Neuropharmacology 62 (2012) 1858–1866

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

The rewarding and locomotor-sensitizing effects of repeated cocaine administration are distinct and separable in mice

Thorfinn T. Riday [a], Barry E. Kosofsky [b,1], C.J. Malanga [a,*]

[a] Laboratory of Developmental Neuropharmacology, Department of Neurology, University of North Carolina at Chapel Hill, USA
[b] Department of Pediatrics, Weill Medical College of Cornell University, The New York Presbyterian Hospital, 525 East 68th St., New York, NY 10021, USA

ARTICLE INFO

Article history:
Received 14 October 2011
Received in revised form
2 December 2011
Accepted 8 December 2011

Keywords:
Intracranial self-stimulation
Brain stimulation reward
Sensitization
GluR1
GluR2
CREB

ABSTRACT

Repeated psychostimulant exposure progressively increases their potency to stimulate motor activity in rodents. This behavioral or locomotor sensitization is considered a model for some aspects of drug addiction in humans, particularly drug craving during abstinence. However, the role of increased motor behavior in drug reward remains incompletely understood. Intracranial self-stimulation (ICSS) was measured concurrently with locomotor activity to determine if acute intermittent cocaine administration had distinguishable effects on motor behavior and perception of brain stimulation-reward (BSR) in the same mice. Sensitization is associated with changes in neuronal activity and glutamatergic neurotransmission in brain reward circuitry. Expression of AMPA receptor subunits (GluR1 and GluR2) and CRE binding protein (CREB) was measured in the ventral tegmental area (VTA), dorsolateral striatum (STR) and nucleus accumbens (NAc) before and after a sensitizing regimen of cocaine, with and without ICSS. Repeated cocaine administration sensitized mice to its locomotor-stimulating effects but not its ability to potentiate BSR. ICSS increased GluR1 in the VTA but not NAc or STR, demonstrating selective changes in protein expression with electrical stimulation of discrete brain structures. Repeated cocaine reduced GluR1, GluR2 and CREB expression in the NAc, and reductions of GluR1 and GluR2 but not CREB were further enhanced by ICSS. These data suggest that the effects of repeated cocaine exposure on reward and motor processes are dissociable in mice, and that reduction of excitatory neurotransmission in the NAc may predict altered motor function independently from changes in reward perception.

1. Introduction

Although more than a century of clinical observations have described tolerance to the euphoric effects of cocaine with compulsive use in humans, animal studies consistently show sensitization to its behavioral effects with repeated exposure. Sensitization to the locomotor-stimulating effects of psychostimulants has been suggested as a behavioral mechanism that may model aspects of human drug addiction, particularly drug craving (Robinson and Berridge, 1993). While some studies have differentiated stimulant effects on reward from their effects on learning mechanisms (Chen et al., 2008), fewer have attempted to examine cocaine effects on both operant and Pavlovian behaviors in the same animals, and investigate cellular adaptations occurring in those animals, during the course of repeated cocaine exposure.

Intracranial self-stimulation (ICSS) is an operant behavior in which animals perform a task to deliver electrical stimulation directly to brain reward circuitry (Carlezon and Chartoff, 2007; Kenny, 2007; Kornetsky and Bain, 1992; Olds and Milner, 1954; Wise, 1998). Drugs of abuse, regardless of pharmacological class, potentiate the rewarding value of brain stimulation-reward, or BSR (Kornetsky and Bain, 1992; Kornetsky and Duvauchelle, 1994). Unlike their effects on locomotor behavior, the effects of psychostimulants (Bauco and Wise, 1997; Frank et al., 1988; Gilliss et al., 2002; Kenny et al., 2003; Wise and Munn, 1993) and opioids (Bauco et al., 1993; Esposito and Kornetsky, 1977) on BSR do not appear to sensitize with repeated exposure.

Cocaine exposure alters synthesis and membrane trafficking of AMPA-sensitive glutamate receptor (AMPAR) subunits in an anatomically specific manner. Increased AMPAR function and GluR1 synthesis in dopaminergic neurons of the mesencephalic ventral tegmental area (VTA) are well-characterized early responses to psychostimulants (Carlezon and Nestler, 2002;

* Corresponding author. Department of Neurology, University of North Carolina at Chapel Hill, Physicians' Office Bldg, 170 Manning Dr., CB 7025, Chapel Hill, NC 27599-7025, USA. Tel.: +1 919 966 1683; fax: +1 919 843 4576.
E-mail addresses: bar2009@med.cornell.edu (B.E. Kosofsky), malangacj@neurology.unc.edu (C.J. Malanga).
[1] Tel.: +1 212 746 3278; fax: +1 212 746 8137.

