

Effects of circadian disruption on methamphetamine consumption in methamphetamine-exposed rats

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Abstract

Rationale A substantial number of clinical studies indicate associations between sleep abnormalities and drug abuse; however, the role played by the circadian system in the development of addiction is largely unknown.

Objective The aim of this study was to examine the effects of experimentally induced chronic jet lag on methamphetamine consumption in a rat model of methamphetamine drinking.

Methods Male Sprague–Dawley rats ($n=32$) were housed in running wheel cages in a 12:12 h light:dark cycle. One group of rats ($n=16$) was given 2 weeks of forced methamphetamine consumption (0.01 % in drinking water; meth pre-exposed) while a second group ($n=16$, not pre-exposed) received water only. This was followed by a 2-week abstinence period during which half of the animals from each group were exposed to four consecutive 6-h advancing phase shifts of the light:dark cycle, while the other half remained on the original light:dark cycle. Methamphetamine consumption was assessed in all rats following the deprivation period using a two-bottle choice paradigm.

Results Methamphetamine consumption was initially lower in methamphetamine pre-exposed versus not pre-exposed rats. However, during the second week following abstinence, consumption was significantly higher in phase-shifted rats of the methamphetamine pre-exposed group compared to all other groups.

Conclusions These data reveal an effect of circadian rhythm disturbance on methamphetamine consumption and suggest that dysregulation of the circadian system be considered in the etiology of relapse and addiction.

Keywords Addiction · Circadian · Jet lag · Methamphetamine · Relapse · Reward

Introduction

The circadian timing system maintains synchrony among an organism's 24-h behavioral and physiological rhythms, including the sleep/wake cycle, and the external day. In humans, clinical studies have shown that disruption of normal circadian rhythmicity can adversely affect health. Exposure to non-traditional work schedules such as night or rotating shifts, and frequent international travel by airline crews, have been linked to increased risk of various types of cancers (Davis et al. 2001; Kubo et al. 2006; Schernhammer et al. 2003), as well as obesity and diabetes (Karlsson et al. 2001; Morikawa et al. 2005), stroke (Virtanen and Notkola 2002), and heart attack (Haupt et al. 2008; Knutsson et al. 1999). The association between sleep disruption and substance use has also recently become evident. Alcohol and drug abuse is more prevalent in patients with sleep complaints than in the general population (Teplin et al. 2006), and up to 50 % of recovering alcoholics report sleep problems prior to alcohol dependence onset (Currie et al. 2003). In addition, sleep disruptions such as insomnia have been found to precipitate relapse to addiction (Brower et al. 2001; Pace-Schott et al. 2005). In fact, Brower et al. (2001) found that the presence of insomnia was the most significant factor for predicting relapse in alcoholic patients entering treatment. Two studies also implicate abnormal circadian function as a vulnerability factor for addiction: increased alcohol consumption has been reported in international business travelers experiencing jet lag, a circadian rhythm sleep disorder (Rogers and Reilly 2002), and nurses working rotating shifts and night shifts show increased

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smoking, alcohol, and other drug use compared to those working normal schedules (Trinkoff and Storr 1998).

Despite these studies correlating sleep disruption and substance use, there is a lack of data directly examining the effects of systematic manipulation of the circadian system. Results from preclinical studies, however, suggest that the circadian system may play a critical role in drug addiction. For example, several studies have demonstrated that animals with circadian clock gene mutations show altered responses to drugs of abuse. Increases in cocaine sensitization, conditioned place preference, and self-administration have been observed in *Clock* and *Per2* mutant mice (Abarca et al. 2002; McClung et al. 2005; Ozburn et al. 2012), and *Per2* mutant mice show increased alcohol consumption compared to wild-type mice (Spanagel et al. 2005). In contrast, loss of *Per1* has been shown to result in loss of cocaine sensitization (Abarca et al. 2002). Furthermore, the existence of daily and circadian rhythms of cocaine self-administration in rats (Baird and Gauvin 2000; Bass et al. 2010; Roberts et al. 2002), the effects of suprachiasmatic nucleus (SCN) lesions on cocaine-seeking behavior in rats (Sleipness et al. 2007), and the prevalence of daily variation in the sensitivity to many drugs of abuse (for review, see Falcon and McClung 2009) argue for clock control of reward behaviors. The transition to short photoperiod (i.e., short day length) has been found to reduce cocaine-induced reinstatement of conditioned place preference in rats (Sorg et al. 2011). Several other studies, also conducted in rats, have examined the effects of constant darkness, constant light, and photoperiod on ethanol consumption (Burke and Kramer 1974; Geller 1971; Sinclair and Geller 1972), and Gauvin et al. (1997) reported increases in ethanol intake in response to phase shifts of the light cycle. However, although a considerable literature exists in the circadian field on the effect of methamphetamine in revealing a SCN-independent oscillator in rats and mice (i.e., the methamphetamine-sensitive circadian oscillator or MASCO; for review, see Honma and Honma 2009), the effect of circadian disruption on methamphetamine consumption has not been tested.

In this study, we disrupted the circadian system using a chronic jet lag (CJL) model in which animals are exposed to light cycles that are shifted every 4 days to mimic a 6-h eastward time zone shift. The CJL model has been widely used to reproduce the effects of shift work and jet lag (Davidson et al. 2006; Filipowski et al. 2004; Penev et al. 1998; Preuss et al. 2008). Rhythms of clock gene expression in the SCN and other tissues are altered or abolished in response to CJL (Castanon-Cervantes et al. 2010), indicating that CJL is an effective circadian disrupter. Moreover, CJL is advantageous in that, unlike bright constant light, another light manipulation frequently used to produce circadian arrhythmicity, CJL has only minimum effects on sleep, and does not induce other hormonal or behavioral measures of stress (Castanon-Cervantes et al. 2010; Iwamoto et al. 2014; Sei et al. 2003). Here, we

examine the effect of chronic jet lag in methamphetamine-treated rats on resumption of methamphetamine drinking after abstinence. We test the hypothesis that animals exposed to CJL will show an increase in methamphetamine consumption following abstinence compared to non-shifted rats.

