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# Enhancement of cue-induced reinstatement of alcohol seeking by acute total sleep restriction in male Wistar rats

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ABSTRACT

Clinical studies suggest that sleep impairment is a barrier to successful treatment in alcohol use disorder (AUD) patients, with sleep disruption associated with relapse to alcohol taking. To date, no preclinical study has evaluated the relationship between impaired sleep and alcohol relapse. In the present study, we used a self-administration model to investigate the effects of sleep restriction on reinstatement induced by alcohol-paired environmental cues. Using a sucrose fading protocol, male Wistar rats ($N = 8$) were trained to self-administer alcohol under a fixed-ratio 2 schedule of alcohol delivery such that completion of every second response resulted in the delivery of the alcohol solution and activation of the alcohol-paired cue light. Once self-administration was stable, behavior was extinguished by omitting delivery of the alcohol solution and the alcohol-paired cues. When responding reached low, stable levels, alcohol seeking was induced by re-presentation of the alcohol-paired cues but with no alcohol solution available for self-administration. To evaluate the effects of sleep restriction on cue-induced alcohol seeking, reinstatement tests were conducted after 6-h of total (slow wave + rapid eye movement [REM]) sleep restriction using the gentle handling method or after 6-h of REM sleep-only restriction using the flower pot method. Relevant control conditions also were evaluated. The results showed that acute restriction of total sleep, but not REM sleep primarily, significantly augmented cue-induced reinstatement of alcohol seeking. This increase was specific to total sleep restriction conditions and cannot be attributed to differences in alcohol intake, responding, or days to extinction. Our findings imply that acute slow wave sleep restriction is necessary and/or sufficient for the enhancement of cue-induced alcohol seeking and, further, suggest that decreased slow wave sleep in AUD patients places individuals at a unique risk for relapse.

## 1. Introduction

Alcohol use disorder (AUD) is a highly prevalent psychiatric disease and a major global public health concern. The World Health Organization has reported that alcohol consumption contributes to more than 200 types of diseases and injury-related health problems (World Health Organization, 2014). AUD also elicits a significant societal burden, with it being the third leading preventable cause of death in the U.S. (Centers for Disease Control and Prevention, 2017). In 2018, 14.4 million U.S adults were diagnosed with AUD (SAMHSA, 2018). AUD is accompanied by a high rate of relapse among patients seeking treatment (Grant et al., 2017), who report stressful events, re-exposure to alcohol itself, and re-exposure to environmental cues associated with past alcohol use as triggers for relapse (for review, see Weiss, 2010). Sleep impairment also has garnered attention as a potential contributor to relapse in patients seeking treatment for AUD (Brower, 2015; Miller et al., 2017).

Human studies suggest that the relationship between AUD and sleep complications is likely bi-directional, with acute and chronic alcohol use inducing disruptions in sleep that, in turn, predispose patients to continued alcohol use, poor treatment response, and relapse (for review, see Koob and Colrain, 2020). Alcohol is known to increase slow wave sleep (SWS) in the first half of the night and decrease SWS in the second half of night (MacLean and Cairns, 1982; Williams et al., 1983; Chan et al., 2013). In addition, while consumption of relatively low doses of alcohol increases rapid eye movement (REM) sleep duration in the first half of the night (MacLean and Cairns, 1982; Van Reen et al., 2006), consumption of relatively higher doses suppresses REM sleep (Feige et al., 2006; Arnedt et al., 2011; Sagawa et al., 2011). The negative

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effects of alcohol use on sleep persist even during abstinence, with patients showing decreased SWS and increased REM sleep after ceasing alcohol use (Lester et al., 1975; Wagman and Allen, 1975; Feige et al., 2007; Irwin et al., 2016; Singh et al., 2018). Of importance, studies have shown repeatedly that both subjective reports of impaired sleep (Skoloda et al., 1979; Brower et al., 1998; Foster and Peters, 1999; Conroy et al., 2006) and impaired sleep determined via polysomnography in AUD patients (Brower et al., 1998; Drummond et al., 1998) predict the return to alcohol use after prolonged abstinence. For instance, Drummond et al. (1998) showed that disrupted sleep at 5 months of abstinence predicted relapse at 14 months in AUD patients and, further, that some sleep impairment persists even after 27 months of abstinence. Therefore, sleep impairment in the context of AUD represents an important barrier to successful treatment of patients.