0028-3908/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2011.12.011

Churchill et al., 1999; Fitzgerald et al., 1996; Saal et al., 2003; Ungless et al., 2001; White et al., 1995; Zhang et al., 1997) but see (Lu et al., 2002).Repeated non-contingent cocaine exposure and withdrawal increases AMPAR synthesis (Churchill et al., 1999; Lu et al., 2003), surface expression (Boudreau and Wolf, 2005) and function (Pierce et al., 1996) in nucleus accumbens (NAc) medium spiny neurons that reverses upon re-exposure to cocaine (Kourrich et al., 2007; Thomas et al., 2001) due to receptor internalization (Boudreau et al., 2007).The transcription factor CRE binding protein (CREB) is a key regulator of cellular processes affecting neuronal excitability and synaptic plasticity in response to drugs of abuse (Carlezon et al., 1998; Dong et al., 2006), and may act in part by regulating expression of glutamate receptor subunits (Olson et al., 2005).

The goal of these experiments was to determine if measurement of locomotor activity and BSR threshold in the same animal could distinguish the effects of repeated, intermittent cocaine exposure on motor behavior and reward processes, respectively. In addition, to determine if BSR leads to similar adaptive changes in neuronal activity we measured expression of proteins involved in glutamatergic transmission (GluR1 and GluR2) and regulation of cellular activity (CREB) in VTA, NAc and dorsolateral striatum (STR), three brain regions implicated in both motor behavior and reward, after repeated ICSS with and without concurrent repeated cocaine exposure.

2. Materials and methods

2.1. Animal Care and Handling

All experimental animal procedures were carried out according to the NIH *Guide to the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill.

2.2. Intracranial self-stimulation (ICSS)

Sixty-two male white Swiss-Webster mice (Taconic Labs) P50-P60 were anesthetized (ketamine/xylazine 120/18 mg/kg i.p.; Sigma) and stereotaxically implanted with insulated monopolar steel electrodes (0.28 mm diameter; Plastics One, Roanoke VA) to the right median forebrain bundle in the lateral hypothalamus using coordinates from Paxinos and Franklin (1996): bregma −2.0 mm (a/p), sagital +0.8 mm (m/l) and depth −4.5 mm (d/v). A steel screw (electrical ground) and the electrode assembly were secured to the skull with dental cement. After recovery mice were individually housed with food and water freely available.

Mice were divided into four experimental groups (A, B, C, and D). Groups A and B mice were used for ICSS experiments while Groups C and D mice served as non-ICSS controls (see below). One week after implantation, mice for intracranial self-stimulation (ICSS) experiments (Groups A and B, Fig. 1) were trained on a continuous (FR-1) schedule of reinforcement for brain stimulation-reward (BSR) in a 16 × 14 × 13 cm operant chamber with a wheel manipulandum and a house light (MedAssociates, St. Albans VT). Each quarter-turn of the wheel earned a 500 ms train of unipolar cathodal square-wave current at a frequency of 158 Hz (pulse width = 100 μs) and activated the house light for 500 ms; subsequent responses during the 500 ms pulse train earned no additional stimulation. Optimal stimulus intensity to sustain responding ≥40 responses/min was determined for each animal (−90 to −220 μA) and was kept constant for all experiments. Mice were then trained with a series of stimulus frequencies in descending order from 158 Hz to 19 Hz in 0.05 $\log_{10}$ increments (i.e., $\log_{10}[112\ \text{Hz}] = 2.05$; $\log_{10}[100\ \text{Hz}] = 2.00$, etc.). At each frequency, five non-contingent priming stimuli were followed by 50 s FR-1 access to BSR during which responses were measured. A 5 s time-out period followed each trial frequency during which responses earned no additional stimulation. Mice were trained to complete four series of 15 trial frequencies (i.e., 1 h daily). The frequency range was adjusted for each mouse such that only the highest 4–6 frequencies sustained responding. For each series of 15 frequencies, operant response rate for BSR was plotted (i.e., the rate-frequency curve). BSR threshold (θ_0) defined as the X-intercept of the least-squares regression line through frequencies that sustained responding at 20, 30, 40, 50, and 60% of the maximal response rate in each series was calculated automatically by custom-designed software. This determination of reward threshold is less sensitive to changes in response rate than other calculations, e.g., EF50 (Miliaressis et al., 1986). Saline injections began when BSR thresholds varied <±10% over three days. During training, sham-ICSS (Group D) mice were placed in the operant chambers, attached to the stimulation apparatus for 1 h daily; and spinning the wheel had no consequences; while surgical control (Group C) mice remained in their home cages and were handled daily.

