1	-163 to 246.6	40.56 to 320.2
p value	0.09	0.17	0.655	0.0165
Stat used for comparison	One sample T test	One sample T test	One sample T test	One sample T test
	8 mg/kg		15 mg/kg	
	noSD Coc, SD Sal	SD Coc, noSD Sal	noSD Coc, SD Sal	SD Coc, noSD Sal
N	12	12	12	12

Mean+/-SEM	126.4+/-83.62	348.7+/-62.95	257.9+/-65.4	284.9+/-94.67
95% CI	-57.64 to 310.5	210.1 to 487.2	114 to 401.9	76.5 to 493.2
p value	0.1588	0.0002	0.0023	0.0119
Stat used for comparison	One sample T test	One sample T test	One sample T test	One sample T test
group comparison	0 mg/kg	3 mg/kg	8 mg/kg	15 mg/kg
Diff btw means	-2.521 ± 49.26	-138.5 ± 108.5	-222.3 ± 104.7	-26.93 ± 115.1
95% CI	-104.7 to 99.63	-365.6 to 88.46	-439.3 to -5.173	-265.6 to 211.7
p value	0.9596	0.11	0.0226	0.4085
Stat used for comparison	Unpaired T test, two tailed	Unpaired T test, one tailed	Unpaired T test, one tailed	Unpaired T test, one tailed

Table 2: Cocaine CPP, Study 2

Study 2	3 mg/kg		8 mg/kg		15 mg/kg	
	noSD	SD	noSD	SD	noSD	SD
N	12	11	13	11	12	12
Mean+/-SEM	128.3+/-76.91	187.4+/-94.94	141.3+/-49.55	256+/-42.39	281.8+/-72.06	179.6+/-100.1
95% CI	-41.02 to 297.5	-24.19 to 398.9	33.35 to 249.3	161.6 to 350.5	123.2 to 440.4	-40.84 to 400
p value	0.1236	0.0767	0.0146	0.0001	0.0024	0.1005
Stat used for comparison	One sample T test	One sample T test	One sample T test	One sample T test	One sample T test	One sample T test

group comparison	3 mg/kg	8 mg/kg	15 mg/kg
Diff btw means	-59.11 ± 121.3	114.7 ± 66.55	102.3 ± 123.4
95% CI	-311.3 to 193.1	-23.31 to 252.7	-153.6 to 358.1
p value	0.3155	0.0494	0.2081
Stat used for comparison	Unpaired T test, one tailed	Unpaired T test, one tailed	Unpaired T test, one tailed

Table 3: Cocaine CPP, Study 3

Study 3	3 mg/kg		8 mg/kg		
	noSD Veh Coc, SD SB Sal	SD SB Coc, noSD Veh Sal	noSD Veh Coc, SD SB Sal	SD SB Coc, noSD Veh Sal	noSD SB Coc, noSD Veh Sal
N	11	12	11	11	11
Mean+/-SEM	48.12+/-48.24	70.25+/-93.15	282+/-68.44	246.8+/-37.69	141.8+/-102.1
95% CI	-59.36 to 155.6	-134.8 to 275.3	129.5 to 434.5	162.8 to 330.7	-85.82 to 369.4
p value	0.342	0.4666	0.0021	<0.0001	0.1953

Stat used for comparison	One sample T test	One sample T test	One sample T test	One sample T test	One sample T test
group comparison	3 mg/kg noSD Veh Coc, SD SB Sal vs SD SB Coc, noSD Veh Sal	8 mg/kg noSD Veh Coc, SD SB Sal vs SD SB Coc, noSD Veh Sal	8 mg/kg noSD Veh Coc, SD SB Sal vs noSD SB Coc, noSD Veh Sal		
Diff btw means	22.13 ± 107.8	35.25	140.2		
95% CI	-202.1 to 246.4	-211.9 to 282.4	-106.9 to 387.4		
p value	0.8394	0.9321	0.3467		
Stat used for comparison	Unpaired T test, two tailed	One way ANOVA with Sidak correction for multiple comparisons	One way ANOVA with Sidak correction for multiple comparisons		

