Behavioural Brain Research 236 (2013) 78–89

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Research report

A rodent "self-report" measure of methamphetamine craving? Rat ultrasonic vocalizations during methamphetamine self-administration, extinction, and reinstatement

Stephen V. Mahler*, David E. Moorman, Matthew W. Feltenstein, Brittney M. Cox, Katelyn B. Ogburn, Michal Bachar, Justin T. McGonigal, Shannon M. Ghee, Ronald E. See

Department of Neurosciences, Medical University of South Carolina, 173 Ashley Ave, Charleston, SC, 29425 173 Ashley Ave, Charleston, SC, 29425, United States

HIGHLIGHTS

- Rat ultrasonic vocalizations (USVs) were measured during methamphetamine seeking.
- Reinstatement elicited more high frequency USVs than other behaviors.
- Different types of reinstatement elicited different types of USVs.
- "22 kHz" USVs were not elicited by a pharmacological stressor.
- USVs contain complex information, and are a useful measure for addiction studies.

ARTICLE INFO

Article history:
Received 27 April 2012
Received in revised form 5 August 2012
Accepted 16 August 2012
Available online 24 August 2012

Keywords:
Addiction
Ultrasonic vocalizations
Reinstatement
Methamphetamine
Cues
Stress

ABSTRACT

Rats emit ultrasonic vocalizations (USVs) in a variety of contexts, and it is increasingly clear that USVs reflect more complex information than mere positive and negative affect states. We sought to examine USVs in a common model of addiction and relapse, the self-administration/reinstatement paradigm, in order to gain insight into subjective states experienced by rats during various types of methamphetamine seeking. We measured three subtypes of "50 kHz" USVs [flats, trills, and non-trill frequency modulated (FM) USVs], as well as long and short duration "22 kHz" USVs, during self-administration and extinction training, and during reinstatement elicited by cues, a methamphetamine prime, cues + prime, or the pharmacological stressor yohimbine. During self-administration and extinction, rats emitted many flats and FMs, (and short duration "22 kHz" USVs on day 1 of self-administration), but few trills. In contrast, methamphetamine priming injections potently enhanced FMs and trills, and trill production was correlated with the degree of methamphetamine + cue-elicited reinstatement. Cues alone yielded increases only in flat USVs during reinstatement, though a subset of rats displaying strong cue-induced reinstatement also emitted long duration, aversion-related "22 kHz" USVs. Although yohimbine administration caused reinstatement, it did not induce "22 kHz" USVs in methamphetamine-experienced or methamphetamine-naïve rats (unlike footshock stress, which did induce long duration "22 kHz" USVs). These findings demonstrate heterogeneity of rat USVs emitted during different types of methamphetamine seeking, and highlight their potential usefulness for gaining insight into the subjective states of rats in rodent models of drug addiction and relapse.

1. Introduction

In clinical studies, subjects can be queried about the subjective effects of drugs, as well as the affective states and craving they experience during situations that cause relapse to drug use, such as exposure to drug-associated cues, stressors, or "priming" doses of the drug itself [1–3]. In contrast, an inherent limitation of animal models of addiction has been the lack of similar indices of subjective drug effects and drug seeking states.

Behavioral neuroscientists using animal models have access to a wide range of tasks modeling addictive behavior and relapse. One prominent model is the self-administration/reinstatement paradigm. As in humans, drug cues, stressors, and drug primes cause reinstatement of drug seeking in rats [4–6], and therefore can be used to examine a key aspect of addiction—its chronic relapsing nature. However, considerable ambiguity exists regarding the subjective states experienced during drug seeking and relapse, especially in animals. In humans, exposure to drug cues in the

* Corresponding author. Tel.: +1 834 792 5289; fax: +1 843 792 4423.
E-mail address: mahler@musc.edu (S.V. Mahler).

0166-4328/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2012.08.023

absence of drug availability causes both pleasurable, drug-like effects [7–10], as well as subjective distress and negative affect [11–17]. In contrast, the affective states experienced by rodent subjects remain unclear. This issue is of considerable theoretical importance for understanding the psychological substrates of relapse, given that some have emphasized the role of negative affect as a relapse risk [18,19], while others have instead emphasized the incentive motivational properties of drug cues, which need not entail stress-like states [20–24].

Measurement of spontaneously emitted ultrasonic vocalizations (USVs) could offer an opportunity to examine the subjective states of rats in addiction paradigms. Adult rats emit USVs in many situations, which have traditionally been grouped into two main categories—"50 kHz" (usually associated with positive affective or motivational states), and "22 kHz" (usually associated with negative affective states) [25–30].

However, mounting evidence suggests that USVs are much more than simple appetitive and aversive signals, but are in fact complex affective and communicative signals that can reflect motivation, anticipation, aggression, social communication, aspects of sexual behavior, aversion, pain, drug withdrawal, and many other states [27,30–35]. "50 kHz" vocalizations occur in at least 14 different subtypes [33], though differences between the functions of most of these are unknown. This said, there is evidence that "50 kHz" USVs varying little in frequency over time (flats), and those characterized by frequency modulation over time (either with or without a rapidly oscillating "trill" pattern) are differentially produced based upon behavioral context and due to experimental manipulations. For example, administration of psychostimulant drugs preferentially elicits trills and other non-trill frequency-modulated vocalizations (FMs), production of which sensitizes with repeated drug administration [33,36–39], and they have been proposed to reflect positive affective states [27,28]. FMs and trills are also emitted preferentially in "sign tracking" animals during a cocaine conditioned place preference task, and therefore may reflect incentive salience of rewards and their cues [40]. In addition, "22 kHz" USVs are emitted in both long and short durations, which may reflect stronger vs. weaker aversion, respectively [32,41,42].