Although several clinical studies have indicated a relationship between impaired sleep and relapse in AUD patients, to our knowledge no study to date has investigated the effects of sleep deprivation in preclinical models of alcohol relapse. Therefore, the goal of the present study was to evaluate the effects of acute mild total sleep (SWS + REM) restriction versus REM sleep restriction only on alcohol seeking using a cue-induced reinstatement procedure in rats. Because of the pattern of sleep alterations observed in abstinent AUD patients (i.e., disrupted SWS and REM sleep, e.g. Wagman and Allen, 1975; Irwin et al., 2016), we hypothesized that total sleep restriction immediately prior to reinstatement testing would have a greater effect on cue-induced reinstatement of alcohol seeking compared to REM sleep restriction only.

## 2. Materials and methods

### 2.1. Subjects

Adult male Wistar rats (Envigo, Indianapolis, IN; $N = 8$), 70 days old and weighing 260–300 g at the start of the experiment, were pair-housed in standard shoebox cages under a 12/12-h light/dark cycle (lights on at 12:00 AM) with food and water available *ad libitum*. All animals were maintained and experiments were conducted in accordance with the University of Mississippi Medical Center's Institutional Animal Care and Use Committee and were in accordance with the National Research Council's Guide for Care and Use of Laboratory Animals (8th edition, 2011).

### 2.2. Cue-induced reinstatement of alcohol seeking

Self-administration sessions occurred in custom-made operant conditioning chambers (Gerbrands Corporation; Arlington, MA; h × w × l: 19 cm × 23.5 cm × 22 cm). One wall of the chamber was equipped with two levers (one designated active, one designated inactive; Gerbrands Corporation), a stainless steel liquid reservoir located below each lever, and a white stimulus light located above each lever. A flashing, amber, jeweled stimulus light was mounted in the center of the panel, between the white stimulus lights. A syringe pump (Razel Scientific, St Albans, VT) controlled the delivery of the solution from a 30-ml syringe, through a ~25.4 cm segment of polyethylene tubing connected to a metal spout that was fixed to the liquid reservoir. A Macintosh computer equipped with custom interface and software controlled all events in the experimental session and recorded data.

Rats were trained to self-administer sweetened alcohol using a sucrose-fading procedure (Samson, 1986; Chandler et al., 2019). Rats initially were trained to respond in order to obtain a 10% (*w/v*) sucrose solution. Briefly, in the presence of the white light located above the active lever, the completion of every second response (FR 2) on the active lever resulted in the delivery of 0.1 ml of solution and activation of the jeweled, flashing, amber cue light (1.66-s duration; i.e., the alcohol-paired cue). A 1-s time-out period followed the delivery of solution and its paired cue, during which all lights were off and responses had no scheduled consequences. Once responding stabilized for sucrose (no upward or downward trends in number of solution deliveries over three consecutive days), alcohol (190 proof ethyl alcohol diluted in tap water; Ultra Pure, Darien, CT) was gradually added to the sucrose and the sucrose was gradually decreased in the following sequence (S, sucrose; E, alcohol; % w/v): 10S, 10S/2E, 10S/5E, 10S/10E, 5S/10E, 4S/10E, 3S/10E, 5S/15E, 2S/15E. During training, subjects moved to the next step in the sequence when they met the a priori criterion of self-administering a dose of ≥0.5 g/kg alcohol during three consecutive sessions. This target dose was chosen because it produces pharmacologically relevant levels of alcohol when ingested in a 30-min session (Besheer et al., 2013). Additionally, at the start of each self-administration session, the equivalent of one delivery of solution (0.1 ml) was available in the liquid reservoir below the active lever. This amount of alcohol is not pharmacologically relevant and functioned to provide additional odor/taste cues (cf. Bäckström et al., 2004; Cannady et al., 2013). Self-administration training continued until the subjects reliably self-administered a 2% sucrose/15% alcohol (*w/v*) solution. All experimental sessions took place during the first 2 h of the dark phase (i.e., 12:00–2:00 pm). Self-administration sessions occurred 5 days per week, lasted 30 min, and continued until stability criteria (three consecutive days with a self-administered dose >0.5 g/kg with no upward or downward trends; cf. Besheer et al., 2013) were met.

