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Research article

Sleep deprivation precipitates the development of amphetamine-induced conditioned place preference in rats

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ABSTRACT

Sleep deprivation (SD) and amphetamine use are commonly associated conditions. SD shares similar neurobiological effects with psychostimulants, playing an important role in drug addiction, especially through conditioning manipulations. The aim of the present study was to investigate the effects of SD on the development of amphetamine-induced conditioned place preference (CPP) in a protocol with a reduced number of conditioning sessions. Male adult Wistar rats were submitted to 4 conditioning sessions (2 sessions/day) in the CPP apparatus, half with saline (non-drug-paired compartment) and half with 2 mg/kg amphetamine (drug-paired compartment) after control (home-cage maintained) or SD (6 h gentle handling method) conditions. Control animals did not express a preference for the amphetamine-paired compartment, showing that 2 conditioning sessions with the drug were not sufficient to establish CPP. On the other hand, animals submitted to SD during the conditioning sessions expressed a preference for the amphetamine-paired compartment, which was maintained across the entire test session. SD precipitated the development of CPP to amphetamine, showing that lack of sleep can contribute to the establishment of a conditioning between the drug effect and environmental cues.

1. Introduction

Amphetamines are largely consumed in late-night parties and for recreational purposes, and as a solution to remain awake due to professional necessities [1]. Thus, amphetamine-like stimulants are most frequently consumed during nighttime, what has prompted researchers to investigate relationships between sleep and drug abuse [2].

Sleep deprivation (SD) has been proposed to share plastic mechanisms with psychostimulant addiction, potentiating the dopaminergic system function [3–5] and particularly contributing to its conditioned component. Previous studies have shown that SD potentiates amphetamine-induced behavioral sensitization in a context-dependent manner [6] and impairs the extinction of cocaine-induced environmental conditioning in mice [7].

Despite the evidence suggesting that sleep loss seems to exert its effects over the course of drug addiction through manipulations in the conditioned component of substance abuse, no study to date had evaluated the effects of SD on the generation of drug-environment conditioning. We designed the present study to investigate if SD would play a fundamental role on the development of amphetamine-induced conditioned place-preference.

2. Experimental procedures

2.1. Subjects

Three-month-old Wistar male rats (250–300 g, CEDEME, UNIFESP) were used in the experiments. Animals were housed 4–5 per cage under controlled temperature (22–23 °C) and lighting conditions (12 h light/12 h dark; lights on at 7a.m.) with free access to food and water. The protocols were in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals, and were approved by the Institutional Ethical Committee of UNIFESP (#6451010714).

2.2. Drug

Amphetamine (Sigma®) was dissolved in 0.9% saline, to the final concentration of 0.2 mg/mL, and administered at the dose of 2 mg/kg. All solutions were administered intraperitoneally at a volume of 10 ml/kg.

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2.3. Sleep deprivation (SD)

Rats were subjected to total SD through the gentle handling method, which consists of keeping the animals awake by gentle manipulations whenever behavioral signs of sleep are observed [8]. Animals were sleep-deprived for 6 h, starting at 8 a.m., immediately before behavioral tasks.

2.4. Conditioned place preference (CPP)

The CPP apparatus consisted of 2 conditioning compartments of equal size (30 × 30 × 60 cm), 1 black with a grid floor and 1 white with a black smooth floor and a black rectangular figure in the wall, connected by a sliding door that remained closed during the conditioning sessions.

2.4.1. Conditioning

An unbiased design was used with animals being randomly assigned to an experimental group and to a 'drug-paired compartment', in a counterbalanced fashion, with each compartment being drug-paired for half of the animals. Conditioning sessions were performed on 2 consecutive days. Two sessions, one with drug and the other with saline, were performed each day with a 3-h interval. The order of drug or saline administration was counterbalanced in a group. Immediately after injections, rats were confined to the assigned compartment for 10 min. Animals that were conditioned to the drug-paired compartment on the 1st session of the day were placed to the non-drug-paired compartment on the 2nd session, and vice versa, with the order switched in the following day for each animal.

2.4.2. CPP test

Animals were positioned in the apparatus with the door opened for 15 min. No injections were administered on the test day. Time spent in each compartment and number of transitions between compartments were registered in 3 different time-points (cumulative time): 5′, 10′, 15′.