Table 4: Cocaine CPP, Study 4

Study 4	8 mg/kg	
	noSD SB	SD SB
N	12	12
Mean+/-SEM	237.4+/-57.03	152.2+/-50.88
95% CI	111.8 to 362.9	40.17 to 264.1
p value	0.0016	0.0123

Stat used for comparison	One sample T test	One sample T test
group comparison	8 mg/kg	
Diff btw means	-85.21 ± 76.42	
95% CI	-243.7 to 73.28	
p value	0.2769	
Stat used for comparison	Unpaired T test, two tailed	

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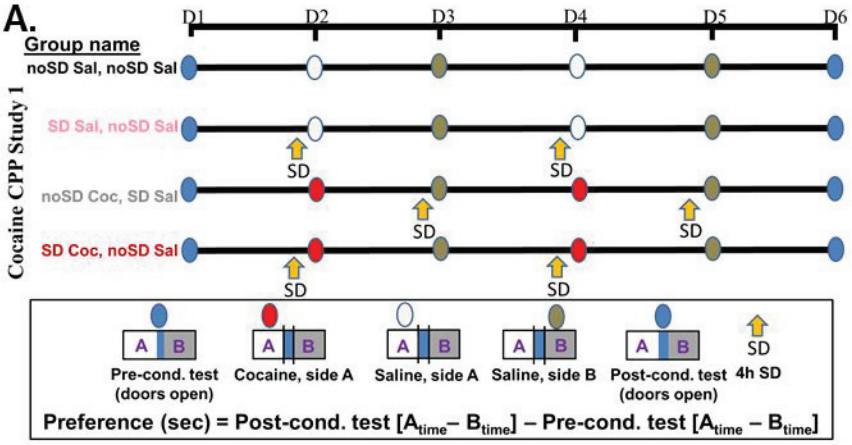

