Behavioural Brain Research 305 (2016) 218–222

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Short communication

The medial preoptic area modulates cocaine-induced locomotion in male rats

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HIGHLIGHTS

- Cocaine-induced locomotion is mediated by the medial preoptic area.
- Lesions of the medial preoptic area facilitate cocaine-induced locomotion.
- Mediation of cocaine-induced locomotion by the medial preoptic area requires input from both hemispheres.

ARTICLE INFO

Article history:
Received 7 January 2016
Received in revised form 23 February 2016
Accepted 1 March 2016
Available online 4 March 2016

Keywords:
Cocaine
Locomotion
Dopamine
Medial preoptic area
Nucleus accumbens
Lesions

ABSTRACT

Cocaine-induced locomotion is mediated by dopamine in the nucleus accumbens (NAc). Recent evidence indicates that the medial preoptic area (mPOA), a region in the rostral hypothalamus, modulates cocaine-induced dopamine in the NAc. Specifically, rats with lesions of the mPOA experienced a greater increase in dopamine following cocaine administration than rats with sham lesions. Whether the mPOA similarly influences cocaine-induced locomotion is not known. Here we examined whether radiofrequency or neurotoxic lesions of the mPOA in male rats influence changes in locomotion that follow cocaine administration. Locomotion was measured following cocaine administration in male rats with neurotoxic, radiofrequency, or sham lesions of the mPOA. Results indicate that bilateral lesions of the mPOA facilitated cocaine-induced locomotion. This facilitation was independent of lesion type, as increased locomotion was observed with either approach. These findings support a role for the mPOA as an integral region in the processing of cocaine-induced behavioral response, in this case locomotor activity.
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In 1976, Kelly and Iversen [1] introduced the idea that dopamine in the nucleus accumbens (NAc) stimulates locomotion after discovering that selective lesions of dopamine fibers in the NAc significantly reduced cocaine-induced locomotor activity. Extensive analyses by Costall and colleagues followed, showing that dopamine administration directly into the NAc increases locomotion [2,3]. This effect is specific to dopamine, as increased primary hyperactivity occurs following microinjections of dopamine into the NAc, but not norepinephrine, serotonin, GABA, or acetylcholine [2]. Cocaine increases locomotion through activation of this system.

Cocaine-induced behavioral changes are dependent on dopamine in the NAc [4]. A host of studies, employing microinjections or microdialysis, support this connection. For example, freely moving rats experience a five-fold increase in levels of extracellular dopamine in the NAc following cocaine administration [5]. Cocaine-induced increases in dopamine follow either experimenter-administered or self-administered drug [6]. Microinjection experiments similarly support the connection between cocaine, dopamine in the NAc, and increased locomotion, by showing that cocaine, but not other anesthetics, directly administered into the NAc causes motor activation in rats [7].

While the preponderance of evidence justifiably points to the mesolimbic dopamine system as the necessary neurobiological endpoint when describing cocaine's behavioral effects, this system does not operate in isolation, and input from other structures may play an equally critical role in cocaine-induced behaviors. We recently provided evidence describing a novel estradiol-dependent input into the mesolimbic system that modulates cocaine-induced neural, neurochemical, and behavioral activity in female rats [8,9]. Our findings demonstrated that neurotoxic lesions of the medial preoptic area (mPOA) increased cocaine-induced dopamine release

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http://dx.doi.org/10.1016/j.bbr.2016.03.002
0166-4328/Published by Elsevier B.V.

in the NAc, that many of the neurons projecting from the mPOA to the ventral tegmental area (VTA) are sensitive to estradiol, and that estradiol microinjections into the mPOA 24-h prior to cocaine administration increased cocaine-induced dopamine release in the NAc [9]. These results are in line with additional work from our laboratory revealing that lesions of the mPOA enhanced cocaine-induced conditioned place preference (CPP) and cocaine-induced cellular activity in the NAc [8]. Together, these results point to the mPOA as an integral region in the processing of cocaine-induced response. However, whether the mPOA similarly affects cocaine-induced behaviors like locomotion in male rats was hitherto unknown. To help answer this question, we examined whether radiofrequency or neurotoxic lesions in the mPOA of male rats influenced cocaine-induced locomotion.

All experimental procedures were in accordance with the National Institutes of Health Guidelines for the Use of Animals and were approved by the Institutional Animal Care and Use Committee at the University of Texas at Austin. Male Sprague Dawley rats (Harlan, Indianapolis), weighing 300–400 g, were double housed in large plastic cages on a 12/12 h light-dark cycle, lights off at 10am, with free access to food and water. Subjects were randomly assigned to surgical condition (sham or lesion) as well as drug administration order (saline first or cocaine first), and were then housed such that cage mates received all the same treatments (surgery and injections).

Stereotaxic surgeries were performed three weeks before behavioral testing. All animals received isofluorane (2–5%) anesthesia. For both radiofrequency and neurotoxic lesions, bilateral boreholes were drilled in the skull above the central region of the mPOA (AP, −0.4 mm; ML, ±0.5 mm; DV, −8.2 mm; according to coordinates from [10]). To produce radiofrequency lesions, a TCZ thermo-coupled electrode (0.25 mm exposed tip) was lowered into the mPOA and a Radionics radiofrequency lesion generator was used to heat the electrode to 80 °C (±3 °C) for 20 s. Sham radiofrequency animals underwent the same procedure without the electrode being heated. For neurotoxic lesions, injections of *N*-methyl-D-aspartate (NMDA; 25 ug/ul) in 0.1 M PBS into each hemisphere of the mPOA were used. NMDA was injected into the mPOA with a 1 ul Hamilton syringe at 0.1 ul/min for 2 min for each hemisphere. The syringe was then left in the mPOA for 5 min before being removed. The same procedure was followed for sham NMDA lesions except vehicle was microinjected in place of NMDA. Lesion placement was verified histologically after behavioral testing was complete. For verification, methyl green was used for subjects with radiofrequency lesions whereas an antibody against NeuN was used to evaluate NMDA lesions.

