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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.

Keywords

cocaine; CPP; PVT; inactivation; addiction

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Contributors

Jenny Browning and Barbara Sorg designed the study and wrote the protocol. Jenny Browning undertook the statistical analysis and wrote the first draft of the manuscript. Barbara Sorg and Heiko Jansen edited the manuscript prior to submission. All authors contributed to and have approved the final manuscript.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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 300-350g were individually housed in a reversed 12:12 light/dark cycle in a temperature- and humidity-controlled colony room at 21±1°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 (NIH Publications, 2011), 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 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 50nl 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 0.5mm 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 grey 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 sec) 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 was 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 hr (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 retested 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 ($p < 0.05$). Statistical analyses were performed using Prism 5 (Graphpad Software Inc., La Jolla, CA).

3. RESULTS

Inactivation of the PVT by the high dose of bac-mus completely inhibited the expression of cocaine CPP [$F(2,15) = 13.79$, $P < 0.001$] (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 self-administration. A similar study found that injection of a kappa opioid receptor antagonist into the PVT blocked contextual reinstatement (Marchant et al., 2010). Additionally, the presentation of cocaine-associated cues during cue-induced reinstatement induced Fos in the PVT, and the magnitude of Fos expression was positively correlated with the level of reinstatement (James et al., 2011). Inactivation of the PVT by tetrodotoxin injection attenuated cocaine-induced reinstatement (James et al., 2010) and injection of cocaine- and amphetamine-regulated transcript (CART) into the PVT also attenuated cocaine-induced reinstatement (James et al., 2010).

In the present study, we investigated the role of the PVT in another model commonly used in addiction research, the CPP model. While the self-administration model is considered the model with the highest level of face validity for the study of addiction, the CPP model has a high degree of predictive validity in that animals show place preference for most drugs that are abused in humans (Bardo and Bevins, 2000; Tzschentke, 1998, 2007). Furthermore, the self-administration model relies on operant conditioning, while the CPP model more closely resembles a classical conditioning model in which place preference indicates that the drug is “rewarding” rather than “reinforcing” because the behavioral output, preference, is not reinforced. In the present study, we did not assess the role of the PVT on the acquisition of place preference but rather on the expression of previously-acquired place preference. Typically, manipulations that decrease the expression of CPP are considered to decrease

drug craving (Sanchis-Segura and Spanagel, 2006). Decreased responding in the self-administration reinstatement model may indicate a decrease in drug craving, motivation, or even simply compulsive learned behavior elicited by a cue or context paired with the drug or the drug itself. Only one study to our knowledge has assessed the role of the PVT in CPP, reporting that lesion of the entire medial dorsal nucleus of the thalamus, including the PVT, prevented sucrose CPP acquisition (McAlonan et al., 1993). Thus, our study demonstrates that inactivation of the anterior PVT blocks the expression of a Pavlovian conditioned response and is in agreement with the studies showing a role for the PVT in the reinstatement of drug-seeking behavior that relies on instrumental responding.

The PVT projects to and/or receives projections from much of the circuitry involved in reward and addiction, including the medial prefrontal cortex, nucleus accumbens, and amygdala (Chen and Su, 1990; Moga et al., 1995; Vertes and Hoover, 2008). The anatomical connections between the PVT and addiction-related brain regions provide many possible mechanisms by which the PVT exerts control over addictive behavior. For example, the PVT sends glutamatergic projections to the nucleus accumbens (Christie et al., 1987), and PVT stimulation increases glutamate levels in the nucleus accumbens (Jones et al., 1989; Parsons et al., 2007), which is associated with drug-intake and reinstatement in animal models (Cornish and Kalivas, 2000; Rassnick et al., 1992). In support of this, Hamlin et al. (2009) found that contextual reinstatement increased activation of nucleus accumbens projecting PVT neurons. Of note, our placements were confined to the *anterior* portion of the PVT. Recently, Li and Kirouac have reported that there are distinct projections to (Li and Kirouac, 2012) and from (Li and Kirouac, 2008) the anterior vs. the posterior PVT. Specifically, the anterior PVT contains far more projections to and from the ventral subiculum of the hypothalamus and denser projections to the PFC, whereas the posterior PVT receives greater input from the PFC and projects more extensively to the extended amygdala, including the central amygdala and the bed nucleus of the stria terminalis. Both the anterior and posterior PVT send extensive projections to the nucleus accumbens shell, but only the posterior PVT sends a large number of projections to the nucleus accumbens core. Due to the differences in neuroanatomical connections of the anterior vs the posterior PVT, the present results may be specific to the anterior portion of the PVT. Further research is needed to distinguish the role of the middle and posterior portions of the PVT in the expression of CPP. Of note, in a tract tracing study using a anterograde/retrograde tracer cocktail injected into the same coordinates used in the present study, our lab found dense axonal and cell staining in the ventral subiculum and PFC and dense axonal staining in the nucleus accumbens shell (data not yet published).

By using a transient inactivation paradigm, we show that inactivation of the PVT with bac-mus abolishes the expression of CPP only while the drug is on board and that this effect is reversed when the animals are given either vehicle or a low dose of bac-mus. In addition, we show that placements outside the PVT do not elicit the same effect indicating that the effect is specific for the PVT. Further research is needed to determine the mechanism(s) by which the PVT contributes to the expression of CPP.

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Figure 1. Inactivation of the PVT inhibits the expression of cocaine conditioned place preference in rats

Data are expressed as mean preference score $\pm$ SEM. (A) Preference scores of rats with cannulae placements in the PVT. (B) Preference scores of rats with cannulae placements outside of the PVT. Grey 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 0.05, ** P 0.01, *** P 0.001).

Figure 2. Inactivation of the PVT does not affect locomotor activity during the test session
Data are expressed as mean $\pm$ SEM of photocell beam breaks (**A**). All animals had confirmed cannulae placements in the PVT. Grey 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.*Placement of cannulae within the PVT are shown in (**B**) Cannula tips of “PVT hits” are represented by black dots. Cannula tips of “PVT misses” are represented by grey pluses. Note: PVA represents the anterior paraventricular nucleus.