Extinction training followed and continued until responding declined and stabilized at the a priori criterion of ≤10% of the number of lever responses during active self-administration for two consecutive sessions. During extinction sessions, the light above the active lever was illuminated but lever presses had no scheduled consequences (i.e., no amber light flash, no pump operation, and no solution delivery). Cue-induced reinstatement tests followed. During tests, the operant conditioning chamber returned to the self-administration configuration (i.e., 0.1 ml of the sweetened alcohol solution was available in the liquid reservoir at the session's start); however, lever presses resulted only in the activation of the cue light and syringe pump without delivery of the sweetened alcohol solution.

### 2.3. Experimental design

All subjects ($N = 8$) initially underwent a cue-induced reinstatement test to ensure that the experimental conditions induced alcohol-seeking behavior (i.e., "baseline" reinstatement cycle). Next, the effects of total (SWS + REM sleep) and REM sleep restriction on alcohol cue-induced reinstatement were assessed in a pseudo-random order. That is, animals were submitted to the total sleep restriction (TSR) and TSR control tests (order randomized between animals), followed by the REM sleep restriction (REMSR) and REMSR control conditions (order randomized between animals). This experimental design resulted in 4 different testing sequences for sleep restriction: (1) TSR, TSR-Control, REMSR, REMSR-Control; (2) TSR, TSR-Control, REMSR-Control, REMSR; (3) TSR-Control, TSR, REMSR, REMSR-Control; (4) TSR-Control, TSR, REMSR-Control, REMSR. Two rats were assigned to each specific testing sequence. Finally, at the completion of all sleep restriction tests, a final cue-induced reinstatement test was conducted under "baseline" conditions to assess the degree to which alcohol seeking remained stable across time. Between each test, subjects were returned to active self-administration sessions to reestablish stable responding and criterion level intake, and behavior was subsequently extinguished as described above.

### 2.4. Total sleep restriction (TSR)

Subjects were submitted to TSR for 6 h using the "gentle-handling method" (cf. Castillo-Ruiz and Nunez, 2011). This method decreases both SWS and REM sleep in rats (Oonk et al., 2016). TSR periods began halfway through the light phase i.e., (at 6:00 AM) and ended immediately before cue-induced reinstatement tests. TSR sessions were conducted in the animals' home-cages in the presence of their cage mates.

Subjects had access to water and food *ab libitum* and were continuously monitored and stroked with a soft paint brush (Michigan Brush, Detroit, MI) whenever behavioral signs of sleep, such as closed eyes or sleep posture, were observed. TSR-Control conditions consisted of maintaining the animals in the same room where the sleep deprivation procedure was being performed, but animals under the control conditions remained undisturbed in their home-cages.

### 2.5. REM sleep restriction (REMSR)

Subjects were submitted to REMSR for 6 h using the "flower pot method" (cf. Cohen and Dement, 1965). In this model, animals are positioned on top of small platforms inside a water tank, and the general muscle relaxation associated with REM sleep causes the animals to fall into the water and wake up. This method has been validated in rats using EEG technology and, under conditions identical to those proposed in this study, REM sleep is suppressed selectively with no change in SWS (Mendelson et al., 1974; Porkka-Heiskanen et al., 1995). The flower pot method is considered a good method for REMS restriction, especially for in vivo behavioral studies (Mehta et al., 2018). REMSR periods began halfway through the light phase (i.e., at 6:00 AM) and ended immediately before cue-induced reinstatement tests (i.e., for a total of 6 h). This portion of the light phase is associated with the highest number of REM cycles and, consequently, the highest percentage (~16%) of REM sleep in the typical 12:12 light/dark cycle for rats (Vivaldi et al., 2005). The REMSR apparatus consisted of 3 inverted flower pots (6.35 cm in diameter) placed inside a plastic tank (Sterilite, Townsend, MA: 87.9 × 47.6 × 32.1 cm) filled with water up to 1 cm of the top of the flower pots, allowing the animals to move around by leaping from one flower pot to another. The REMSR-Control condition consisted of 3 larger inverted flower pots (12.7 cm in diameter) placed inside the plastic tank and also filled with water up to 1 cm of the top of the flower pots. Water temperature in both conditions was maintained at 20 °C. During REMSR sessions and REMSR-Control sessions, subjects had access to water and food *ab libitum* via a food hopper and a volumetric tube filled with water affixed to the side of the tank. Subjects were monitored frequently to ensure that subjects spent the majority of the time on the flower pots.