Of the animals receiving radiofrequency lesions, 11 were removed because they had lesions outside the mPOA, presented with tumors, or experienced seizures. Of the animals receiving sham-radiofrequency lesions, 4 were removed for similar reasons. Of the animals receiving neurotoxic lesions, 12 were removed with failed lesions. Of the remaining animals with neurotoxic lesions, 13 had unilateral and 7 had bilateral lesions; thus, bilateral and unilateral lesions of the mPOA were treated as separate levels of surgery during analysis. After exclusions, the four groups contained the following number of animals: radiofrequency-sham lesions (n = 15), radiofrequency lesions (n = 13); neurotoxic-sham lesions (n = 18), neurotoxic-unilateral lesions (n = 13), neurotoxic-bilateral lesions (n = 7).

Med Associates open field boxes (44 cm × 44 cm × 30 cm) equipped with photodiodes (2.5 cm apart on each side of the box; 16 per side) were used to measure locomotor activity. Locomotion was operationalized as beam breaks, with a beam break defined as breaking every 5th photodiode. Locomotor activity was recorded in 5-min bins using Activity Monitor 5 software (Med Associates Inc.).

All locomotion tests were carried out during the dark cycle. Subjects were removed from the colony in their home cage and brought to the behavioral testing room; during transportation, a dark sheet was draped over the home cage. On the first day of testing, all subjects were allowed 25 min to habituate to the locomotion test chamber before being returned to the colony. The following day, subjects were placed in the chamber for 5 (radiofrequency experiment) or 10 (neurotoxic experiment) minutes to establish baseline locomotion and then given an intraperitoneal (IP) injection of either saline (0.9% NaCl) 0.5 mL/kg or cocaine 15 mg/kg/0.5 mL. After 20 min, subjects were removed from the test chamber and returned to the colony. Six days later, all subjects underwent a second 25-min habituation session, and received a counterbalanced test session; the following day, subjects that received cocaine first received saline and vice-versa (see Fig. 1 for summary).

All subjects received an overdose of Euthasol (130 mg/kg), were tested for deep anesthesia, and were then transcardially perfused with 50 mL of PBS and 250 mL of 4% paraformaldehyde. Brains were removed and stored in 4% paraformaldehyde for 1-h before being transferred to 30% sucrose and stored at 4 °C overnight; after 24-h brains were transferred to another 30% sucrose solution and stored at 4 °C until sectioning. Brains were sliced at 40 μm and stored in cryoprotectant (30% ethylene glycol, 30% sucrose, and 0.0002% sodium azide in 0.1 M phosphate buffer (PB)) at −20 °C.

In order to determine neurotoxic lesion placement, immunohistochemistry was performed to visualize NeuN, a neuronal marker. Brain slices containing the mPOA were washed in 0.1 M PB and incubated in a monoclonal mouse anti-NeuN antibody (1:15000, Millipore, MAB337) overnight. Tissues were further incubated in an anti-mouse biotinylated antibody (1:1000, Vector) for 1-h and enhanced with avidin/biotin (1:1000, ABC kit, Vector). NeuN immunoreactivity was visualized by incubating tissues in a 3,3′Diaminobenzidine (DAB) for 10 min. Between all incubations tissue was washed in 0.1 M PB 4 × 5 min. Slices were then mounted, dehydrated, and cover slipped in preparation for bright-field microscopy.

Radiofrequency lesion placement was determined by staining tissue with methyl green. Brain slices containing the mPOA were mounted on charged slides and soaked in methyl green solution for 5 min. Slides where then dehydrated and cover-slipped in preparation for bright field microscopy.

All statistics were performed using R, version 3.2.2. In order to account for within subject variability, all locomotion data was analyzed as a percent change from baseline (time point prior to the administration of saline/cocaine). For subjects with radiofrequency lesions, a 4 × 2 × 2 mixed factorial ANOVA was performed with surgery (sham/lesion) as a between subject factor, and drug (saline/cocaine) and time (four 5-min intervals after drug administration) as within-subject factors. A two-way interaction between surgery and drug was then decomposed using Welch's two sample *t*-tests (holding drug constant) and paired *t*-tests (holding surgery constant). For subjects with neurotoxic lesions, a 4 × 3 × 2 mixed factorial ANOVA was performed with surgery (sham, bilateral, or unilateral lesion) as a between-subjects factor and drug (saline/cocaine) and time (four 5-min intervals after drug administration) as within-subject factors. A two-way interaction between surgery and drug was then decomposed using one-way ANOVAs (holding drug constant) followed by Tukey's HSD and paired *t*-tests (holding surgery constant). For subjects with either radiofrequency or neurotoxic lesions, a two way interaction between time and drug was decomposed using a repeated measures ANOVA (holding drug constant) followed by Tukey's HSD and a series of paired *t*-tests (holding time constant).

Results indicate that lesions of the mPOA facilitated cocaine-induced locomotor activity in male rats. Specifically, a 4 × 2 × 2 mixed factorial ANOVA performed on subjects receiving radiofre-