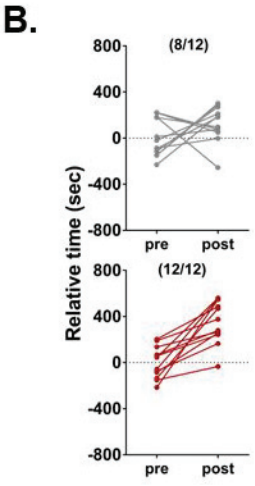

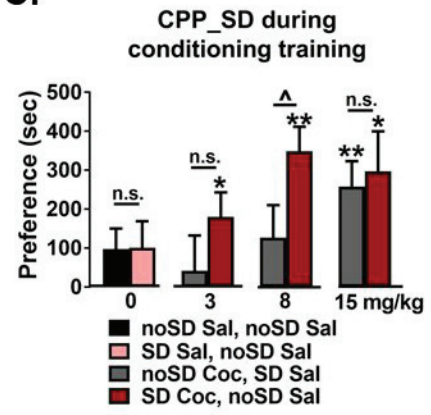

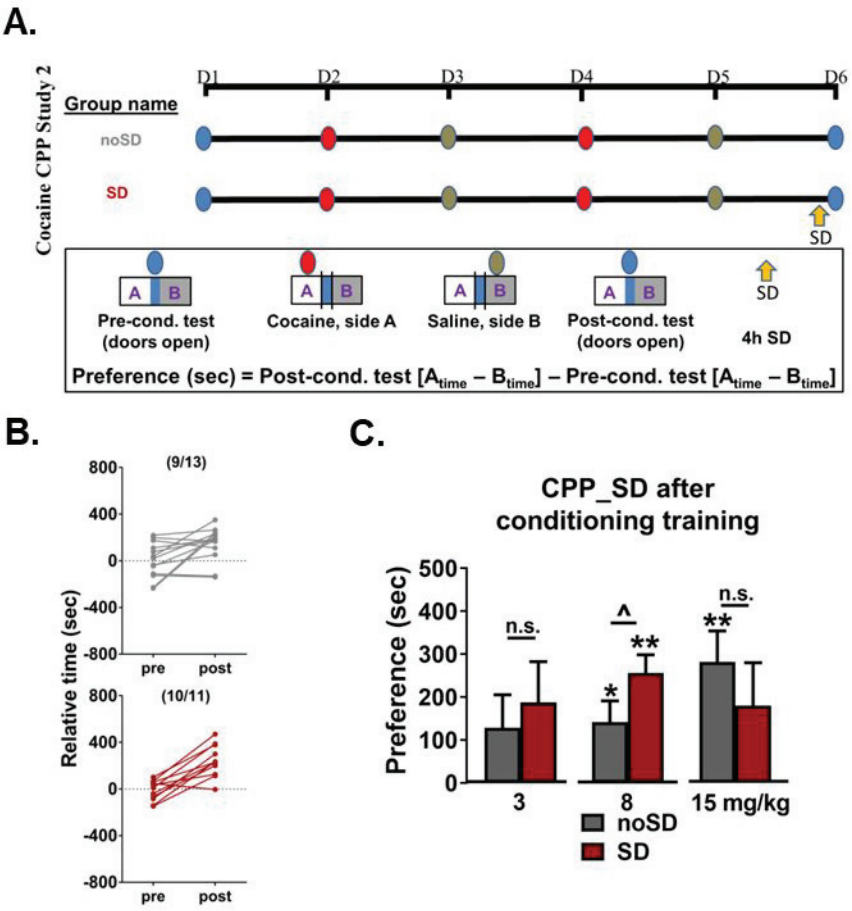

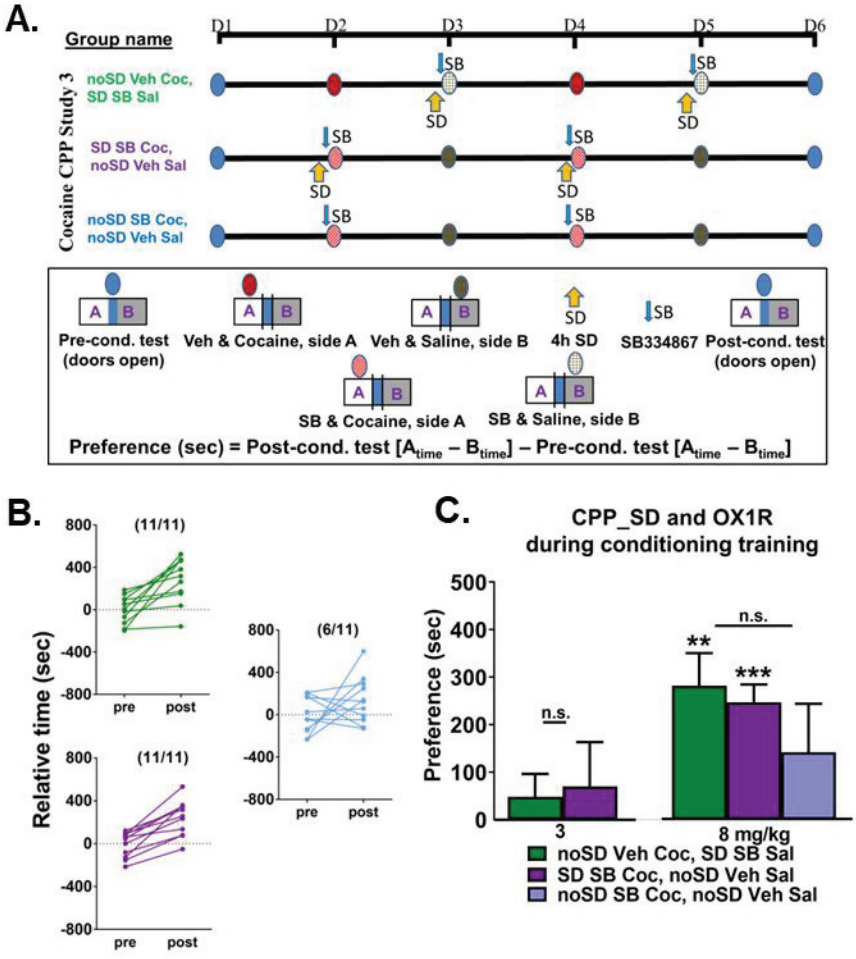

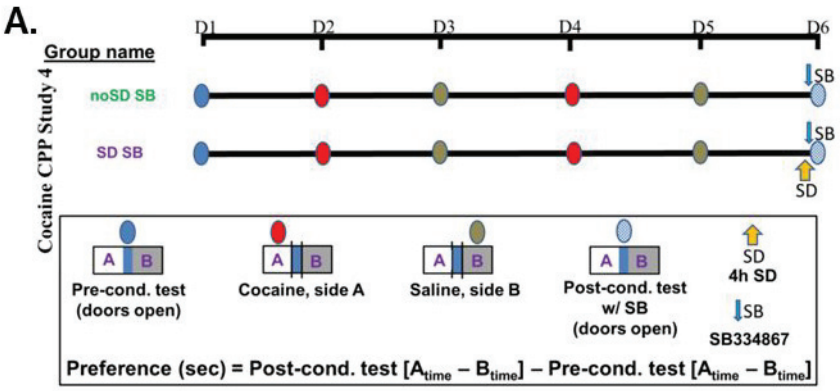

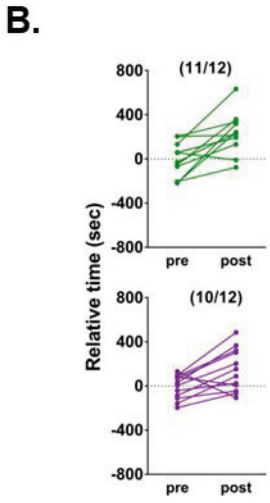

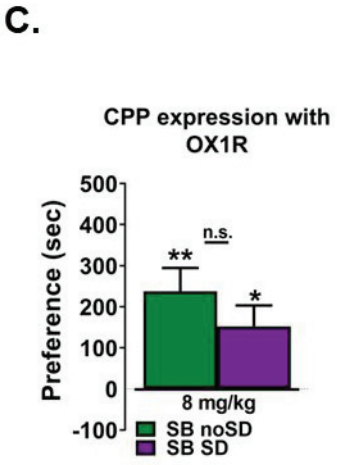

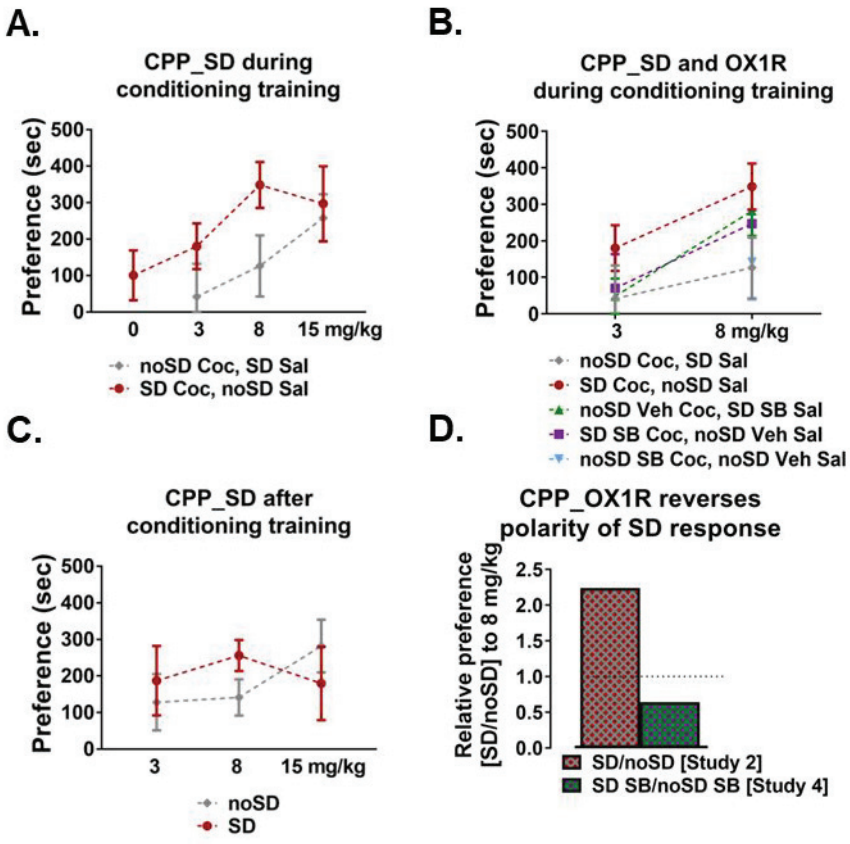

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95% CI	-17.73 to 213.4	-49.92 to 251.1	-163 to 246.6	40.56 to 320.2
p value	0.09	0.17	0.655	0.0165
Stat used for comparison	One sample T test	One sample T test	One sample T test	One sample T test
	8 mg/kg		15 mg/kg	
	noSD Coc, SD Sal	SD Coc, noSD Sal	noSD Coc, SD Sal	SD Coc, noSD Sal
N	12	12	12	12
Mean+/-SEM	126.4+/-83.62	348.7+/-62.95	257.9+/-65.4	284.9+/-94.67
95% CI	-57.64 to 310.5	210.1 to 487.2	114 to 401.9	76.5 to 493.2