### 2.6. Statistical analysis

Acquisition data for the group of rats are presented as mean (± SEM) number of sessions to reach stability criteria at each sucrose/ethanol solution. Data were analyzed with a one-way repeated measures ANOVA, followed by all pairwise multiple comparisons using Bonferroni *t*-tests. Initial and final, "baseline" cue-induced reinstatement results are presented as mean (± SEM) active lever responses during the last 3 days of self-administration, during the last 2 days of extinction, and during the reinstatement test. Data were analyzed with a one-way repeated measures ANOVA followed by all pairwise multiple comparisons using Bonferroni *t*-tests. A paired *t*-test comparing levels of alcohol seeking at the start and the end of the study was used to assess stability of the behavior.

To assess the extent to which the sleep restriction protocols (or their control conditions) altered cue-induced alcohol seeking, data from test sessions were converted to a percentage of baseline cue-induced responding. Baseline responding was calculated to be the average number of responses from the initial and final cue-induced reinstatement tests. Data were analyzed with a one-way repeated measures ANOVA followed by all pairwise multiple comparisons using Bonferroni *t*-tests. Similarly, inactive lever responding during the sleep restriction conditions were expressed as a percentage of active self-administration responses and analyzed with a one-way repeated measures ANOVA.

To assess stability of self-administration and extinction performance across the study, alcohol intake (g/kg), number of responses during active self-administration, and days to extinction across cycles (first to last) were analyzed with a one-way repeated measures ANOVA. Statistical analyses were conducted using SigmaPlot 13 software and the alpha level for all tests was set at $p \leq 0.05$.

## 3. Results

### 3.1. Acquisition of self-administration and cue-induced reinstatement under baseline conditions

Once subjects reliably self-administered a 10% (*w/v*) sucrose only solution, it took an average of 51.88 ± 2.17 (SEM) days after the introduction of ethanol to complete the sucrose-fading procedure (Fig. 1A). As the proportion of ethanol increased and of sucrose decreased, there was a very gradual increase in the number of days to achieve stability. At the terminal solution (i.e., 2S/15E), however, there was a significant increase in the number of days to achieve stability ($F(7, 49) = 16.502$, $p < 0.001$; Bonferroni t-tests $p < 0.001$).

A baseline cue-induced reinstatement test was conducted initially to ensure that the experimental conditions induced alcohol seeking. As shown in Fig. 1B, active lever presses during the last three sessions of self-administration prior to extinction ranged from 40.0 to 107.0 (group mean = 61.42 ± 7.6) and alcohol intake ranged from 0.6 to 1.7 g/kg (group mean = 0.90 ± 0.12). Subjects took an average of 9.63 ± 0.8 days (range = 5–12 days) to meet extinction criteria (≤ 10% of baseline responses), reaching an average number of responses per session of 3.69 ± 0.4 (range = 0–7). During the reinstatement test, responding on the active lever increased to 20.2 ± 0.7 responses/session (range = 11–26). A one-way repeated measures ANOVA indicated a significant difference for active lever presses between active self-administration, extinction, and reinstatement tests (Fig. 1B; $F(2, 14) = 45.451$, $p < 0.001$). Bonferroni's multiple-comparison test revealed that active lever presses during extinction were significantly lower compared to active self-administration (Fig. 1B, $p < 0.05$). Responding during the baseline cue-induced reinstatement test also was significantly lower compared to active self-administration (Fig. 1B, $p < 0.05$). However, responding during the reinstatement test was significantly higher compared to extinction (Fig. 1B, $p < 0.05$), indicating that the alcohol-paired cue induced significant responding during the reinstatement test session.

At the conclusion of the sleep restriction experiments, another cue-induced reinstatement test was conducted under baseline, non-sleep restricted conditions to assess the stability of the reinstatement effect. As shown in Fig. 1C, self-administration, extinction and reinstatement behavior at the end of the study was similar to that at the start of the study (i.e., Fig. 1B). A one-way repeated measures ANOVA indicated a significant difference for active lever presses between active self-administration, extinction, and reinstatement tests (Fig. 1C; $F(2, 14) = 39.152$, $p < 0.001$). Bonferroni's multiple-comparison test revealed that active lever presses during extinction were significantly lower compared to active self-administration (Fig. 1C, $p < 0.05$). Responding during the baseline cue-induced reinstatement test also was significantly lower compared to active self-administration (Fig. 1C, $p < 0.05$). However, responding during the reinstatement test was significantly higher compared to extinction (Fig. 1C, $p < 0.05$), indicating that the alcohol-paired cue continued to induce significant responding during the final reinstatement test session. Further, the level of alcohol seeking observed during the final test was not different from that observed during the initial test ($t(7) = -0.715$, $p = 0.498$).

