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Short communication

Involvement of the pineal gland in diurnal cocaine reward in mice

Murat Kurtuncu, Ahmet D. Arslan, Mustafa Akhisaroglu, Hari Manev, Tolga Uz*

Department of Psychiatry, Psychiatric Institute, University of Illinois at Chicago, 1601 West Taylor Street, M/C 912, Chicago, IL 60612, USA

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Abstract

Contribution of circadian mechanisms to the psychostimulant-induced behaviors has been suggested. The pineal gland is important component of circadian mechanisms. Using pinealectomized mice and sham-operated controls, we tested the contribution of pineal gland to the rewarding effects of cocaine in conditioned place preference test. Experiments were performed both during the day and at night. Controls with intact pineal glands demonstrated significant decrease in cocaine-induced conditioned place preference at night compared to daytime, whereas pinealectomized mice did not show any diurnal differences. Circadian mechanisms regulated by the pineal gland thus appear critically involved in cocaine-induced reward.

Keywords: Cocaine; Conditioned place preference; Mouse; Circadian rhythm; Pinealectomy

1. Introduction

Recent work on fruit flies and rodents suggests that circadian mechanisms are important for the development of psychostimulant-induced behaviors such as locomotor sensitization and reward (Andretic et al., 1999; Gaytan et al., 1999; Uz et al., 2002; Abarca et al., 2002). One of the important components of circadian mechanisms, "clock" genes, has been proposed, first in fruit flies and more recently in mice, as a new pathway in the development of addictive behaviors; flies and mice mutant for these genes including *Period* (i.e., *Per1*), do not become sensitized to cocaine (Andretic et al., 1999; Abarca et al., 2002). Using the conditioned place preference paradigm, it has also been reported that *Per1* knockout mice show a lack of cocaine reward behavior (Abarca et al., 2002).

The pineal gland and its products *N*-acetylserotonin and melatonin are considered important regulators of circadian mechanisms (i.e., clock genes) in the brain. It has been shown that in the pars tuberalis, removal of pineal gland (pinealectomy) abolishes the circadian rhythm of *Per1* (Messager et al., 2001; von Gall et al., 2002). We recently found that pinelactomized mice show a lack of circadian PER1 protein rhythms in the striatum, an area important for the development and maintenance of drug-induced behaviors such as sensitization (Uz et al., 2003).

Since cocaine reward requires circadian mechanisms including the clock gene *Per1* (Abarca et al., 2002) and the rhythm of *Per1* is regulated by pineal products such as melatonin (Uz et al., 2003), we tested the involvement of the pineal gland and its products in cocaine reward using a model of pinealectomy and a standard conditioned place preference paradigm during the day and at night.

2. Materials and methods

2.1. Animals and drug treatment

Pinealectomized and sham-operated C3H mice, 8 weeks old and weighing 25–30 g were purchased from Taconic (Germantown, NY, USA). Animals (24 sham-pinealectomized and 24 pinealectomized) were housed in groups of three and had free access to laboratory chow and water except during behavioral experiments. They were kept in a temperature-controlled room under conditions of 14-h light/10-h dark cycle (lights on at 5 am; Zeitgeber time

* Corresponding author. Tel.: +1-312-413-4580; fax: +1-312-413-4569.
E-mail address: uz@psych.uic.edu (T. Uz).

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00 [ZT00]). Cocaine hydrochloride (Sigma, St. Louis, MO, USA) was dissolved in sterile physiological saline (0.9% NaCl) and administered intraperitoneally (10 mg/kg; i.p.) in an injection volume of 0.05 ml/25 g body weight. Experiments were carried out both during the day (10 am; ZT05) and at night (1 am; ZT20). The time of injection was chosen based on circadian PER1 protein rhythms in the brain: high levels during the day and low levels at night (Uz et al., 2003). The experiment room was illuminated by normal fluorescent light (about 150 lx at the bench level). The nighttime drug administrations were done under dim red light (0.1 lx at the bench level). The experimental protocol was approved by the Institutional Animal Care and Ethics Committee.

2.2. Conditioned place preference procedure

Conditioned place preference was assessed in Plexiglas chambers that were divided into two compartments of equal size (22 × 22 × 22 cm), the first one with black walls and floor, the second one with white walls and floor. Each compartment had different floor textures, smooth for the white one and rough for the black. The two unique compartments were separated by a Plexiglas wall with a door (10 × 5 cm) that could be lifted during pre- and post-conditioning test sessions allowing access to both compartments. During the conditioning sessions, the door was kept closed, thereby limiting the access to one compartment. The time spent in each compartment was recorded with a ceiling-mount camera with night vision (i.e., infrared) to record the nighttime experiments. Conditioned place preference was assessed by determination of chamber side preference in three phases either during the day (daytime group) or at night (nighttime group). Initial preference (i.e., pre-conditioning) was determined as the compartment in which a mouse spent more than 450 s out of a 15-min (900-s) trial. Conditioning was conducted over a 6-day period in which cocaine was administered when the animal was restricted for 30 min to the initially non-preferred compartment and saline was administered when the animal was restricted to the preferred compartment for 30 min. Animals received one conditioning session per day, counterbalanced between saline and cocaine, for a total of three saline pairings and three cocaine pairings. Twenty fours after the last conditioning, final chamber preference was measured for 15 min (i.e., post-conditioning). Conditioned place preference was evaluated as differences in post-conditioning vs. pre-conditioning time spent in the drug-paired compartment.

2.3. Statistical analysis

The difference in time spent in the drug-paired compartment between the post- and pre-conditioning test sessions is reported as the "conditioned score" (mean ± S.E.M.). Results were analyzed by one-way analysis of variance (ANOVA) with Scheffe's post-hoc test. Significance was accepted as $P < 0.05$.

