Neuroscience Letters 608 (2015) 34–39

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

Transient inactivation of the posterior paraventricular nucleus of the thalamus blocks cocaine-seeking behavior

A. Matzeu*, F. Weiss, R. Martin-Fardon

Molecular and Cellular Neuroscience Department, The Scripps Research Institute, La Jolla, CA, USA

HIGHLIGHTS

- The paraventricular nucleus of the thalamus (PVT) has recently gained attention because of its involvement in the modulation of drug-directed behavior.
- Cue-induced reinstatement of cocaine seeking is more sensitive to temporary inactivation of the pPVT than cue-induced reinstatement of natural reward seeking.
- The pPVT plays an important role in neuronal mechanisms that drive cocaine-seeking behavior.

ARTICLE INFO

Article history:
Received 27 July 2015
Received in revised form 1 October 2015
Accepted 5 October 2015
Available online 9 October 2015

Keywords:
Paraventricular nucleus of the thalamus
Cocaine
Natural reward
Conditioned reinstatement

ABSTRACT

Originally studied for its role in energy homeostasis, the paraventricular nucleus of the thalamus (PVT) has recently gained attention because of its involvement in the modulation of drug-directed behavior. The posterior part of the PVT (pPVT) is connected with brain structures that modulate motivated behavior, and we tested whether the pPVT plays a pivotal role in cocaine seeking. The aim of the present study was to investigate whether transient inactivation of the pPVT prevents cue-induced reinstatement of cocaine seeking but not natural reward seeking. Male Wistar rats were trained to associate a discriminative stimulus (S^+) with the availability of cocaine or a highly palatable conventional reinforcer, sweetened condensed milk (SCM). Following extinction, the cocaine S^+ and SCM S^+ elicited comparable levels of reinstatement. Intra-pPVT administration of the γ-aminobutyric acid-A ($GABA_A$) and $GABA_B$ receptor agonists muscimol and baclofen (0.06 and 0.6 mM, respectively) prior to the presentation of the cocaine or SCM S^+ completely prevented the reinstatement of cocaine seeking, with no statistically significant effects on SCM seeking. These data show that the pPVT plays an important role in neuronal mechanisms that drive cocaine-seeking behavior.

1. Introduction

The high relapse rate and long-lasting vulnerability to relapse in abstinent individuals are among the challenges encountered in the effective treatment of drug addiction [17,25,27,28]. One hypothesis that can explain the long-lasting nature of drug-seeking behavior is that the neuronal circuits that mediate the control of drug-seeking and drug-taking behaviors have common motivational neuronal substrates that are not specific to addiction-related processes but are robustly activated by drugs.

The thalamus was recently proposed to be included in the neurocircuitry of addiction [15,24]. The paraventricular nucleus of the thalamus (PVT), considered a communication point between the ventral and dorsal striatum and lateral hypothalamus, has drawn attention because it plays a key role in energy homeostasis, arousal, endocrine regulation, and reward [3,14,30,35] and has been reported to be engaged in the effects of drugs (*e.g.*, cocaine and ethanol). The posterior part of the PVT (pPVT) projects to the nucleus accumbens (NAC), extended amygdala, and medial prefrontal cortex (mPFC) [18], placing the pPVT at a pivotal point to modulate motivated behavior.

Earlier findings showed that the PVT is activated by the presentation of cocaine-paired cues [5]. Recent findings demonstrated that transient inactivation of the PVT prevented cocaine prime-induced reinstatement [12] and the expression of conditioned place preference [6]. Moreover, data from this laboratory demonstrated a positive correlation between cocaine-seeking behavior that was

* Corresponding author at: Molecular and Cellular Neuroscience Department, The Scripps Research Institute, 10550 North Torrey Pines Road, SP30-2120, La Jolla, CA 92037, USA. Fax: +1 858 784 7146.
E-mail address: amatzeu@scripps.edu (A. Matzeu).

http://dx.doi.org/10.1016/j.neulet.2015.10.016
0304-3940/

induced by cocaine-related stimuli and PVT activation (measured by Fos-expressing neurons), whereas presentation of the stimulus that was predictive of a potent food reward (SCM S^+) elicited reinstatement but induced only nonspecific Fos expression that was not correlated with behavior, confirming that the PVT, as a whole, is recruited during the conditioned reinstatement of cocaine seeking [22].

Based on the previous observations that the PVT is differentially recruited by stimuli that are conditioned to the availability of cocaine *vs.* palatable food reward and that the pPVT is specifically connected to brain structures that are involved in the regulation of motivated behavior, the aim of the present study was to evaluate the importance of pPVT integrity in cocaine-seeking behavior *vs.* behavior that is motivated by stimuli that are conditioned to a highly palatable conventional reinforcer (sweetened condensed milk [SCM]). This was achieved by transiently inactivating the pPVT using the γ-aminobutyric acid-A ($GABA_A$) and $GABA_B$ receptor agonists muscimol and baclofen, respectively, administered together before conditioned reinstatement.