### 3.2. Acute sleep restriction and alcohol cue-induced reinstatement

Fig. 2 shows the average lever responses during cue-induced reinstatement tests after rats experienced a sleep restriction protocol or its control condition, expressed as percentage of baseline cue-induced alcohol seeking. Panel 2A shows alcohol-seeking behavior under TSR control and test conditions; panel 2B shows alcohol-seeking behavior under REMSR control and test conditions. A one-way repeated measures ANOVA revealed a significant treatment effect for sleep conditions (F

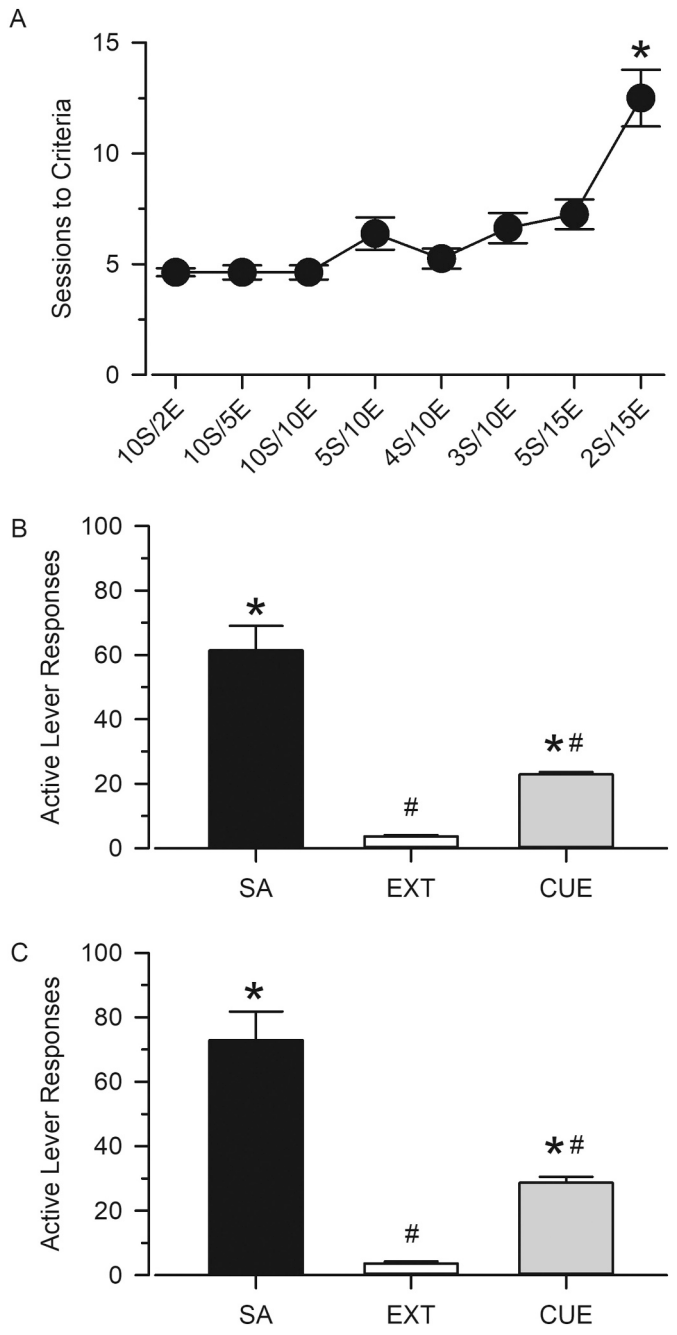


**Fig. 1.** A. Acquisition of ethanol self-administration. Data are mean ± SEM number of sessions to reach criteria at each step of the sucrose fade procedure in $N = 8$. *indicates $p < 0.05$ compared to all other steps. B. Active lever responses during self-administration (last three days of self-administration preceding extinction; "SA"), extinction (last 2 days; "EXT"), and initial cue-induced reinstatement test ("CUE"). C. Active lever responses during self-administration (last three days of self-administration preceding extinction; "SA"), extinction (last 2 days; "EXT"), and final cue-induced reinstatement test ("CUE"). Data are mean + SEM active lever responses in N = 8. * indicates p < 0.05 compared to extinction, # indicates p < 0.05 compared to self-administration.