Methods and materials

Animals

Three-month-old adult male Sprague–Dawley rats ($n=32$) purchased from Charles River Labs and weighing 360–420 g were used in this study. Rats were used based on their history of use in circadian studies of oral methamphetamine consumption, and the Sprague–Dawley strain was chosen based on the use of this strain in previous work examining effects of phase shifting and manipulation of light cycles on ethanol consumption. Rats were divided into four groups ($n=8$ rats per group), and were housed in individual ($20.3 \times 25.4 \times 45.7$ cm) running wheel cages on corn cob bedding in light-tight, temperature- and humidity-controlled boxes (21 °C/ 50 % relative humidity) and maintained on a 12:12 h light:dark cycle (lights on at 0500, off at 1700; mean light intensity $40 \mu\text{W}/\text{cm}^2$). Animals had unrestricted access to food (Harlan Teklad 8664 rodent diet) and water (or methamphetamine-water) throughout the study. Locomotor activity was measured continuously as the number of wheel revolutions recorded in 1-min bins, and was analyzed using Clocklab software (Actimetrics, Wilmette, IL). Food, water, and methamphetamine intake were measured daily by weighing of bottles and food. Body weights were obtained two times per week throughout the study. All procedures were approved by the University of Virginia Animal Care and Use Committee and are in compliance with the NIH Guide for the Care and Use of Laboratory Animals.

Drugs

Methamphetamine hydrochloride (Sigma-Aldrich, St. Louis, MO) was dissolved to a concentration of 100 mg/L in tap water. The concentration of methamphetamine in water (0.01 %) was held constant across all subjects. This concentration was selected based on its history of use in circadian studies (Honma et al. 1989; Ruis et al. 1990; Tataroglu et al. 2006), where it has been shown to induce robust behavioral effects on activity without affecting neuronal activity rhythms in the SCN (Moriya et al. 1996) or rhythms of clock gene expression in the SCN (Masubuchi et al. 2000). Methamphetamine solutions were made fresh weekly and were delivered in bottles identical to those containing water.

Experimental procedure

Figure 1 depicts the experimental timeline and groups used in this study. After 1 week of baseline recording of feeding, drinking, and activity in all rats ($n=32$), half of the animals (groups 1 and 2) underwent 2 weeks of forced methamphetamine consumption during which 0.01 % methamphetamine was added to the drinking water bottle to induce dependence. The other half of the animals (groups 3 and 4) received water only. A 2-week abstinence period followed, during which half of the methamphetamine pre-exposed and half of the not pre-exposed rats (groups 1 and 3) were exposed to four consecutive phase shifts of the 12:12 h light:dark cycle, such that a 6-h advance was effected on day 1 of abstinence, and every 4 days thereafter (days 5, 9, and 13 of abstinence). The fourth phase shift, which occurred 2 days before the end of the abstinence period, returned the shifted animals to the original light:dark cycle. A 2-week abstinence period was selected based on previous work demonstrating that the neuroadaptations leading to the development of an addicted phenotype, i.e., the enhanced motivation to obtain drug following abstinence in animals with extended versus short exposure, develop over a 10–14-day abstinence period (Roberts et al. 2007; Ramôa et al. 2014). We hypothesized that circadian disruption during this critical period might disrupt these neuroadaptations and lead to changes in subsequent methamphetamine consumption. At the end of this abstinence period, consumption of methamphetamine was measured in all animals for 2 weeks using a two-bottle choice paradigm. Each animal was presented with two bottles, one containing water and one containing 0.01 % methamphetamine in water. The concentration of methamphetamine used for two-bottle choice testing was the same as was used for forced consumption in order to facilitate direct comparisons between forced and free consumption. Bottle positions were switched two times per week to control for position preferences. Sipper tubes were used that contained ball bearings at the tip to prevent leakage. In our preliminary testing, these bottles dripped less

than 0.75 ml per day. All procedures in the dark were carried out with the aid of infrared viewers.

Withdrawal scoring

In order to establish that methamphetamine dependence had been induced by the forced access procedure, animals were screened for behavioral signs of withdrawal. Behavioral observations were conducted at 12, 24, 36, 48, 60, and 72 h following the removal of methamphetamine. Each rat was observed in its cage for 15 min by two independent observers, blind to the animals' treatment. Inter-observer scores did not differ by more than 15 % and thus were averaged. Observers counted occurrences of withdrawal signs utilizing a checklist adapted from a model of cocaine withdrawal scoring developed by Malin et al. (2000) for quantitating spontaneously emitted behaviors following termination of exposure to drug. Withdrawal signs included ptosis, gasps/body writhes, teeth chatter/chews, head shakes/tremors, and less frequent signs such as scratches and genital licks/ejaculations. The presence of rhinorrhea, epiphora, piloerection, and abnormal posture (hunching) were counted no more than once per 5 min. Other signs were counted at each occurrence. Each rat's overall withdrawal score was recorded as the total number of withdrawal signs summed across all categories.