2. Materials and methods

2.1. Animals

Forty-two male Wistar rats (Charles River, Wilmington, MA, USA), weighing 200–225 g upon arrival, were housed two per cage in a temperature- and humidity-controlled vivarium on a reverse 12 h/12 h light/dark cycle with *ad libitum* access to food and water. All of the procedures were conducted in strict adherence to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

2.2. Drugs

Cocaine hydrochloride (COC; National Institute on Drug Abuse, Bethesda, MD, USA) was dissolved in 0.9% sodium chloride (Hospira, Lake Forest, IL, USA; 2.5 mg/ml). Sweetened condensed milk (Nestlé, Solon, OH, USA) was diluted 2:1 (v/v) in water. Muscimol and baclofen (M/B; Tocris Bioscience, Bristol, United Kingdom) were dissolved in 0.9% sodium chloride at concentrations of 0.06 and 0.6 mM, respectively.

2.3. Self-administration, conditioning, and extinction

Behavioral training and testing were conducted as previously described [20,21] (Fig. 1). Rats that were designated for COC self-administration training were surgically prepared with indwelling silastic catheters that were inserted in the right jugular vein. Rats that were designated for testing with the highly palatable food reward, SCM, were not subjected to surgical procedures. Following 7 days of postsurgical recovery, the rats began self-administration training. Each session was initiated by extending two retractable levers into the operant conditioning chamber. The self-administration of COC (0.25 mg per 0.1 ml infusion, delivered over 4 s) or SCM (0.1 ml delivered into a 0.2 ml receptacle) began on a fixed-ratio 1 (FR1) schedule of reinforcement in daily 120 min (COC) or 40 min (SCM) sessions, 5 days per week. Responses at the right, active lever were reinforced, followed by a 20 s timeout (20 s TO) period that was signaled by illumination of a cue light above the active lever. During this time, the lever remained inactive to prevent accidental overdosing with COC. To maintain identical training and experimental conditions, the 20 s signaled TO period was implemented during SCM self-administration. Responses at the left, inactive lever were without programmed consequences. Following 10 days of COC or SCM self-administration training, a contingency was introduced whereby responses at the active lever were differentially reinforced in the presence of discriminative stimuli (S^D) that signaled reward availability *vs.* non-availability. Constant 70 dB white noise served as a discriminative stimulus (S^+) that signaled availability of the reinforcer (COC or SCM), whereas illumination of a 2.8 W house light located at the top of the chamber's front panel served as a discriminative stimulus (S^-) that signaled non-availability of the reinforcer (*i.e.*, saline solution instead of COC or no consequence instead of SCM). Each session was initiated by presenting the respective S^D and extending the levers into the chambers. The S^D remained present until termination of the session by retraction of the levers. In the presence of the S^+, responses at the right, active lever were reinforced by COC or SCM on an FR1 schedule, followed by a 20 s TO period that was signaled by illumination of a cue light above the lever. In the presence of the S^-, responses at the right, active lever were followed by an intermittent tone, during which the lever remained inactive for 20 s. Three daily sessions (each lasting 1 h for the COC group and 20 min for the SCM group), separated by 30 min intervals, were conducted, with two S^+ (reward) sessions and one S^- (non-reward) session sequenced in random order. The SCM sessions were restricted to 20 min to avoid satiety by the excessive ingestion of SCM and to ensure that the number of responses were comparable during the first and second S^+ sessions [20,21]. Three days after the beginning of the conditioning training, the rats were implanted with a guide cannula (23-gauge, 15 mm, Plastics One, Roanoke, VA, USA) aimed at the pPVT (anterior/posterior, −3.3 mm; medial/lateral, ±2.72 mm from bregma; dorsal/ventral, −2.96 mm from dura, at an angle of 25° [31], positioned 3.5 mm above the target injection point; Fig. 2). After 7–10 days of recovery, the animals resumed conditioning training for an additional 7 days. Following a total of 10 days of conditioning training (*i.e.*, a total of 20 S^+ and 10 S^- sessions), both the COC and SCM groups were placed on extinction (EXT) conditions in daily 1 h sessions. Each EXT session was initiated by extension of the active and inactive levers in the absence of either S^D. During an EXT session, the rats were allowed to lever press, but they did not receive reinforcement. The EXT criterion was less than five responses per session for 3 consecutive days.

2.4. Conditioned reinstatement

On the last day of EXT training, every rat received a sham injection (SHAM) for habituation to the microinjection (Fig. 1). Twenty-four hours later, both groups of animals (SCM and COC) underwent the reinstatement test with reintroduction of the S^-. Three days later, the animals received an intra-PVT microinjection of the $GABA_A$/$GABA_B$ agonists (M/B) or vehicle (VEH; Fig. 1) using a microinfusion pump (Harvard 22 Syringe Pump, Holliston, MA, USA) and injectors that extended 3.5 mm beyond the guide cannula. Injections were made at a flow rate of 0.5 μl/min over 1 min, followed by an additional 1 min with the injector in place to allow for drug diffusion. Reinstatement tests were conducted under extinction conditions but with reintroduction of the S^+ only.

2.5. Histology

Upon completion of the reinstatement test, the rats were euthanized by CO_2 inhalation, and their brains were collected and snap frozen. The brains were then sliced in 40 μm coronal sections, and injector placements within the pPVT were verified (Fig. 2).

2.6. Statistical analysis

The data were analyzed using one- or two-way analysis of variance (ANOVA). Significant main effects or interactions were followed by the Protected Least Significant Difference (PLSD) *post*