(3,21) = 4.116, $p < 0.05$). Bonferroni multiple comparisons tests indicated that active lever responding engendered by the alcohol-paired cue was significantly increased after TSR conditions compared to TSR-Control conditions (Fig. 2A; $p < 0.05$). Specifically, active lever responding after TSR was approximately 140% of baseline cue-induced reinstatement levels, compared to only 93% of baseline levels after TSR-Control conditions. In contrast, REMSR did not augment alcohol cue-induced seeking behavior compared to control (Fig. 2B). That is, active lever responses during reinstatement following REMSR condition did not differ significantly when compared to the REMSR-Control condition. Finally, during all reinstatement tests following sleep restriction conditions, inactive lever responding remained low (i.e., 0–13% of baseline reinstatement) and did not differ as a function of condition (F(3, 21) = 1.964, n.s.).

### 3.3. Stability of self-administration and extinction behavior

Subjects underwent six cycles of self-administration, extinction and reinstatement testing across the course of this study. Table 1 shows alcohol intake, number of self-administration responses, and number of days to extinction for each cycle. With respect to alcohol intake, a one-way repeated measures ANOVA revealed stable levels of alcohol drinking across the study (F(5, 35) = 1.051, n.s.). Some variability was evident in the number of responses during self-administration, but the differences did not reach significance (F(5, 35) = 2.066, n.s.). Finally, the number of days required to meet the extinction criterion did change significantly with cycle (F(5, 35) = 2.613, $p < 0.05$). However, no post hoc comparison was significant.

## 4. Discussion

The high prevalence of sleep impairment in AUD (Brower, 2015; Miller et al., 2017), as well as reports suggesting restricted or disrupted sleep as a predictor of relapse (Skoloda et al., 1979; Brower et al., 1998; Foster and Peters, 1999; Conroy et al., 2006), indicate an important role for sleep in AUD. However, no study to date has directly demonstrated that sleep impairment induces relapse to alcohol seeking. Sleep deprivation studies in humans are complex, particularly in the broader context of AUD, and sleep in humans can be influenced by a variety of confounding factors also related to AUD and/or abstinence, such as stress, irregular work schedules and poor sleep habits (Kalmbach et al., 2018). Preclinical studies are useful because investigators can reduce the influence of confounding variables and systematically evaluate the effects of sleep restriction on different abuse-related effects of alcohol. Here, we investigated the effects of acute total sleep (SWS + REM) restriction and REM sleep only restriction in a rat model of alcohol relapse.

In our study, subjects were trained to respond in order to obtain delivery of an alcohol solution under conditions in which every alcohol delivery was paired with a flashing light cue. Following establishment of self-administration and subsequent extinction of responding, alcohol-seeking behavior was reinstated by the reintroduction of the alcohol-paired cues (stimulus light, pump motor noise, alcohol olfactory stimulus). Importantly, cue-induced alcohol seeking remained stable across the course of the study. The reinstatement model of alcohol seeking mimics conditions that are reported to increase subjective reports of craving and trigger relapse in AUD patients (Epstein et al., 2006; Marchant et al., 2019). Acute drug re-exposure and exposure to drug-associated cues are key contributors to relapse in humans (Childress et al., 1993; De Wit, 1996; Grüsser et al., 2004). Although not explicitly studied here, stress also is known to induce relapse in humans (Blaine and Sinha, 2017), and stress-induced reinstatement has been repeatedly demonstrated in rodent alcohol self-administration studies (for review, see Mantsch et al., 2016). In the present study, we use the reinstatement model to investigate the potential influence of acute sleep restriction on alcohol-seeking behavior induced by alcohol-paired environmental cues.

Our findings show that 6 h of total sleep (SWS + REM sleep) restriction during the second half of the inactive (light) phase led to a significant increase in alcohol seeking behavior. This result is specific to the sleep restriction protocol and cannot be attributed to differences in self-administration (alcohol intake or responding) or length of extinction period, as these factors remained relatively stable across the course