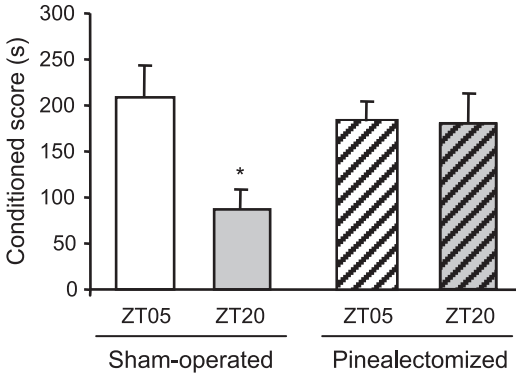

Fig. 1. Diurnal differences in cocaine reward and the role of the pineal gland. Cocaine-induced place preference was measured both during the day (ZT05) and at night (ZT20). Each bar represents the time spent in the drug paired compartment during 15-min-long post-conditioning session minus the time spent in the drug paired compartment during 15-min-long pre-conditioning session (i.e., conditioned score, mean ± S.E.M.; $n = 12$). Please note the significant decrease in cocaine-induced place preference that occurred only in nighttime sham-operated group (i.e., high *N*-acetylserotonin/melatonin, low PER1 protein levels). The zeitgeber time 00 (ZT00) is defined as lights on. *$P < 0.05$ in comparison with the corresponding daytime-conditioned scores (Scheffe's test).

3. Results

Cocaine's reward and reinforcing properties were assessed with the commonly used conditioned place preference paradigm (Miner, 1997). The initial side preferences prior to cocaine conditioning were similar among all groups tested. Daytime cocaine conditioning resulted in a significant change in preference in favor of the drug-paired side in the *N*-acetylserotonin/melatonin proficient sham-operated group (Fig. 1). When the animals were conditioned to cocaine at night, there was a significant decrease in drug-induced place preference in sham-operated group (Fig. 1; one-way ANOVA: $F(3, 44) = 3.607$, $P < 0.05$). On the other hand, pinealectomized (i.e., *N*-acetylserotonin/melatonin-deficient) mice showed a similar preference for the drug-paired side regardless of cocaine conditioning time (both during the day and at night; Fig. 1).

4. Discussion

In this study, we confirmed that cocaine reward occurs in a circadian fashion in mice with regular *N*-acetylserotonin/melatonin rhythms, as was earlier suggested (Abarca et al., 2002). Then, employing a model of pinealectomy, we demonstrated for the first time that circadian rhythms or the presence of pineal products such as melatonin are critical for diurnal cocaine reward. Psychostimulants, such as cocaine and amphetamine, induce positive conditioned place

preference responses (i.e., reward) in mice (Miner, 1997; Seale and Carney, 1991; Cunningham et al., 1999). Conditioned place preference is a commonly used behavioral paradigm for drug seeking behavior in which subjects demonstrate learned association to specific environmental stimuli (i.e., cues) with the positively rewarding or aversive effects of a drug. It has been found that *Per1* knockout mice lack cocaine reward (Abarca et al., 2002). We have recently reported that the PER1 protein has a circadian rhythm with peak values during the day in the striatum (Uz et al., 2003). Here we demonstrate that mice conditioned to cocaine at the time when PER1 protein levels are the lowest (i.e., nighttime) present significantly low cocaine reward compared to daytime (i.e., high PER1 protein levels) cocaine conditioning (Fig. 1). This is in line with the abolished cocaine reward observed in *Per1* mutant mice (Abarca et al., 2002).

The function of clock genes in mammals has been studied extensively in the suprachiasmatic nucleus due to their "master clock" properties. The mammalian clock system is believed to be hierarchical, with a "master clock" located within the suprachiasmatic nucleus (Reppert and Weaver, 1991). The daily light–dark cycle entrains the suprachiasmatic nucleus pacemaker to the 24-h day primarily through the retinohypothalamic pathway, and the suprachiasmatic nucleus, via superior cervical ganglion, entrains the rhythmic production of pineal hormones *N*-acetylserotonin and melatonin in the pineal gland with low levels during the day and high levels at night (Goto et al., 1989). The expression of clock genes demonstrates a region-specific internal rhythm throughout the rest of the brain, which is thought to be responsible for the regional, so-called "non-clock" function of clock genes (Masubuchi et al., 2000; Abe et al., 2001). Since this rhythm is different than the one in the suprachiasmatic nucleus and is abolished in *N*-acetylserotonin and melatonin deficiency (e.g., pinealectomy), it is believed that the pineal products, *N*-acetylserotonin and melatonin, control the rhythm of clock gene expression for the rest of the brain. It has been reported that under the conditions in which *Per1* rhythm in the striatum and hypophyseal pars tuberalis has either shifted or been abolished, the rhythm in the suprachiasmatic nucleus remained the same (Messager et al., 2001; von Gall et al., 2002; Abe et al., 2001).

Using models of pinealectomy and circadian rhythms, we have recently demonstrated that the pineal gland and its products such as melatonin are crucial for the existence of clock gene *Per1* rhythms in the striatum (Uz et al., 2003). In this study, we report that pinealectomy (i.e., abolished *Per1* rhythm) restored place preference after nighttime cocaine conditioning (Fig. 1). Regardless of the time of conditioning, pinealectomized mice strongly preferred the cocaine-paired side (Fig. 1), suggesting that the rhythm of clock gene *Per1* is equally important as the presence or absence of its expression.

Further research is needed to understand how pineal *N*-acetylserotonin and melatonin (i.e., melatonin receptors) participate in regulating *Per1* expression and clock-controlled genes. We expect this line of research to help us understand the underlying mechanisms of addictive behavior and to develop new treatment strategies to block the rewarding properties of cocaine.

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