Data analyses

Periods of activity rhythms were determined by Chi-square periodogram analysis using Clocklab software, with a significance level of $p=0.01$. The number of days to reach stable entrainment was calculated by counting the number of days of transients of the activity rhythm following the final shift of the light:dark cycle and preceding entrainment of the rhythm to a 24-h period for at least 5 days. Student's t test was used to compare group means. Methamphetamine consumption in milligrams per kilogram was calculated by using

Group 1 Meth pre-exposed Shift	Baseline	Forced Methamphetamine Consumption	Abstinence				2-Bottle Choice
	1 Week		2 Weeks	Shift 1	Shift 2	Shift 3	
Group 2 Meth pre-exposed No-Shift	Baseline	Forced Methamphetamine Consumption	Abstinence				2-Bottle Choice
	1 Week		2 Weeks	No Phase Shifting			
Group 3 Not pre-exposed Shift	Baseline	Water	Abstinence				2-Bottle Choice
	1 Week		2 Weeks	Shift 1	Shift 2	Shift 3	
Group 4 Not pre-exposed No-Shift	Baseline	Water	Abstinence				2-Bottle Choice
	1 Week		2 Weeks	No Phase Shifting			

Fig. 1 Timeline of experimental procedures. After 1 week of baseline recording of feeding, drinking, and activity, two groups of rats (1 and 2) underwent 2 weeks of forced methamphetamine consumption, while two groups (3 and 4) received water only. This was followed by a 2-week abstinence period, during which four discrete phase shifts of the light:dark

cycle were administered to half of the methamphetamine pre-exposed and not pre-exposed rats (groups 1 and 3) while the other half (groups 3 and 4) remained on the original light:dark cycle. All groups underwent two-bottle choice testing for 2 weeks following abstinence

methamphetamine consumed and body weights. Preference was calculated for two-bottle choice consumption by dividing the volume of methamphetamine-containing solution consumed by total fluid intake. The not pre-exposed no-shift group was used as a comparison group to determine the effects of chronic jet lag with percent change calculated by dividing the difference between the experimental and control groups by control values and multiplying by 100. Data were analyzed using the Statistical Package for Social Sciences (SPSS, Version 20). Repeated measures ANOVA was used to determine group differences in withdrawal signs, body weight, consumption, and preference, with exposure (methamphetamine pre-exposed, not pre-exposed) and shift (shift, no-shift) as between-subjects factor and days as the within-subjects factor. Following a significant three-way interaction of day x methamphetamine pre-exposure x shift, or a significant two-way interaction of day x methamphetamine pre-exposure or day x shift, data were collapsed on exposure and/or shift and further examined using repeated measures ANOVA. Subsequent group comparisons across days were made using univariate ANOVA. Univariate ANOVA was also used for percent change from baseline analyses, with the not pre-exposed no-shift group used as the control and set to 0. When appropriate, post hoc comparisons were conducted using a Bonferroni test. Statistical significance was set at $p < 0.05$.

Results

Behavioral and physiological effects of forced methamphetamine consumption

Forced methamphetamine consumption

Figure 2a illustrates mean (\pm SEM) methamphetamine intake (mg/kg) during the 14-day period of forced consumption. As expected, methamphetamine intake escalated over the 14-day

period, with repeated measures ANOVA revealing a significant main effect of day [$F(13,182)=7.02, p < 0.001$]. Importantly, there was no significant difference in methamphetamine intake in the groups designated to receive the shift or no-shift manipulation during abstinence nor was there a day by shift interaction, indicating that methamphetamine consumption was equivalent between the two methamphetamine pre-exposed groups during the period of forced consumption prior to abstinence and phase-shifting.

Effects on body weight

Body weights, measured approximately twice a week over the course of the 7-week long experiment, are shown in Fig. 2b. Repeated measures ANOVA revealed significant overall effects of day [$F(14,392)=185.44, p < 0.001$], and methamphetamine pre-exposure [$F(1,28)=4.59, p < 0.05$], and a significant interaction of day by methamphetamine pre-exposure [$F(14,392)=11.62, p < 0.001$]. No overall or interactive effects of shift were observed (p 's > 0.05), and thus subsequent analysis of the effect of methamphetamine pre-exposure on body weight was examined across days collapsed across shift. This analysis revealed significant overall effects of day [$F(14,420)=189.53, p < 0.001$] and methamphetamine pre-exposure [$F(1,30)=10,641.15, p < 0.05$], and a significant interaction of day by methamphetamine pre-exposure [$F(14,420)=11.85, p < 0.001$]. Further analysis of differences between methamphetamine pre-exposed groups on each day using univariate ANOVA revealed significant differences between methamphetamine pre-exposed and not pre-exposed groups during weighing sessions 5 through 8 (sessions 5, 6, and 7, p 's < 0.001 ; session 8, $p < 0.01$), with trends for a significant difference for sessions 9 through 11 (session 9 and 10, $p < 0.1$; session 11, $p = 0.06$). Thus, during the period of forced methamphetamine consumption (i.e., weighing sessions 5 through 8), body weights of the methamphetamine pre-exposed animals were significantly lower than those of the

