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Short communication

Inactivation of the paraventricular thalamus abolishes the expression of cocaine conditioned place preference in rats

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ABSTRACT

Background: The paraventricular thalamus (PVT) is rapidly becoming recognized as part of the addiction circuitry. In addition to its strong anatomical connection to most of the brain regions underlying addiction, such as the nucleus accumbens, prefrontal cortex, amygdala, and hippocampus, the PVT has recently been shown to contribute to cocaine sensitization and reinstatement. In the present study, we examined the role of the PVT in the expression of cocaine conditioned place preference (CPP).
Methods: We tested the impact of PVT inactivation by baclofen/muscimol (bac-mus) microinjection on the expression of cocaine-induced CPP in rats. Rats were implanted with guide cannulae into the PVT. Bac-mus ($GABA_B$–$GABA_A$ agonists) or saline was injected into the PVT prior to CPP testing.
Results: Inactivation of the PVT by bac-mus prevented the expression of CPP, while placements outside the PVT did not affect CPP. Intra-PVT injections of bac-mus did not affect locomotor activity during the session.
Conclusions: In the present study, we contribute to the growing body of research supporting a role for the PVT in addiction by demonstrating that the PVT is necessary for the expression of cocaine CPP.
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1. Introduction

Recently, it has been suggested that the PVT may play a critical role in addictive behaviors and thus should be included as part of the reward circuitry (James and Dayas, 2013; Martin-Fardon and Boutrel, 2012). Mounting evidence supports the role of the PVT in addictive behaviors. For example, the PVT sustains electrical self-stimulation (Clavier and Gerfen, 1982). Furthermore, Young and Deutch (1998) found that lesion of the PVT in rats prevents cocaine-induced locomotor sensitization. Also, lesion of the PVT inhibits context-dependent reinstatement of alcohol-seeking behavior using the self-administration paradigm (Hamlin et al., 2009), while injection of tetrodotoxin (TTX) into the PVT prevents cocaine-induced reinstatement of cocaine-seeking behavior (James et al., 2010). Recent studies support a role for CART, dynorphin and orexin in the reinstatement of drug-seeking by the PVT (James et al., 2010; Marchant et al., 2010; Martin-Fardon and Boutrel, 2012).

Although several studies have examined the role of the PVT in self-administration reinstatement, few have assessed the role of the PVT in CPP. One such study found that lesions of the entire medial dorsal thalamus, including the PVT, prevented the acquisition of sucrose CPP (McAlonan et al., 1993), but to our knowledge, no one has assessed the role of the PVT in the expression of CPP. In the present study, we assessed the role of the PVT in the expression of previously acquired cocaine-induced CPP in rats by temporarily inactivating the PVT with baclofen-muscimol ($GABA_A$ & $GABA_B$ agonists) prior to CPP testing.

2. Methods

2.1. Animals

Male Sprague Dawley rats ($n = 29$; Simonsen Laboratories, Gilroy, CA) weighing 300–350 g were individually housed in a reversed 12:12 light/dark cycle in a temperature- and humidity-controlled colony room at $21 \pm 1\,^{\circ}C$ and were handled daily. Care of the animals was in accordance with the National Institute of Health guide for the care and use of laboratory animals, and all procedures were approved by the Institutional Animal Care and Use Committee of Washington State University. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

Cocaine hydrochloride (Sigma–Aldrich, St. Louis, MO) was dissolved at room temperature in 0.9% sodium chloride and filtered through a 0.2 μm filter. Baclofen and muscimol were dissolved together in saline at room temperature. Two doses

