Published in final edited form as:

Behav Brain Res. 2010 December 6; 214(1): 75–79. doi:10.1016/j.bbr.2010.04.057.

Cocaine deprivation effect: Cue abstinence over weekends boosts anticipatory 50-kHz ultrasonic vocalizations in rats

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Abstract

In drug dependence studies, rats are often tested daily with short breaks (such as weekends) spent untested in their home cages. Research on alcohol models has suggested that breaks from continuous testing can transiently enhance self-administration (termed the “alcohol deprivation effect”). The present study explored whether the salience of cocaine-access cues is increased after skipping weekend cocaine and cue exposures. Ultrasonic vocalizations (USVs) of the 50-kHz class are emitted by rats exposed to intravenous cocaine and have been shown to increase with repeated drug exposure at the same dose level (sensitization). The present study found that over the course of several weeks of cocaine self- or yoked-administration *pre*-drug cues signaling forthcoming access or delivery of cocaine elicited marked amounts of anticipatory 50-kHz USVs, and that weekend deprivation from cues and cocaine exaggerated further the level of calling (more calls on Mondays compared to Fridays). Anticipatory USVs extinguished less rapidly when weekend access to unreinforced cues was denied. The results may have clinical implications, in that intermittently avoiding cues or context may enhance drug cue salience and resistance to extinction.

Keywords

addiction; craving; cocaine; ultrasonic vocalization; deprivation effect; expectancy

INTRODUCTION

Cocaine is a highly addictive drug, especially in people vulnerable to overuse. Cues that reliably signal impending drug availability can lead to craving, particularly with extended stimulant drug-taking experience [6, 16, 17, 24, 30–32] and can trigger relapse in abstinent cocaine addicts [37]. In rats, cues that are paired with reinforcing drugs can increase synaptic dopamine and facilitate drug seeking (e.g., lever pressing) and psychomotor

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activity [2, 10–12, 35]. Furthermore, these effects have been shown to persist after cue extinction training [9, 25, 29, 38, 39].

Unlike in people [6], assessing the degree of drug craving in the absence of drug seeking in animals has been challenging. However, accumulating evidence suggests that rat ultrasonic vocalization (USV) in the 35–80 kHz range, termed “50-kHz” USVs, may reflect ongoing positive hedonic affect as well as cue-related anticipation of upcoming highly reinforcing events. For example, sexually experienced male rats reliably emit 50-kHz USVs when exposed to female odors in the absence of the female, and these calls occur also in response to cues that signal other positive reinforcers such as food, social contacts and drugs of abuse [4, 18, 19, 23, 26, 40]. These studies suggest that USVs can be an indicator of a state of “looking forward to” impending reward and perhaps even an index for the level of drug expectation and wanting.

The present study confirmed that anticipatory 50-kHz USVs increase in rats repeatedly exposed to cues that predict upcoming intravenous cocaine access and delivery. In addition, this study showed that these calls increase further on the day following a 2-day period in which the rats were not exposed to the cues or the drug but instead remained in their home cages (deprivation of drug and cue/context).

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (Charles River Laboratories, Inc., Wilmington, MA), 9–10 weeks old, were well-handled and housed in polypropylene cages under a reversed light/dark cycle (8 p.m. – 8 a.m.). Food and water were provided *ad libitum*. Animals were tested in the dark cycle, 5 days per week with weekends off.

Apparatus

One-lever operant chambers (28 cm × 22 cm × 21 cm) were used for lever response training, cocaine conditioning and extinction sessions. To limit external light and noise these chambers were set in sound-attenuating boxes (Med-Associates, St. Albans, VT). For cocaine sessions, the rat’s indwelling intravenous catheter was connected to tubing from a syringe mounted on a motorized pump (Razel Scientific Instruments, Model A, St. Albans, VT). The syringe contained either a solution of cocaine (0.75 mg/kg/injection) or sterile saline. The operant chamber was also equipped with a house light and a stimulus light above the lever. After each lever press, cocaine or saline was administered over a 6-sec infusion time and the stimulus light above the lever illuminated for the duration of the infusion. An ultrasonic microphone (PCB Piezotronics, Buffalo, NY) was positioned over the center of the chamber and USVs were digitally recorded and stored. Photocells were positioned 5 cm from each sidewall and in the middle of the chamber and photobeam interruptions were recorded in 10-min intervals to assess locomotor activity within the chamber in correspondence with USV recordings.