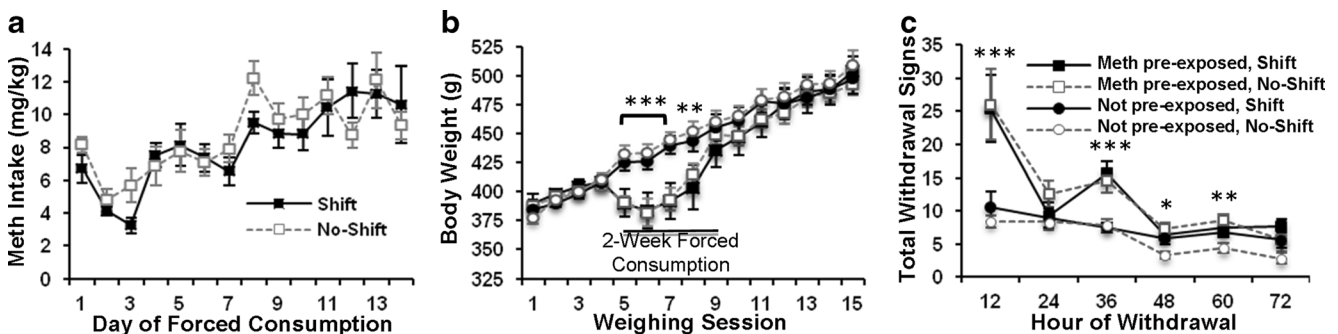


Fig. 2 Physiological and behavioral responses to forced methamphetamine consumption and abstinence. **a** Mean (\pm SEM) daily methamphetamine intake during the 2-week period of forced methamphetamine consumption prior to abstinence, showing escalation of intake. **b** Mean (\pm SEM) body weights, measured every 3–4 days over the course

of the 6-week long experiment. $n=8$ animals/group. **c** Mean (\pm SEM) overall withdrawal signs, recorded every 12 h for 72 h following removal of methamphetamine bottles. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, indicate significant differences between methamphetamine pre-exposed and not pre-exposed groups

not pre-exposed controls, demonstrating a clear physiological effect of the drug. At the end of the forced consumption period, body weights of the methamphetamine pre-exposed animals rapidly rebounded and did not differ from those of controls for the remainder of the experiment.

Withdrawal

In order to examine signs of physical dependence induced by the forced consumption procedure, we scored withdrawal behaviors every 12 h for 72 h after removal of methamphetamine bottles (Fig. 2c). Repeated measures ANOVA revealed significant overall effects of time [$F(5140)=24.48, p<0.001$] and methamphetamine pre-exposure [$F(1,28)=35.94, p<0.001$], and a significant interaction of time by methamphetamine pre-exposure [$F(5140)=8.68, p<0.001$]. No overall or interactive effects of shift were observed (p 's >0.05), and thus subsequent analysis of the effect of methamphetamine pre-exposure on withdrawal behaviors was examined over time collapsed across shift. Results from the repeated measures ANOVA revealed significant overall effects of time [$F(5150)=25.61, p<0.001$], and methamphetamine pre-exposure [$F(1,30)=36.76, p<0.001$], and a significant interaction of time by methamphetamine pre-exposure [$F(5150)=9.07, p<0.001$]. Further analyses of differences between methamphetamine pre-exposed groups at each withdrawal time using univariate ANOVA revealed significant differences between methamphetamine pre-exposed and not pre-exposed groups at both 12 h ($p<0.001$) and 36 h ($p<0.05$) after removal of methamphetamine bottles. However, at the 24-h time point, withdrawal signs in the methamphetamine pre-exposed groups did not differ from the not pre-exposed

groups. The number of withdrawal signs in the methamphetamine pre-exposed groups remained higher than in the not pre-exposed groups at 48 h ($p<0.05$) and 60 h ($p<0.01$), but did not differ from the not pre-exposed groups by 72 h after removal of methamphetamine bottles.

Locomotor activity

Activity records from one rat of each experimental group, and showing median data records in order to best represent the most common characteristic of each group, are shown in Fig. 3. In rats of groups 1 and 2, methamphetamine treatment during the 2-week period of forced consumption led to changes in the daily activity rhythm that are typical for rodents treated with this moderate-to-high dose of methamphetamine (Ruis et al. 1990; Tataroglu et al. 2006; Honma et al. 1986). In particular, significant increases in the period length of the daily activity rhythm were observed in 13 out of 16 of the animals (Table 1), with 11 out of these 16 demonstrating two activity components; a component that remained synchronized with the light:dark cycle (i.e., the light-entrained SCN circadian oscillator), and another component free-running with a long period similar to that reported for the methamphetamine-sensitive circadian oscillator (Tataroglu et al. 2006). Such variability is to be expected due to interactions between competing oscillators. In contrast, activity rhythms of not pre-exposed control rats given water during this same 2-week period (groups 3 and 4) remained stably entrained by the light:dark cycle, with periods close to 24 h, and activity largely restricted to the dark phase. Upon removal of methamphetamine, the period of the activity rhythm returned to 24 h within 2–3 days in animals that were not

Fig. 3 Running wheel activity records of one representative animal from each experimental group. Records are double-plotted so that each horizontal line represents 48 h, and successive days are plotted below and to the right of the preceding day. The main light:dark cycle (lights on from 0500 to 1700) is represented by black and white bars above the top two records. For animals that received phase shifts, the advance of the onset of the light phase for each shift is highlighted in red within the records