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of baclofen/muscimol (bac-mus) were used in the present study. The high dose consisted of 0.006 nmol/nl baclofen and 0.0006 nmol/nl muscimol. The low dose consisted of 0.001 nmol/nl baclofen and 0.0001 nmol/nl muscimol. All microinjections were 50 nL in volume, which we determined to be confined to the PVT in another experiment using injections of the anterograde tract tracer, 10% biotinylated dextran amine (BDA), into the PVT using the same coordinates (unpublished data). With the exception of occasional retrograde transport, primarily only cell bodies within the injection site are labeled with BDA following one week to allow for anterograde transport to occur. Using this method, we determined that a 50 nL injection of BDA using the coordinates in the present study results in cell staining confined to the PVT. Microinjections were performed with 33G injector needles that extended 0.5 mm past the tip of the guide cannulae. Injections were given over 1.5 min via pressure injection. Microinjection needles were left in place for 1 min following injection to allow for diffusion. Obturators were replaced following injection.

2.3. Surgery

Rats were anesthetized with an intraperitoneal (IP) injection (1 mL/kg) of a solution containing ketamine hydrochloride (87 mg/mL) and xylazine (150 mg/mL) prior to surgery. Animals were placed into a stereotaxic frame (Stoelting, Il) and the head was leveled. A small hole was drilled in the skull. The stereotaxic arm was set at a 10° angle. Guide cannulae (26G with 33G obturators) were placed at the coordinates 7.4 AP,+1.0 ML,−6.1 DV from the interaural line and mounted to the skull using screws and dental acrylic. Animals were given at least one week following surgery to recover prior to the start of cocaine CPP.

2.4. Cocaine CPP training

CPP chambers (Med Associates) contained a white and a black chamber with different flooring that provided two distinct contexts. Each served as either the drug-paired or saline-paired side. There was also a third gray chamber with a distinct floor that connected the two chambers and thereby provided a neutral environment. Animals were placed in the middle chamber at the start of habituation, initial preference determination, and testing. Animals were habituated to the CPP chambers for 15 min prior to the start of CPP. The next day, a test of initial preference was carried out by placing rats in the middle chamber of the CPP box and allowing them to move freely between chambers while time spent in each chamber was recorded using standard MedPC software. The cocaine-paired side was counterbalanced for both initial preference and chamber type (black vs. white chamber). Animals showing a strong preference (≥400 s) for one chamber were automatically given cocaine on the non-preferred side. One day after the test for initial preference, rats began cocaine CPP training. The animals were given 12 mg/kg cocaine injections (IP) or saline (1 mL/kg) prior to the start of the 30 min training sessions. On the first day of training, all animals received cocaine injections and were placed in the cocaine-paired side while access to the middle and saline-paired chambers were blocked by a barrier. The following day, all animals received saline injections and were placed on the saline-paired side. Each day following, for a total of 8 days of training, the animals received alternating injections of cocaine prior to placement in the cocaine-paired side and saline prior to placement in the saline-paired side.

Testing occurred the day following the last day of training. Animals in the first group were given a 50 nL microinjection of either a high dose of bac-mus (see "Drugs" above for exact dosing information) or vehicle (saline) 10–30 min prior to placement in the CPP chambers. Of note, the time course of bac-mus action within the PVT has not been previously determined for the doses used, however, the low variability within treatments between 10 min and 30 min following injection suggests that the drug effect was consistent throughout this time frame, and previous studies have relied on bac-mus effects for up to 2 h (McFarland and Kalivas, 2001). Animals were allowed access to all chambers while movement was recorded. In the first group of animals, only the high dose of bac-mus was tested. Animals were given 2 days between test sessions. On the third day, they were given the opposite treatment (bac-mus or vehicle) and re-tested for cocaine-induced place preference.

Two additional groups of animals were given two doses of bac-mus (high and low dose bac-mus) and a vehicle (saline) injection, counterbalanced for order of administration. Animals were given two days off between test sessions.

Six animals were removed from the study due to a significant decrease in locomotor activity being observed on at least one test day. In addition, three animals were dropped from the study because they did not show place preference. In all nine cases, cannulae placement was outside the PVT.