Self-Administration and Yoked Groups

Rats were divided into 4 groups: Cocaine self-administration (SA), Cocaine yoked (Y), Saline SA and Saline Y. SA rats were first trained to press the lever for sucrose pellets and later allowed to self-deliver cocaine or saline. Y rats were matched for dose with one of the SA rats and passively received an identical amount of food pellets, cocaine or saline infusions as their self-administering counterpart.

Lever Response Training

SA rats were trained to lever press for sucrose pellets (45 mg, P.J. Noyes, Lancaster, NH) on a fixed ratio-1 (FR-1) reinforcement schedule. 10-min lever response training sessions occurred over 12 days. Lever presses illuminated a light above the lever, and was immediately followed by delivery of a sucrose pellet. Yoked animals were treated identically except that no lever was made available.

Jugular Catheterization Surgery

Silastic catheters were implanted in all rats under anesthesia consisting of a mixture of oxygen (0.8 l/min; Airgas Southwest, Corpus Christi, TX) and isoflurane (2.5–4%; AErrane, Baxter Healthcare, Deerfield, IL) delivered via a gas delivery system (VetEquip, Inc, Pleasanton, CA). Catheters were made from 8.5 cm Silastic tubing (0.64 mm o.d.) with one end connected to a cannula endpiece (Plastics One, Roanoke, VA). The catheter was inserted into the jugular vein on one side of the body. The endpiece of the cannula was subcutaneously passed to an incision on the head. Four stainless steel screws and dental acrylic cement were used to anchor the catheter to the dorsal surface of the head. To prevent infection, 3 drops of gentamicin sulfate (50 mg/ml) were applied to the jugular incision before closing the suture. Carprofen (5 mg/kg, s.c.), an anti-inflammatory agent, was also administered immediately after the surgical procedure. One-week recovery from surgery was allowed before starting conditioning sessions. To maintain catheter patency, the catheters were flushed daily with 0.1 ml heparinized saline (1 U/ml heparin). The antibiotic Timentin (100 mg/1.5 ml) was added to the flushing solution during the first week post-surgery.

Conditioning and Extinction Sessions

Chamber lights were turned off during the 10-min pre-drug anticipation intervals signaling impending cocaine or saline availability. Immediately following these intervals, the house light was illuminated and, at an unpredictable time point within a 30 sec span, the lever was presented to SA rats, indicating that they were allowed to lever press for cocaine or saline over a period of 60 minutes. During this session, Y rats received unpredictable cocaine or saline injections according to the rate of lever pressing by their self-administering counterparts. Conditioning and extinction sessions were carried out 5 days per week, with no sessions occurring on weekends. After 4 weeks of conditioning, extinction sessions were conducted for 4 weeks, in which all lever presses yielded delivery of only saline (0.1 ml/infusion) to all groups.

Data Collection

USVs and locomotor activity were recorded every weekday (Monday-Friday) in operant chambers during the 10-min intervals prior to cocaine or saline availability or extinction sessions. Over the 2-day weekends, animals remained in the vivarium environment in their home cages. During experimental sessions, motor activity was recorded by MED-PC software (Med Associates, St. Albans, VT) and USVs were recorded using ultrasonic microphones (PCB Piezotronics, Buffalo, NY; working frequency range: 5–126,000 Hz) mounted in the center of the operant chamber, inaccessible to the rat. The maximum distance of the microphone to any point within the chamber was approximately 26 cm. Therefore, as the rat was freely moving within the chamber at all times, the proximity between the microphone and rat varied during each session, but was always less than 26 cm. RECORDER multi-channel recording software and SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany) were used to record and assess rat USVs. Sample 50-kHz USVs sonograms and slowed-down calls can be viewed and heard at the following web

sites: www.schallertlab.org (Media section) and <http://www.utexas.edu/pharmacy/divisions/pharmtox/faculty/duvauchelle2.html>.

Drug

Cocaine HCl (0.75 mg/kg/infusion) in sterile 0.9% sodium chloride was adjusted daily according to body weight such that cocaine-reinforced lever responses led to delivery of 0.1 ml cocaine solution at the 0.75 mg/kg dose.

RESULTS

USVs and locomotor activity were recorded during the 10-min intervals prior to each Conditioning and Extinction session. Because the primary aim of the present study was to examine whether a 2-day (weekend) respite from exposure to the conditioning chamber affected the number of anticipatory USVs, we analyzed pre-drug session (cue only) data across all Fridays vs. Mondays during Conditioning Sessions and Extinction Sessions. We also compared anticipatory USVs and locomotor activity data on Fridays with those on Tuesdays, Wednesdays and Thursdays and confirmed that each of these values did not significantly differ.