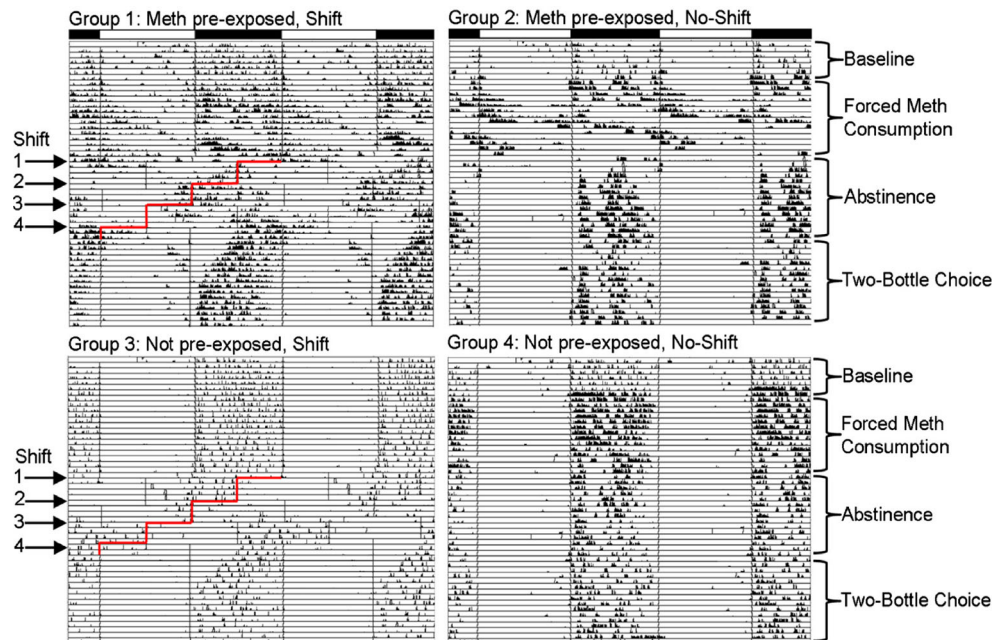


Table 1 Mean circadian period (\pm SEM) as determined by chi-square periodogram analysis (significance level $p=0.01$) for each experimental group during different phases of the experiment

	Baseline	Meth pre-exposure		Chronic Jet Lag (CJL)		Transients	Post-Transient
Meth CJL	24.00 \pm 0.01 ($n=8/8$)	24.07 ($n=1/8$)	L: none	S: 23.38 \pm 0.70* ($n=2/8$)	L: 24.42 \pm 0.17* ($n=2/8$)	23.67 \pm 0.26 ($n=8/8$)	23.98 \pm 0.01 ($n=8/8$)
		~24: 23.99 \pm 0.03 L: 26.67 \pm 0.31* ($n=7/8$)		S: 22.76 \pm 0.39* L: 25.15 \pm 0.26* ($n=4/8$)			
Meth No CJL	23.99 \pm 0.01 ($n=8/8$)	24.15 \pm 0.15 ($n=2/8$)	L: 24.67 \pm 0.08* ($n=2/8$)	23.97 \pm 0.02 ($n=8/8$)	n/a	n/a	23.98 \pm 0.01 ($n=8/8$)
		~24: 24.07 \pm 0.07 L: 27.81 \pm 1.11* ($n=4/8$)					
Water CJL	24.01 \pm 0.01 ($n=8/8$)	23.97 \pm 0.01 ($n=8/8$)	n/a	S: 23.10 \pm 0.06* ($n=3/8$)	L: none	23.36 \pm 0.21 ($n=8/8$)	24.01 \pm 0.01 ($n=8/8$)
				S: 23.04 \pm 0.19* L: 25.12 \pm 0.07* ($n=5/8$)			
Water No CJL	23.99 \pm 0.01 ($n=8/8$)	24.01 \pm 0.01 ($n=8/8$)	n/a	24.00 \pm 0.01 ($n=8/8$)	n/a	n/a	24.00 \pm 0.00 ($n=8/8$)

In the two groups of animals pre-exposed to methamphetamine (meth pre-exposure), most rats displayed activity rhythms during methamphetamine pre-exposure that were split into two distinct components; a 24-h component (~24:) and a long period (L:) component. The other animals displayed either a 24-h period or a long period. Likewise, in the two groups exposed to chronic jet lag (CJL), activity rhythms were often split into two components; a short period component (S:) and a long period component, while the remaining animals displayed either a short period or long period rhythms. Values under the transients heading show free-running circadian period during the time following the chronic jet lag treatment when the activity rhythm is in the process of entraining to the final light:dark cycle. Values under post-transient show the activity rhythm period following stable entrainment to the final light:dark cycle. Asterisk indicates significant difference from baseline period, Student's t test, $p<0.05$

phase shifted. In animals subjected to chronic jet lag (groups 1 and 3), activity rhythms either advanced in the direction of the light:dark cycle shift ($n=5$ of 16) or delayed in the opposite direction ($n=2$ of 16), as evidenced by either short or long period rhythms, respectively (Table 1), and rhythms in 9 of 16 animals split into both a short and a long period component, but in all cases failed to entrain to the 24-h period of the light:dark cycles at any point. Following the final shift of the light:dark cycle, the number of days required to achieve stable entrainment (i.e., the number of days of transients in the activity rhythm) did not differ between methamphetamine pre-exposed (9.6 ± 0.98) and not pre-exposed (7.8 ± 0.76) rats.

Two-bottle choice testing

The effects of chronic phase shifting on methamphetamine consumption and preference were tested in all groups using a two-bottle choice paradigm (Fig. 4). For consumption, results from the repeated measures ANOVA (for consumption) revealed significant effects of day [$F(13,364)=28.09$, $p<0.001$], methamphetamine pre-exposure [$F(1,28)=3.39$, $p<0.05$], day by methamphetamine pre-exposure [$F(39,364)=5.83$, $p<0.001$], and day by shift [$F(13,364)=2.43$, $p<0.01$]. Following these significant interactions of day by methamphetamine pre-exposure and day by shift, data were collapsed on either methamphetamine pre-exposure or shift and further examined. Repeated measures ANOVA

examining effects of pre-exposure on methamphetamine consumption revealed significant overall effects of day [$F(13,390)=26.54$, $p<0.001$], and methamphetamine pre-exposure [$F(1,30)=3.31$, $p<0.05$], and a significant interaction of day by pre-exposure [$F(13,390)=5.50$, $p<0.001$]. Subsequent univariate analysis of the effect of methamphetamine pre-exposure on each of the days revealed significant differences between the pre-exposed and not pre-exposed groups on days 1 ($p<0.001$), 3 ($p<0.01$), 5 ($p<0.001$), 8 ($p<0.01$), 10 ($p<0.05$), 11 ($p<0.01$), and 13 ($p<0.05$). Repeated measures ANOVA examining effects of shift on consumption revealed significant effects of day [$F(13,390)=23.98$, $p<0.001$] and day by shift [$F(13,390)=2.071$, $p<0.05$]. Subsequent analysis of differences between shift groups across days using univariate ANOVA revealed significant differences between shift and no-shift groups on days 9 ($p<0.001$), 11 ($p<0.05$), and 12 ($p=0.01$).