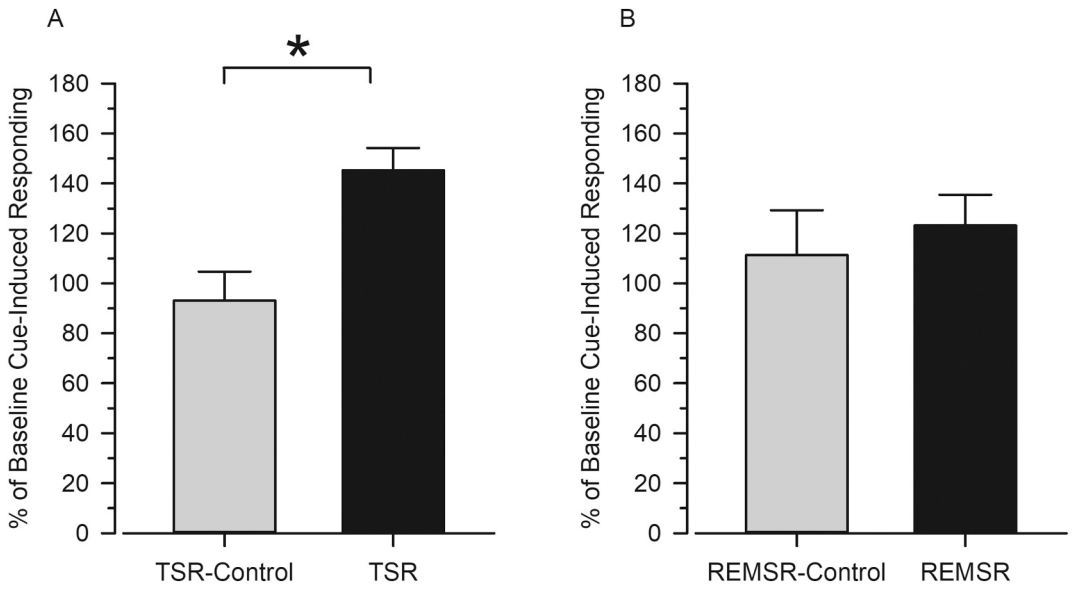


**Fig. 2.** Modulation of cue-induced reinstatement by sleep restriction conditions: A. Total sleep restriction (TSR) and B. REM sleep restriction (REMSR). Data are mean + SEM of active lever responses expressed as a percentage of baseline cue-induced responding in N = 8. * indicates $p < 0.05$ compared to TSR-Control.

**Table 1**
Alcohol intake, number of self-administration responses, and days to extinction across the five reinstatement cycles. Data presented as mean (SEM) in N = 8 rats.

| Variable | Cycle | | | | | | *P* value |
|---|---|---|---|---|---|---|---|
| | First[a] | Second[b] | Third[b] | Fourth[c] | Fifth[c] | Sixth[d] | |
| Intake (g/kg) | 0.90 (0.12) | 0.67 (0.12) | 0.83 (0.10) | 0.82 (0.08) | 0.80 (0.07) | 0.97 (0.16) | 0.404 |
| No. of self-administration responses | 61.42 (7.56) | 54.00 (3.19) | 76.59 (6.36) | 76.29 (7.00) | 68.96 (5.72) | 72.88 (8.91) | 0.093 |
| Sessions to extinction | 9.63 (0.75) | 8.75 (1.01) | 8.38 (1.58) | 6.75 (0.98) | 5.38 (0.65) | 7.00 (1.09) | 0.041* |

[a] Data associated with initial, "baseline" reinstatement cycle.
[b] Data associated with total sleep restriction and total sleep control test cycles.
[c] Data associated with REM sleep restriction and REM sleep control test cycles.
[d] Data associated with final, "baseline" reinstatement cycle.
* $P < 0.05$; one-way repeated measures ANOVA.

of the entire study. Further, this result is not a function of nonspecific increases in responding, as significant increases in responding were evident only on the active lever, and not the inactive lever. Our sleep restriction protocol aimed to mimic, in part, aspects of the sleep conditions reported in dependent AUD patients during abstinence. For example, recently sober AUD patients (30 days of abstinence) display several deficits in sleep measures including a specific decrease in SWS (Feige et al., 2007; Irwin et al., 2016; Singh et al., 2018). Additionally, the reported changes in total sleep time and SWS during abstinence vary between different studies, but tend to be mild (for review, see Koob and Colrain, 2020). For these reasons, we opted for a 6-h sleep restriction protocol (compared to a more typical 12–24-h sleep deprivation protocol; e.g. Franken et al., 1991; Saré et al., 2016) that would be predicted to result in restriction of SWS (cf. Oonk et al., 2016). The sleep restriction ended immediately before a reinstatement test session in order to replicate disrupted sleep conditions that would immediately precede a relapse episode. It is important to note that rodents show a sleep-wake cycle in many respects distinct from that of humans. While humans show a single phase of nocturnal sleep, rodents show several sleep cycles throughout the 24-h day and concentrate most of their sleep during the light phase, spending nearly 60% of the inactive phase in SWS (Clancy et al., 1978). Therefore, our sleep restriction protocol still allowed animals to sleep during the first half of the inactive phase. Our finding showing that a relatively short period of total sleep restriction in rodents can increase alcohol-seeking behavior induced by alcohol-paired cues corroborates human studies indicating sleep impairment as a predictor of relapse in AUD and, further, implies that even mild restriction should be considered a risk factor.