p value	0.1588	0.0002	0.0023	0.0119
Stat used for comparison	One sample T test	One sample T test	One sample T test	One sample T test
group comparison	0 mg/kg	3 mg/kg	8 mg/kg	15 mg/kg
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Table 2: Cocaine CPP, Study 2

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	noSD	SD	noSD	SD	noSD	SD
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Stat used for comparison	Unpaired T test, one tailed		Unpaired T test, one tailed		Unpaired T test, one tailed	

Table 3: Cocaine CPP, Study 3

Study 3	3 mg/kg		8 mg/kg		
	noSD Veh Coc, SD SB Sal	SD SB Coc, noSD Veh Sal	noSD Veh Coc, SD SB Sal	SD SB Coc, noSD Veh Sal	noSD SB Coc, noSD Veh Sal
N	11	12	11	11	11
Mean+/-SEM	48.12+/-48.24	70.25+/-93.15	282+/-68.44	246.8+/-37.69	141.8+/-102.1
95% CI	-59.36 to 155.6	-134.8 to 275.3	129.5 to 434.5	162.8 to 330.7	-85.82 to 369.4
p value	0.342	0.4666	0.0021	<0.0001	0.1953
Stat used for comparison	One sample T test	One sample T test	One sample T test	One sample T test	One sample T test
group comparison	3 mg/kg noSD Veh Coc, SD SB Sal vs SD SB Coc, noSD Veh Sal	8 mg/kg noSD Veh Coc, SD SB Sal vs SD SB Coc, noSD Veh Sal	8 mg/kg noSD Veh Coc, SD SB Sal vs noSD SB Coc, noSD Veh Sal		
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95% CI	-202.1 to 246.4	-211.9 to 282.4	-106.9 to 387.4		
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Stat used for comparison	Unpaired T test, two tailed	One way ANOVA with Sidak correction for multiple comparisons	One way ANOVA with Sidak correction for multiple comparisons		

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