While there has been some limited exploration of rodent USVs emitted during cocaine self-administration [41,43–45] and reinstatement/relapse [43], no studies to date have examined USVs during methamphetamine taking or seeking, or differences between production of USV subtypes in these tasks. As such, we sought to examine the affective states of rats as measured with spontaneously emitted USVs during methamphetamine self-administration, extinction, and different types of reinstatement.

2. Materials and methods

2.1. Subjects

Male Long-Evans rats ($n = 15$, initial weight 250–300 g, Charles River Laboratories, Raleigh, NC, USA) were individually housed upon arrival from the vendor in a temperature- and humidity-controlled vivarium on a reverse 12 h light–dark cycle (lights off at 06:00). All experimental procedures occurred between 07:00 and 16:00. In the home cage, rats had access to water ad libitum and were maintained on a controlled diet (20–25 g/day) of standard rat chow (Harlan, Indianapolis, IN, USA) for the duration of each experiment. All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Medical University of South Carolina and conformed to Federal guidelines as described in the "Guide for the Care and Use of Laboratory Rats" of the Institute of Laboratory Animal Resources on Life Sciences, National Research Council.

2.2. Drugs

Methamphetamine hydrochloride (Sigma Chemical, St. Louis, MO, USA) was used for self-administration and primed reinstatement procedures. Drugs used for anesthesia in rats that underwent self-administration and reinstatement procedures were ketamine (Vedco Inc, St. Joseph, MO, USA), xylazine (Lloyd Laboratories, Shenandoah, IA, USA), Equithesin (sodium pentobarbital 4 mg/kg, chloral hydrate 17 mg/kg, 21.3 mg/kg magnesium sulfate heptahydrate dissolved in 44% propylene glycol, 10% ethanol solution), and ketorolac (Sigma Chemical, St. Louis, MO, USA). After each self-administration session, catheters were flushed with cefazolin (Schein Pharmaceuticals, Florham Park, NJ, USA) and heparin (Elkins-Sinn, Cherry Hill, NJ, USA). Catheter patency was verified with methohexital sodium as needed (Eli Lilly, Indianapolis, IN, USA). Yohimbine hydrochloride (Sigma Chemical, St. Louis, MO, USA) was used for stress-induced reinstatement.

2.3. Surgery

Animals were anesthetized with IP injections of ketamine (66 mg/kg), xylazine (1.3 mg/kg), and equithesin (0.5 ml/kg). Ketorolac (2.0 mg/kg, IP) was given immediately prior to surgery as an analgesic. Chronic indwelling catheters were constructed as described previously [46]. The end of the catheter was inserted into the right jugular vein and was secured to surrounding tissue with sutures. The catheter ran subcutaneously and exited on the rat's back, posterior to the shoulder blades. An antibiotic solution of cefazolin (10 mg/0.1 ml) was given post-surgery and during recovery. During self-administration, rats received an IV infusion (0.1 ml) of 10 U/ml heparinized saline before each session. After each session, catheters were flushed with cefazolin and heparinized saline. As necessary, catheter patency was verified with methohexital sodium (10 mg/ml dissolved in 0.9% physiological saline), a short-acting barbiturate that produces a rapid loss of muscle tone when administered intravenously. No rats were excluded for loss of catheter patency. Methohexital sodium was administered 0–1 times/rat following self-administration sessions (i.e. 22 h prior to the next session) on rare occasions if methamphetamine intake appeared low.

2.4. Behavioral training and testing procedures

Rats lever pressed for methamphetamine in standard Plexiglas self-administration chambers that were individually enclosed in a melamine sound-attenuating chamber with a ventilation fan (Med-Associates Inc., St. Albans, VT, USA), linked to a computerized data collection program (MED-PC, Med Associates). The chambers were equipped with two levers, a white stimulus light above each lever, a tone generator, and a white house light.

Following three 2 h acclimation sessions in which animals were exposed to the operant conditioning chamber (only the house light was illuminated), rats self-administered methamphetamine (20 μg/infusion, dissolved in sterile 0.9% physiological saline) during daily 2 h sessions on a FR1 schedule of reinforcement. At the start of each session, the catheter was connected to a liquid swivel (Instech, Plymouth Meeting, PA, USA) via polyethylene 50 tubing (0.023″ ID × 0.05″ OD) encased in steel spring leashes (Plastics One Inc., Roanoke, VA, USA). A house light signaled the initiation of the session and remained illuminated throughout it. Presses on the active lever resulted in a 2 s activation of the infusion pump (50 μl bolus) and a 5 s presentation of a stimulus complex (white stimulus light above the active lever and activation of the tone generator; 4.5 kHz, 78 dB). After each infusion, responses on the active lever were recorded and included in analyses, but had no consequences during a 20 s time-out period. Inactive lever