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Research report

Increased latencies to initiate cocaine self-administration following laterodorsal tegmental nucleus lesions

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HIGHLIGHTS

- We used a neurotoxin designed to lesion LDTg cholinergic cells.
- LDTg-lesioned rats failed to regularly initiate cocaine self-administration.
- Cocaine priming reduced response initiation latencies in LDTg-lesioned rats.
- Lesions did not significantly alter the rewarding effects of cocaine.
- Lesions likely altered the rats' responsiveness to cocaine-predictive stimuli.

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ABSTRACT

Cholinergic input to the ventral tegmental area (VTA), origin of the mesocorticolimbic dopamine system that is critical for cocaine reward, is important for both cocaine seeking and cocaine taking. The laterodorsal tegmental nucleus (LDTg) provides one of the two major sources of excitatory cholinergic input to the VTA, but little is known of the role of the LDTg in cocaine reward. LDTg cholinergic cells express urotensin-II receptors and here we used local microinjections of a conjugate of the endogenous ligand for these receptors with diphtheria toxin (Dtx::UII) to lesion the cholinergic cells of the LDTg in rats previously trained to self-administer cocaine (1 mg/kg/infusion, i.v.). Lesioned rats showed long latencies to initiate cocaine self-administration after treatment with the toxin, which resulted in a reduction in cocaine intake per session. Priming injections reduced latencies to initiate responding for cocaine in lesioned rats, and once they began to respond the rats regulated their moment-to-moment cocaine intake within normal limits. Thus we conclude that while LDTg cholinergic cell loss does not significantly alter the rewarding effects of cocaine, LDTg lesions can reduce the rat's responsiveness to cocaine-predictive stimuli.

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1. Introduction

While it is widely understood that the mesocorticolimbic dopamine system plays a central role in reward function [1], and that this system is activated by both rewards themselves and by conditioned stimuli that have been associated with rewards [2], little is known about which of the multiple inputs to this system [3,4] carry reward-relevant information. Acetylcholine input to the ventral tegmental area (VTA) is implicated by several lines of study. First, infusions of the cholinergic agonist carbachol directly into the ventral tegmental area (origin of the mesocorticolimbic dopamine system) were found to potentiate the rewarding effects of lateral hypothalamic brain stimulation [5,6]. Second, such infusions were found to establish conditioned place preferences [5]. Third, rats were found to work for microinjections of carbachol directly into the VTA [7]. That VTA acetylcholine might play a role in cocaine reward is suggested by the finding that extracellular VTA acetylcholine levels are elevated during intravenous cocaine self-administration and during cocaine-seeking under unexpected extinction conditions. An initial rapid elevation in VTA acetylcholine at the beginning of a cocaine self-administration session is seen only in rats previously trained to self-administer cocaine and occurs not only when the rat receives the drug but also when it is expecting the drug but gets saline in its place. A relatively slower

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increase and subsequently elevated plateau in VTA acetylcholine levels is seen even in cocaine-naïve animals and is thus due to the cocaine itself. The elevation of VTA acetylcholine due to cocaine itself appears to regulate cocaine taking, as blockade of VTA cholinergic receptors in trained rats increases their hourly cocaine intake [8].

There are two sources of cholinergic input to the VTA, the pedunculopontine tegmental nucleus (PPTg) and the laterodorsal tegmental nucleus (LDTg) [9,10]. Of these, PPTg has been implicated in reward function [11,12] but lesions of the cholinergic cells of PPTg do not affect intravenous cocaine or heroin self-administration or conditioned place preference [13]. In the present study we used diptheria toxin conjugated to the ligand for a receptor expressed selectively on cholinergic neurons to assess the effects of loss of cholinergic LDTg neurons on intravenous cocaine self-administration.

2. Materials and methods

2.1. Animals

Fourteen male Long-Evans rats (Charles River, Raleigh, NC) weighing 275–325 g at the time of surgery were used. They were individually housed under a reverse light–dark cycle (12/12, lights off at 8 am) with free access to food and water throughout. All experiments were performed in accordance with the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Drugs and neurotoxin

Ketamine, Xylazine, and (-)-Cocaine hydrochloride were obtained from the NIH. Cocaine was dissolved in 0.9% sterile saline. Diphtheria toxin conjugated to urotensin-II (Dtx::UII) [14] was obtained from One World Biotech (Warminster, PA).