For percent preference (Fig. 4b), repeated measures ANOVA revealed significant overall effects of day [$F(13,364)=13.12$, $p<0.001$] and shift [$F(1,28)=4.39$, $p<0.05$], and a significant interaction of day by methamphetamine pre-exposure [$F(13,364)=6.12$, $p<0.001$]. Following a significant interaction of day by methamphetamine pre-exposure, data were collapsed on shift, and the effect of methamphetamine pre-exposure on preference was examined. ANOVA revealed significant effects of day [$F(13,390)=13.18$, $p<0.001$] and day by methamphetamine pre-exposure [$F(13,290)=6.15$, $p<0.001$]. Further univariate analyses of the

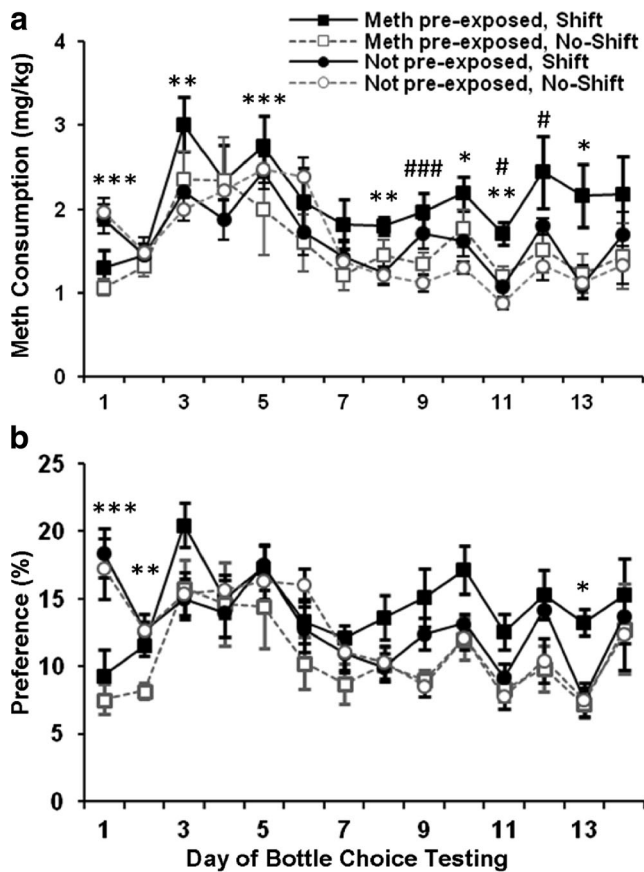


Fig. 4 Mean (\pm SEM) daily methamphetamine intake (a) and daily percent preference (b) over 14 days of two-bottle choice testing. $n=8$ animals/group. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ indicate significant differences between methamphetamine pre-exposed and not pre-exposed groups. # $p<0.05$, ### $p<0.001$ indicate significant differences between shift and no-shift groups

effect of methamphetamine pre-exposure on each of the days revealed significant differences between methamphetamine pre-exposed and not pre-exposed groups on days 1 ($p<0.001$), 2 ($p<0.01$), and 13 ($p<0.05$). Thus, both methamphetamine pre-exposure and phase shifting during abstinence had significant effects on subsequent methamphetamine consumption. Percent preference measures, however, were affected only by methamphetamine pre-exposure.

In order to further examine the effects of day on consumption in the different groups over the 2-week two-bottle choice period, and to further explore a priori hypothesized differences in the effect of circadian disruption in methamphetamine pre-exposed versus not pre-exposed rats, the data were plotted as percent change in methamphetamine consumption from not pre-exposed no-shift controls on day 1, during the first week, and during the second week of two-bottle choice testing (Fig. 5). On day 1 of testing (Fig. 5a), both methamphetamine pre-exposed groups consumed significantly lower levels of

methamphetamine as compared to the two not pre-exposed groups (group effect $F(3,28)=7.27$, $p=0.001$; post hoc comparison between groups showing significant differences between methamphetamine pre-exposed and not pre-exposed groups, $p<0.05$). Over days 1–7 (Fig. 5b), consumption in the methamphetamine pre-exposed groups increased and was no longer significantly different from that of not pre-exposed controls. During days 8–14 (Fig. 5c), however, methamphetamine consumption in phase-shifted rats of the methamphetamine pre-exposed group was higher than in all other groups, with ANOVA revealing a significant effect of group ($F(3,28)=8.16$, $p<0.001$), and post hoc comparisons between groups showing that levels of consumption were significantly higher in phase-shifted methamphetamine pre-exposed rats as compared to methamphetamine pre-exposed animals that had not been subjected to phase shifts ($p<0.01$), not pre-exposed animals that had been phase shifted ($p<0.05$), and not pre-exposed animals that had not received phase shifts ($p<0.001$). Similar results were found when data were analyzed using percent change in preference from not pre-exposed no-shift controls (Fig. 6) with ANOVA revealing a significant group effect [$F(3,28)=6.11$, $p=0.01$], and from post hoc comparison between groups showing significant differences between not pre-exposed and methamphetamine pre-exposed groups on day 1 (Fig. 6a, p 's <0.05), and between the methamphetamine pre-exposed shift and both the methamphetamine pre-exposed no-shift group ($p<0.05$) and the not pre-exposed no-shift control group ($p<0.05$) on days 8–14 (Fig. 6c).