2.5. Experimental design

Twenty-five rats were randomly allocated to 2 groups: CTRL (n = 12) and SD (n = 13). Animals were conditioned as previously described. In each of the 2 conditioning days, rats were kept undisturbed in their home-cages or submitted to the SD protocol starting at 8a.m. After 3 h, rats were submitted to the 1st conditioning session. Promptly after the end of the 10-min conditioning session, animals were returned to the control or SD condition, in which they remained for another 3-h period before being submitted to the 2nd conditioning session on the same day. On day 3, the CPP test was performed. The experimental design is summarized in Table 1.

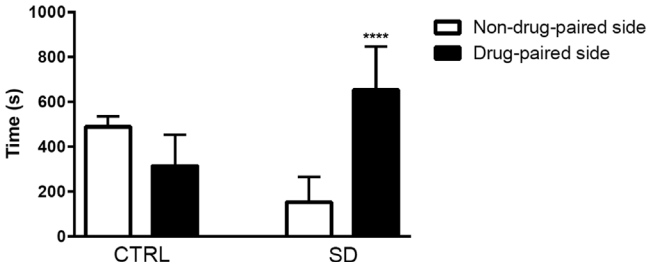

Fig. 1. Time spent in the drug-paired vs non-drug-paired compartments of the conditioned place preference apparatus during the test session. SD: 6 h of sleep deprivation (n = 13); CTRL: home-cage maintained (n = 12). Data are reported as mean ± SEM. ****p < .0001 compared with non-drug-paired compartment.

2.6. Statistical analysis

Within-group comparisons were performed using paired *t*-test. CPP test delta time and transitions across different time-points were analyzed with 2-way repeated measures (RM) analysis of variance (ANOVA) followed by Bonferroni post hoc test. Significance was arbitrated at a probability of $p < .05$.

3. Results

Analysis of the time spent in the non-drug-paired vs drug-paired compartments in the 15-min test session indicated that control animals did not develop amphetamine-induced CPP [paired Student's *t*-test: $t(11) = 2.02$, $p = .26$] (Fig. 1). Rats submitted to SD along with the conditioning sessions expressed a preference for the amphetamine-paired environment in the test session [paired Student's *t*-test: $t(12) = 6.37$, $p < .0001$]. For cumulative delta time, 2-way RM ANOVA revealed a significant interaction effect between group (CTRL or SD) and time (5′, 10′ or 15′) [$F(2,24) = 56.08$, $p < .0001$]. Bonferroni post hoc test showed a significant difference between CTRL and SD groups for 10′ and 15′ time-points (Fig. 2). Analysis of the total number of transitions between the 2 compartments indicated a significant effect of group [$F(1,12) = 7.79$, $p < .05$] and time [$F(2,24) = 12.01$, $p < .001$] factors, but no interaction, with Bonferroni test showing a decrease in the number of transitions in the SD group compared to the CTRL group at both 10′ and 15′ (Fig. 3).

4. Discussion

Our adapted CPP model consisted of 4 conditioning sessions (2 sessions/day), at least half the number of trials usually conducted in classic CPP protocols. We expected that the control group would not develop amphetamine-induced CPP, allowing us to see a potentiating effect of SD in the experimental group. Animals from the control group did not express a preference for the drug-paired environment (Fig. 1),

Table 1
Example of experimental design.

	Timeline		Day 1			Day 2			Day 3
			1st Conditioning Day			2nd Conditioning Day			Test
			Sleep condition (8am–2pm)	Morning Session (11am)	Afternoon Session (2pm)	Sleep condition (8am–2pm)	Morning Session (11am)	Afternoon Session (2pm)	
Groups	CTRL	Animal 1	Home-cage	SAL (black)	AMPH (white)	Home-cage	AMPH (white)	SAL (black)	Post conditioning
		Animal 2	maintained	AMPH (black)	SAL (white)	maintained	SAL (white)	AMPH (black)	Test
	SD	Animal 1	Sleep deprivation	SAL (white)	AMPH (black)	Sleep deprivation	AMPH (black)	SAL (white)	
		Animal 2		AMPH (white)	SAL (black)		SAL (black)	AMPH (white)	

Example of experimental design for 2 different animals in each group, establishing 4 possible schemes of conditioning. All 4 schemes were randomly applied to different animals in both groups. SAL – saline i.p. injection; AMPH – 2.0 mg/kg amphetamine i.p. injection; black k exposure to the black compartment of the apparatus for 10 min; white – exposure to the white compartment of the apparatus for 10 min.