2.5. Data analysis

A preference score (the time spent on the cocaine-paired side minus the initial preference) and locomotor activity were analyzed using a one-way ANOVA followed by Tukey post hoc analysis in the case of a significant difference ($p \leq 0.05$). Statistical analyses were performed using Prism 5 (Graphpad Software Inc., La Jolla, CA).

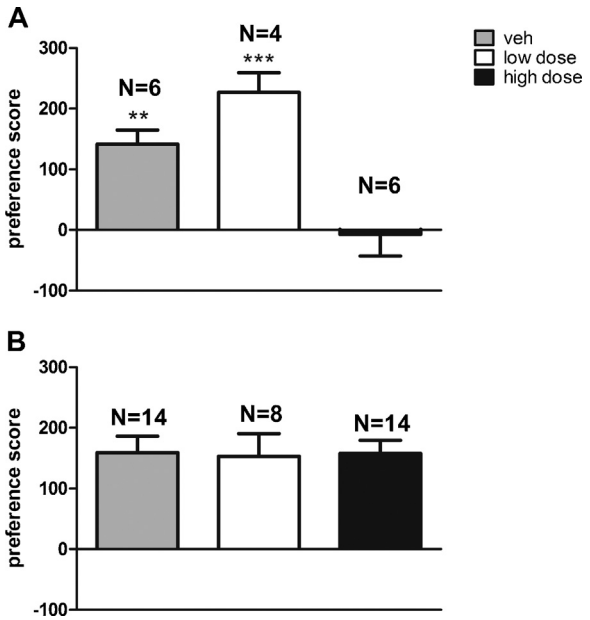

Fig. 1. Inactivation of the PVT inhibits the expression of cocaine conditioned place preference in rats.
Data are expressed as mean preference score ± SEM. (A) Preference scores of rats with cannulae placements in the PVT. (B) Preference scores of rats with cannulae placements outside of the PVT. Gray bars represent animals given the vehicle dose, white bars represent animals given the low dose (0.001 nmol/nl baclofen and 0.0001 nmol/nl muscimol) of bac-mus, and black bars represent animals given the high dose (0.006 nmol/nl baclofen and 0.0006 nmol/nl muscimol) of bac-mus.*Represents a significant difference from vehicle dose (One-way ANOVA, followed by Tukey, $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$).

3. Results

Inactivation of the PVT by the high dose of bac-mus completely inhibited the expression of cocaine CPP [$F_{(2,15)} = 13.79$, $P \leq 0.001$] (Fig. 1A). CPP was not affected in animals with cannulae placements outside the PVT (Fig. 1B). Furthermore, locomotor activity was not affected by bac-mus injections into the PVT (Fig. 2A).

4. Discussion

In the present experiment, we add to the mounting evidence indicating a role for the PVT in addiction by determining that inactivation of the PVT completely abolishes cocaine CPP expression. To our knowledge, we are the first to demonstrate a role for the PVT specifically in the expression phase of previously acquired cocaine-induced CPP.

The contribution of the PVT to addiction is gaining interest, and it has been recently suggested that the PVT should be included as part of the addiction circuitry (James and Dayas, 2013; Martin-Fardon and Boutrel, 2012). Early investigations showed that the PVT supported self-stimulation, indicating that it was a brain region involved in reward (Clavier and Gerfen, 1982; Cooper and Taylor, 1967). Electrolytic lesion of the PVT prevented cocaine-induced locomotor sensitization, and both cocaine and amphetamine injections increased Fos expression in the PVT (Deutch et al., 1998; Young and Deutch, 1998).

More recent studies have focused primarily on the role of the PVT in the reinstatement of drug-seeking behavior in drug self-administration experiments. The PVT has been shown to mediate contextual-, cue-, and cocaine-induced reinstatement of cocaine-seeking behavior. For example, Hamlin et al. (2009) reported that lesion of the PVT prevented contextual reinstatement of alcohol seeking but did not impair acquisition of alcohol