Discussion

In this study, we used a rat model to examine the effects of a 2-week chronic jet lag paradigm on methamphetamine consumption following abstinence. During the second week following abstinence, rats previously exposed to methamphetamine and subjected to circadian disruption during abstinence showed a significantly higher percent change in methamphetamine consumption from not pre-exposed no-shift controls, compared to the methamphetamine pre-exposed no-shift and the not pre-exposed shifted groups. These results suggest that disruption of normal circadian rhythmicity may increase drug-taking behavior, and that normal clock function may be important for maintaining homeostasis within the reward system.

Methamphetamine pre-exposure was provided with 2 weeks of forced consumption of 0.01 % methamphetamine in the drinking water. This treatment resulted in clear

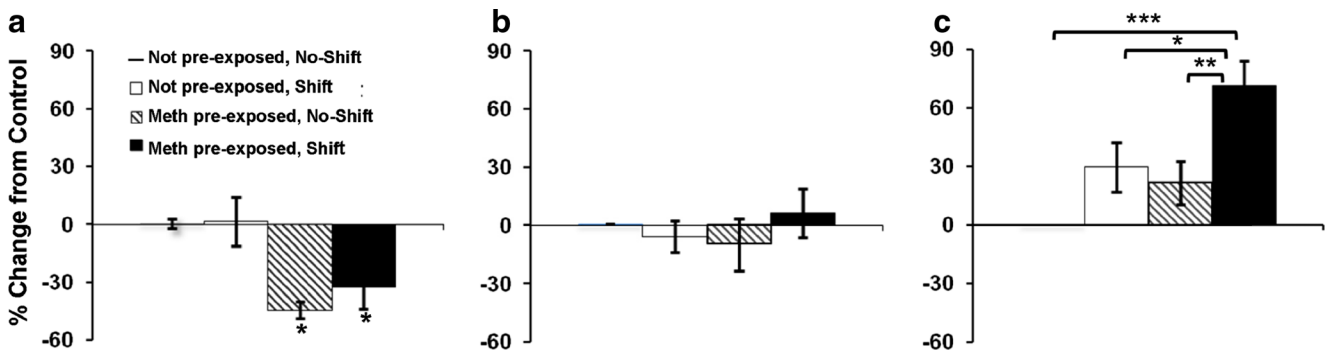


Fig. 5 Mean (\pm SEM) percent change in daily mg/kg methamphetamine consumption from not pre-exposed no-shift controls on day 1 (**a**), days 1–7 (**b**), and days 8–14 (**c**) of two-bottle choice testing. Not pre-exposed no-

shift control level is set at 0. * p <0.05 in (**b**) indicates a significant difference from not pre-exposed no-shift and not pre-exposed shift groups. * p <0.05, ** p <0.01, *** p <0.001. n =8 animals/group

physiological and behavioral effects, including weight loss, changes in activity patterns, and escalation of intake during forced consumption. These effects, in combination with the presence of withdrawal symptoms, suggest physical dependence in the methamphetamine pre-exposed groups. However, although withdrawal behaviors were higher in the methamphetamine pre-exposed groups, we did not find a significant association between levels of withdrawal behavior and methamphetamine consumption (data not shown). This may have been due to an insufficient sensitivity of our visual withdrawal scoring technique and/or the high concentration of methamphetamine used during forced consumption. Further experiments are necessary to definitively show dependence in the methamphetamine pre-exposed rats under this paradigm. Surprisingly, the number of withdrawal signs was significantly lower at the 24-h time point compared to the 12- and 36-h time points, suggesting the possibility of a circadian rhythm of withdrawal symptoms. Although the demonstration of circadian rhythms of withdrawal symptoms in constant conditions has not, to our knowledge, been explored for any drug, Damaggio and Gorman (2014) recently reported that physiological responses to ethanol withdrawal do vary as a

function of circadian phase in animals entrained to a skeleton photoperiod.

We found that methamphetamine pre-exposed rats subjected to a 2-week chronic jet lag procedure showed significantly higher percent change in methamphetamine consumption from not pre-exposed no-shift controls compared to the other experimental groups (i.e., methamphetamine pre-exposed no-shift and not pre-exposed shifted groups) during the second week following abstinence. In a limited access maintenance model of ethanol drinking in rats, Gauvin et al. (1997) reported transient increases in ethanol consumption when single phase advances or delays of the light:dark cycle were administered during the self-administration period. In the same study, alternating high and low ethanol intake was observed during a more slowly changing 7-day “shift work” schedule, depending on which shift was in effect. These alternating levels of drinking may have been due to partial entrainment of the circadian clock to the light:dark cycle during some shifts and not others, and/or differing effects of circadian disruption in non-dependent animals. In the current study, we used a high concentration of methamphetamine that is likely to have induced dependence, and shifted the light:dark