Two-way ANOVAs showed no significant differences in 50-kHz USVs or locomotor activity levels between SA and Y groups during the pre-drug session intervals for either the Cocaine or Saline conditions. Therefore, cocaine-receiving groups were combined and the saline groups were combined (SA + Y Cocaine and SA + Y Saline) for analyses. Two-way ANOVAs compared effects of Treatment (Cocaine vs. Saline) with repeated measures on Days (Friday vs. Monday).

Conditioning Sessions

50-kHz USVs—Two-way repeated measures ANOVA showed significant Treatment ($F(1,23) = 11.49$; $p = 0.003$), Day ($F(1,23) = 9.327$; $p = 0.006$) and Treatment $\times$ Day Interaction effects ($F(1,23) = 6.579$; $p = 0.017$). Posthoc tests revealed that USVs elicited during pre-drug intervals on both days (e.g., Fridays and Mondays) were significantly higher in the “cocaine-expected” condition compared to the “saline-expected” condition. In addition, the cocaine rats showed significant enhancement in the number of USVs on Mondays compared to Fridays ($p < 0.01$), but the saline rats did not (see Figure 1).

Locomotor Activity—Two-way repeated measures ANOVA showed significant effects of Treatment ($F(1,23) = 20.38$; $p = 0.000$), but no significant differences were observed for Day ($F(1,23) = 2.418$; n.s.) or Treatment $\times$ Day interaction ($F(1,23) = 0.44$; n.s.). Thus, exposure to cues/context increased locomotion in the cocaine-expected condition compared to the saline-expected condition, but in contrast to USV results, there was no detectable enhancement after weekend cocaine/cue deprivation (see Figure 2).

Extinction Sessions

50-kHz USVs—Two-way repeated measures ANOVA showed significant effects of Treatment ($F(1,21) = 8.36$; $p = 0.009$) and Day ($F(1,21) = 8.828$; $p = 0.007$), but no significant Treatment $\times$ Day interaction effects ($F(1,21) = 2.82$; n.s.). Extinction training significantly reduced cue-elicited USVs, but the level of calling remained higher in the cocaine-expected condition compared to the saline-expected condition. In addition, the level of calling in the cocaine-expected condition was significantly higher on Mondays compared to Fridays (see Figure 3).

Locomotor Activity—Two-way repeated measures ANOVA showed significant Day ($F(1,21) = 5.519$; $p = 0.034$), but not Treatment ($F(1,21) = 1.32$; n.s.) or Treatment $\times$ Day interaction effects ($F(1,21) = 0.127$; n.s.). As shown in Figure 4, Cocaine and Saline groups showed comparable levels of locomotion during extinction training and the effects of weekend deprivation were minor at best.

DISCUSSION

50-kHz USVs and locomotor activity were increased in anticipation of cocaine exposure and decreased in anticipation of extinction sessions. However, a brief period of home cage confinement (over weekends) during the cocaine conditioning phase substantially enhanced the number of cue-induced USVs (i.e., more calls on Mondays than on Fridays), but not locomotor activity. Thus, under these conditions, anticipatory vocalization counts were a more sensitive measure of the cocaine deprivation effect than were locomotor activity scores. Even during extinction training it was apparent that a 2-day confinement to the home cage potentiated the number of USVs when the animals were placed into the previously drug-paired operant chamber.

A close examination of the data revealed that the potentiation of USVs was significant even on the first Monday following a 2-day absence from the drug-paired environment and did not escalate with each successive abstinence interval. These findings indicate that learning through repeated abstinence experience was not required for the observed USV effect and further suggest that cocaine abstinence may lead directly to enhanced incentive salience of drug context. These data are in line with previous work reporting that alcohol- and nicotine-associated environments provoked relapse behaviors [21, 22, 27]. However, our findings with cocaine conditioning are not fully consistent with conditioned heroin effects, as contextual cues associated with heroin withdrawal did not motivate drug seeking unless heroin reinforcement had been made available in the withdrawal state. Under these circumstances, learned associations between rescue from withdrawal symptoms and/or relief from conditioned withdrawal effects and a heroin-associated context appeared to enhance relapse behaviors with successive returns to the heroin-associated environment [13].