In contrast to results from rats undergoing the total sleep restriction protocol, results from rats subjected to acute REM sleep restriction show that alcohol-seeking behavior induced by alcohol-paired cues was not increased. Although the current study did not independently verify with EEG technology that the flower pot method specifically reduced REM sleep, previous studies have shown evidence supporting this selectivity (e.g., Mendelson et al., 1974; Porkka-Heiskanen et al., 1995). Therefore, our findings suggest that restriction of primarily REM sleep is not sufficient to explain the results obtained with total sleep restriction, and that significant SWS restriction may be either necessary or sufficient to augment cue-induced reinstatement of alcohol seeking. These observations are relevant in the context of AUD, considering that patients in prolonged abstinence tend to display decreased SWS while showing only slight changes in REM sleep (see review: Koob and Colrain, 2020). Because alcohol consumption is known to decrease REM sleep (Gross et al., 1973; Gross and Hastey, 1975; Lester et al., 1975), slight increases in REM sleep during abstinence are likely to represent extended REM sleep rebound after chronic alcohol use. Our findings suggest that a significant decrease in SWS, specifically, may be the key feature mediating sleep impairment-induced risk for relapse to alcohol seeking and, further, may be the more critical change in sleep architecture contributing to the bidirectional relationship between sleep impairment and AUD.

Acute total sleep restriction also has been shown to potentiate the

abuse-related behavioral effects of other drugs of abuse in rodents (Berro et al., 2014a, 2014b, 2018; Saito et al., 2014). These studies suggest that total sleep loss contributes to drug addiction specifically via manipulations in the conditioned component of substance abuse. For instance, in mice, sleep restriction precipitates the development of amphetamine-induced conditioned place preference (Berro et al., 2018), impairs the extinction of cocaine-induced environmental conditioning (Berro et al., 2014a) and only potentiates amphetamine-induced behavioral sensitization if the drug is administered in the test environment (but not in the home-cage) (Frussa-Filho et al., 2004). Drug-environment conditioning has long been proposed to be mediated by the mesolimbic dopamine reward system (Kalivas, 2002), and sleep deprivation amplifies the reactivity of brain reward networks in humans (Gujar et al., 2011) via disinhibition of mesolimbic dopaminergic networks (Volkow et al., 2008, 2009). Importantly, microdialysis studies also show increased dopamine levels in the nucleus accumbens during and immediately following 6 h of total sleep restriction in rats (Zant et al., 2011). Therefore, our findings showing that total sleep restriction also potentiates alcohol seeking in a cue-induced reinstatement model further corroborate the hypothesis that the conditioned component of drug abuse seems to be mediated by total sleep deprivation.

In summary, our findings show that significant SWS restriction is necessary and/or sufficient for the augmentation of cue-induced reinstatement of alcohol seeking by acute sleep restriction and suggest that decreased SWS during AUD abstinence (Feige et al., 2007; Irwin et al., 2016; Singh et al., 2018) places individuals at a unique risk for relapse. Future preclinical studies could investigate sleep parameters in rodents during extinction following chronic alcohol self-administration to investigate whether decreased SWS is observed in alcohol dependent rats as it is in abstinent AUD patients and whether the decrease correlates with cue-induced reinstatement responses. Our findings suggest that this would indeed be the case. Further, our model could be a useful tool to investigate the neurobiological underpinnings of this phenomenon. Finally, our results suggest that future treatment strategies should focus on SWS promotion as an adjunctive treatment for AUD and relapse prevention. Note that benzodiazepines-type drugs, the most commonly prescribed sleep aids, are known to decrease SWS in healthy human volunteers (Arbon et al., 2015) and, for that reason, may not be useful treatments in AUD patients.

## Declaration of competing interest

The authors have no competing interests to disclose.

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