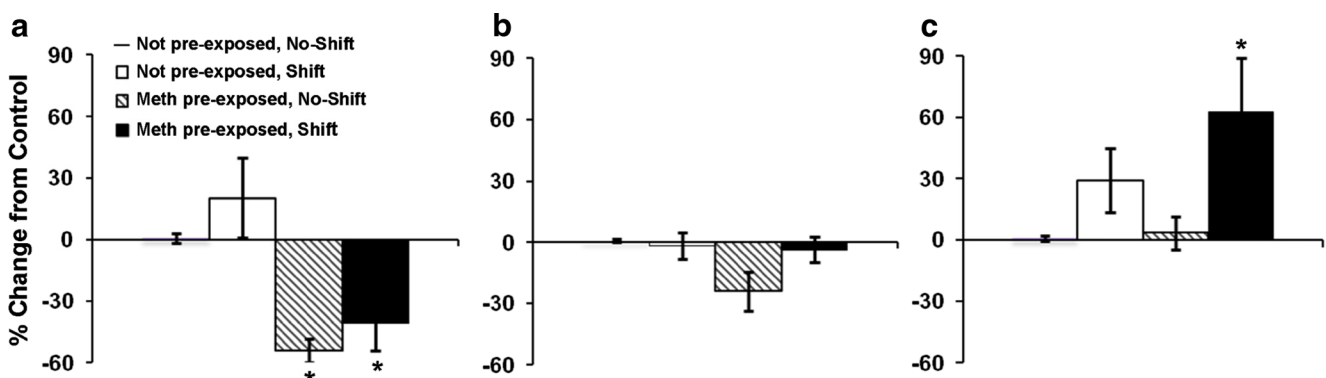


Fig. 6 Mean (\pm SEM) percent change in preference for methamphetamine from not pre-exposed no-shift controls on **a** day 1, **b** days 1–7, and **c** days 8–14 of two-bottle choice testing. * p <0.05 in **a** indicates a significant difference from not pre-exposed shift and not pre-

exposed no-shift groups; * p <0.05 in **b** indicates a significant difference from methamphetamine pre-exposed no-shift and not pre-exposed shift groups. n =8 animals/group

cycle at a rapid rate that did not permit clock entrainment. Importantly, with the exception of the first day of abstinence, percent change in methamphetamine consumption and preference for the methamphetamine pre-exposed no-shift and the not pre-exposed shifted rats was not significantly higher than that of the not pre-exposed non-shifted control group. These results indicate that neither prior exposure to this concentration of methamphetamine nor the 2-week long chronic jet lag procedure were in themselves sufficient to produce increases in consumption following abstinence in the methamphetamine pre-exposed shifted rats, and raise the possibility that there may be a combined effect of circadian disruption and prior methamphetamine exposure on enhancing drug-induced neuroadaptations. Such neuroadaptations are believed to lead to increased drug-seeking and to mediate prolonged relapse vulnerability following abstinence (Grimm et al. 2001). However, it is also possible that additional mechanisms such as physiological stresses induced by forced methamphetamine exposure may produce downstream effects that interact with circadian disruption to increase subsequent methamphetamine consumption. The circadian clock regulates daily rhythms of glucocorticoids, and is itself influenced by them (for review, see Dickmeis et al. 2013). Thus, alterations in glucocorticoid levels resulting from forced methamphetamine consumption prior to phase shifting may have enhanced the level of circadian disruption in the methamphetamine pre-exposed phase-shifted group. In addition, the effects of phase shifting during the period of rapid body weight gain immediately following forced methamphetamine consumption might produce effects that alter later methamphetamine intake. Future studies will be necessary to elucidate the neurobiological mechanism (s) underlying the possible effects of circadian disruption on methamphetamine consumption.

Repeated exposure to methamphetamine is known to result in hypersensitivity, or sensitization, to the psychomotor and incentive motivational effects of the drug following an abstinence period (Ito et al. 1997; McDaid et al. 2006), and sensitization is believed to reflect neurobiological changes related to addiction. In the current study, our measures of mg/kg consumption, percent preference, and percent change show that both methamphetamine pre-exposed groups consumed significantly less methamphetamine than not pre-exposed groups on the first day of two-bottle choice testing. Although this initial low level of consumption is consistent with a sensitized response to methamphetamine in which a lower dose would be required to elicit the same level of psychomotor stimulation, it is also possible that it indicates a developed aversion for methamphetamine, an interpretation supported by the low preference scores. There were no significant differences in percent change in methamphetamine consumption from not pre-exposed no-shift controls between the two methamphetamine pre-exposed groups on the first day and during the first week of two-bottle choice testing.

However, during the second week of testing, the methamphetamine pre-exposed shifted rats showed significantly higher percent change in methamphetamine consumption from not pre-exposed no-shift controls, compared to the other experimental groups. The mechanism behind this delayed effect is unclear but, interestingly, this period of time corresponds to the time when the rats had stably re-entrained to the 12:12 h light:dark cycle. It is possible that the transient clock phase during the first week of testing obscured group differences, and only after stabilization of clock phase did these differences become apparent. The combined effects of methamphetamine exposure and circadian disruption in maintaining a high level of methamphetamine drinking may have been due to either an increase in the rewarding effects or a decrease in the aversive effects of methamphetamine compared to the control groups. In either case, our findings support the idea that circadian disruption may enhance the drug-induced neurobiological changes that lead to addiction.

Numerous clinical studies indicate a correlation between the presence of sleep and/or circadian disorders and the onset of substance use and abuse (Currie et al. 2003; Rogers and Reilly 2002; Teplin et al. 2006; Trinkoff and Storr 1998; Wong et al. 2009). In addition, sleep problems have been shown to be a predominant factor predicting relapse (Brower et al. 2001; Pace-Schott et al. 2005). The current study indicates that methamphetamine pre-exposed rats consume greater amounts of methamphetamine following abstinence when subject to disruptions of their circadian system during the abstinence period. These results underscore the importance of maintaining circadian regularity in human substance abuse patients, and suggest that circadian therapies may significantly decrease relapse vulnerability in substance abuse patients. They further suggest that alterations in the circadian timing system, such as those occurring in the shift workers who constitute almost 15 % of full-time workers in the USA (Bureau of Labor Statistics 2005), may be a vulnerability factor in the development of addiction.

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Conflict of interest All authors report no potential conflicts of interest.

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