Rats given free-access to alcohol followed by a period of imposed abstinence show a transient but pronounced increase in alcohol intake upon renewed alcohol availability, commonly termed the “alcohol deprivation effect” [34]. A similar effect may have been found with nicotine reinforcement [28]. Although no anticipatory behaviors were assessed in these studies, repeated cycles of deprivation and access to drugs increased the magnitude and duration of reinstated consumption [3, 33]. These data have led to the paradoxical suggestion that, in drug-dependent individuals, avoiding drug cues or context when trying to quit may actually promote craving or obsession and perhaps impede continued drug abstinence. The expression of the alcohol deprivation effect is proposed to be an animal model of alcohol craving [15] and has previously been used to investigate pharmacological treatments for preventing relapse [14]. Therefore, the measurement of USVs elicited during cocaine or other drug cue presentation may be useful for testing craving in animal studies and for exploring potential neural mechanisms of plasticity underlying learning and memory of hedonic events.

50-kHz USVs are also emitted in response to cues that have come to predict the opportunity to mate or self-stimulate the medial forebrain bundle [4, 5, 7, 8], as well as during exposure to the side of a chamber in which morphine or amphetamine was delivered (conditioned place preference) [19]. This class of USVs also increases *during* drug exposure and remains high persistently with repeated experience, perhaps reflecting sensitization and an enhancement of the reinforcing properties of the drug and/or its context [1, 26]. It is

important to note that long 22-kHz calls, which have been linked to aversive events [20], were not detected in any group of rats, not even during extinction sessions.

Drug obsession is an important characteristic of drug dependence [36]. To the extent that the number of 50-kHz USVs in rats parallels drug cue salience and the potency of drug cues and context to engage drug expectation and incentive motivation, inclusion of this measure in pre-clinical studies may help to improve their relevance in translational drug dependence research.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH Grants DA014640 and DA014640-05S1, The University of Texas VP Research Office (C.L.D.), NIDA Drug Supply Program, and The University of Texas Waggoner Center for Alcohol and Addiction Research Bruce-Jones Graduate Fellowships and NIH Training Grant T32AA07471 (E.Y.M., A.M.A.). We thank Leah McAleer and Mohamed Abdalla for their help in running the experiments and Neha Thakore, Tiffany Nguyen, Hunter Owen, Proy Phongsawad, Helen Reed, Byron Barksdale, Tian Tian, Linda Ju, Rachel Chavana and Rosie Maddox for their assistance in USV data analyses. The data were collected from the same animals included in recent publication [23], although different data were extracted.

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Figure 1. Conditioning Sessions: Cocaine and Cue Abstinence-Enhanced USVs

Expectation of forthcoming cocaine exposure, compared with saline expectation, was associated with a marked increase in USVs. This effect was significantly enhanced on Mondays (relative to Fridays) following a weekend of abstinence in the home cage. Thus, keeping rats away from cues and context for 2 days was sufficient to boost the level of calling once they returned to the conditioning chambers. (** = significantly greater number of USVs on Monday compared to Friday; $p < 0.01$; Paired Samples T-test); ^ = significantly greater number of USVs compared to matched Saline Day at $p < 0.01$; Independent Samples T-test).

Figure 2. Conditioning Sessions: Influence of Cocaine and Cue Abstinence on Locomotor Activity

Cocaine-experienced animals showed significantly higher locomotor activity than the Saline group on both Friday and Monday pre-drug intervals (^^ = significant difference compared to matched day Saline values at $p > 0.01$; Independent Samples T-test). However, locomotor activity in the Cocaine-experienced rats did not significantly differ as the result of two days of cocaine and cue abstinence.

Figure 3. Extinction Sessions: Effects of Cue and Cocaine Abstinence on USVs

Extinction training reduced the number of cocaine-anticipatory USVs, but there were significantly more USVs on both Fridays and Mondays compared to the Saline rats (^, ^^ = significant difference compared to matched day Saline values at $p < 0.01$ and 0.05 , respectively; Independent Samples T-test). In addition, enhancement of calling on Mondays relative to Fridays following weekend home cage confinement was still significant for the Cocaine-experienced animals (** = significant difference at $p < 0.01$; Paired Samples T-test).

Figure 4. Extinction Sessions: Effects of Cue Abstinence on Locomotor Activity

During Extinction training, overall activity between the Cocaine and Saline groups were not significantly different, but the cocaine-expected condition had slightly enhanced locomotor activity following two days of cue and drug abstinence (* = significant difference at $p < 0.05$; Paired Samples T-test).