On each test day, three rate-frequency curves were acquired before and four after injection with saline vehicle or cocaine (Sigma). The first series (warm-up) was discarded; θ_0 and maximum rate from the second and third rate-frequency curves were averaged and used as daily baselines for each animal. Cocaine (15 mg/kg as the free base) was administered in normal saline by intraperitoneal injection. BSR thresholds and maximum response rates were measured for four 15-min series after cocaine or saline injections and expressed as percent changes from baseline. Group D (novelty control) mice were placed in the ICSS chambers for 45 min, injected with saline or cocaine, and returned to the chambers for an additional hour, mimicking testing conditions for ICSS mice (Groups A and B). On experimental day 7, ten ICSS mice (5 each from Groups A and B) and six non-ICSS surgical control mice (Group C) were euthanized and brains were processed for protein determinations. During cocaine abstinence (Days 19–32) Group A and B mice were tested with ICSS and saline injections on alternate days and Group D mice were injected with saline on the same schedule in the ICSS chambers.

2.3. Locomotor behavior

Locomotion was measured in transparent activity monitoring boxes (28 × 28 cm) separated by opaque barriers in a darkened room. Beam breaks of a 16 × 16 infrared array were quantified by commercial software (MedAssociates) and expressed as total distance traveled (cm). All mice were placed in the locomotor boxes for 15 min (habituation), removed and injected with either saline or cocaine (15 mg/kg i.p. as the free base), immediately returned to the boxes, and monitored for 30 min. On experimental day 33 (see Fig. 1) locomotor activity was measured in all non-ICSS mice (Groups C and D) and in half of the ICSS mice (Groups A and B) after cocaine challenge (15 mg/kg i.p.); cocaine effects on BSR were measured in the other half of the Group A and B mice. On day 34, locomotor activity was measured after cocaine in the half of Group A and B mice in which ICSS was measured on day 33, and cocaine effects on BSR in the half that were challenged in the locomotor apparatus on day 33. For each animal, locomotor sensitization to cocaine was

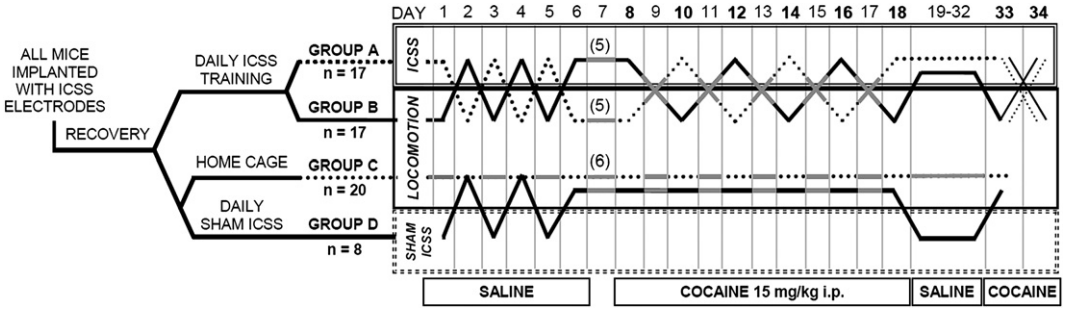

Fig. 1. Diagram of the experimental design for alternating measurement of intracranial self-stimulation (ICSS) and locomotion in mice habituated to saline injections (days 1–6) and given a repeated cocaine regimen (days 8–18) followed by withdrawal (days 19–32) and cocaine challenge. For example, Group A mice received saline and ICSS was measured on Day 1; received saline and locomotion was measured on Day 2; etc. ICSS mice (Groups A and B) received saline and ICSS was measured every other day during cocaine withdrawal (days 19–32). Cocaine challenge (15 mg/kg i.p.) was measured in half of Group A and Group B mice with ICSS on day 33 and with locomotor activity on day 34; the other half were challenged with locomotion measurement (day 33) then ICSS (day 34). Solid gray lines (days 7, 9, etc.) indicate experimental days that mice remained in their home cages. Days numbered in bold (days 8, 10, etc.) indicate experiments in which mice received cocaine. Numbers in parentheses on day 7 indicate mice removed from the protocol for protein determinations shown in Fig. 3. See Materials and methods for details.