2.3. IV catheter implantation

Fourteen rats were each implanted with an intravenous microrenathane catheter (Braintree Scientific, Braintree, MA) inserted into the right external jugular vein. Each rat was first anesthetized with a combination of ketamine and xylazine (57 mg/kg and 9 mg/kg i.p., respectively) followed by isoflurane (2–3% in 1 L/min Oxygen). Catheter tubing was attached to a cannula adaptor backmount assembly at the other end. The backmount assembly was positioned so that it exited the animals back just caudal to the scapulae. Catheters were flushed daily with heparin (10 USP/ml in sterile saline), containing gentamicin (0.08 mg/ml).

2.4. LDTg Dtx::UII infusion

Following the completion of cocaine self-administration training, each rat was anesthetized and secured in a stereotaxic apparatus and three injections of 200 nl each of either Dtx::UII (42 ng protein per infusion, 126 ng total per hemisphere) or 0.01 M phosphate-buffered saline (vehicle) were made into each LDTg using a syringe controlled by an automated infusion pump (UMP 3; World Precision Instruments; Sarasota, FL) attached to one of the stereotaxic manipulator arms. The injector needle was lowered into each LDTg from the front along an angled trajectory of 30° from vertical. The three injections per hemisphere were aimed at the following coordinates according to the rat brain atlas of Paxinos & Watson [15] relative to Bregma: (1) A-P −8.7, M-L ±1.13, D-V −7.0, (2) A-P −8.5, M-L ±1.13, D-V −6.8, (3) A-P −7.0, M-L ±1.13, D-V −6.4. The needle tip was lowered to the most caudal coordinate and the first injection was made. The needle was then retracted to the second coordinate and a second injection was made. The needle was then retracted to the third coordinate and a third injection was made. Following each infusion, the injector was left in place for five minutes to allow for diffusion of the injected solution from the needle tip. Successive bilateral LDTg ibotenic acid lesions on the same day are associated with high mortality rates [16,17]. For this reason injections of toxin or vehicle into the left and right LDTg were separated by 48 h, with the order of injections counter-balanced. No rats died during the post-lesion recovery period.

2.5. Drug self-administration

2.5.1. Drug self-administration apparatus

Operant conditioning chambers, measuring 25 cm × 27 cm × 30 cm were used. Each chamber was equipped with a retractable lever and a stationary lever, a red house light, and a white cue light located above the retractable lever and was housed within a sound-attenuating enclosure equipped with a fan that provided both ventilation and a constant source of masking noise.

2.5.2. Drug self-administration procedure

Each rat was housed and trained in a dedicated operant chamber throughout the experiment. Daily 4-h intravenous drug self-administration training sessions began 7–10 days after i.v. catheter implantation and lasted for 13–15 days. Each rat was placed into its dedicated chamber immediately prior to the first self-administration session. For each rat the backmount catheter was connected to an infusion line attached to a liquid swivel that allowed for free movement of the rat within the operant chamber. The beginning of each daily training session was signaled by illumination of the red house light and insertion of the response lever into the operant chamber. No priming infusions were given during training. Each rat was allowed to self-administer cocaine (1.0 mg/kg/infusion, i.v.) under a fixed ratio 1 schedule of reinforcement with a 20-s time-out period accompanied by illumination of a white cue light located above the retractable lever. Lever presses during the time-out period were recorded but were not reinforced. Presses on the stationary lever were recorded but had no programmed consequence. At the end of the training session the retractable lever was withdrawn from the chamber and the house light was turned off. During the training phase rats were required to reach a criterion of three consecutive days of stable responding defined as less than 10% variability across days with a minimum of 30 cocaine injections per 4-h session. Once the rats met the training criteria, they were randomly assigned to either a LDTg toxin lesion or a sham lesion treatment group and underwent the intracranial injection procedure. Self-administration testing was resumed 4 days after the second LDTg lesion.

2.6. Cocaine priming

Priming injections of cocaine (10 mg/kg, i.p.) or saline (1 ml/kg, i.p.) were introduced five minutes prior to the beginning of the 12th and 13th post-lesion sessions in lesioned rats; the order of treatments was counterbalanced across rats. Each rat was allowed to earn cocaine infusions normally for the duration of the test sessions. Normal testing – testing without priming injections – resumed on post-lesion test day 14.

2.7. Cocaine dose–response testing

Because lesioned rats did not initiate cocaine self-administration reliably in response to lever-insertion, cocaine priming injections (10 mg/kg, i.p) were given 5 min prior to the beginning of each of the next 9 daily testing sessions. The dose of cocaine was set to 0.5, 1, or 2 mg/kg/infusion for each session. Each