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Repeated Intravenous Cocaine Experience: Development and Escalation of Pre-Drug Anticipatory 50-kHz Ultrasonic Vocalizations in Rats

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Abstract

Ultrasonic vocalization (USV) in the 50-kHz range occurs in rats immediately upon first-time exposure to cocaine or amphetamine, and rapidly increases with repetitive drug exposure at the same dose. This sensitized positive-affect response to these drugs of abuse is persistent in that the peak level of USVs again appears when the drug is reintroduced after several weeks of drug discontinuation. The present study explored whether with enough experience USVs might be elicited, and gradually escalate, in anticipation of impending drug delivery. Rats were trained to self-administer (SA) cocaine intravenously by lever pressing 5 days per week for 4 weeks. Yoked rats received experimenter-delivered cocaine matching that of SA rats. USVs and locomotor activity were recorded during each 10-min period prior to 60-min drug access sessions. Extinction trials in which drug access was denied were then carried out over an additional 4-week period. After about a week of cocaine experience, both the SA and Yoked groups began to progressively increase USVs when placed in an environment that predicted forthcoming drug exposure. Extinction of anticipatory calls and locomotion occurred over days after drug access ended. USVs may be a useful model for specifically investigating the neural basis of drug anticipation and aid in developing and assessing new addiction treatment strategies for reducing craving and relapse.

Keywords

addiction; craving; cocaine; ultrasound; sensitization; self-administration

INTRODUCTION

Drug dependence is characterized by a pathological obsession to consume a drug. In susceptible individuals, predisposing vulnerability factors that facilitate stimulus-reward associative

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learning may influence the progression from controlled social drug use to compulsive drug abuse [23,37]. Pairing environmental stimuli with stimulant drugs can lead to marked strengthening of drug-cue associations [35,36]. With extended experience, exposure to drug-paired cues alone can increase activity in midbrain and forebrain dopamine systems, enhance locomotion [15,49] and promote drug self-administration [32,50]. In addition, drug cues can acquire incentive-motivational value [43]. For example, cues previously associated with cocaine can become conditioned reinforcers, meaning that rats will lever press for presentation of the cue alone even when it is no longer accompanied by the drug [14,16]. Learned drug-cue associations elicit strong ratings of craving in human cocaine dependents and have been reported to trigger relapse during abstinence [48]. This suggests that the formation of drug-cue associations has a high impact on the development, progression and treatment of cocaine dependence.

In humans, drug craving and/or anticipatory attention very often occur, and increase, even in the absence of overt drug seeking behaviors (e.g., when trying to quit). Their occurrence and intensity can be estimated by verbal or checklist responses, but have been difficult to model adequately in animals. In rodents, increases in locomotor activity, rotational behavior (in the unilateral DA depletion model) and repetitive stereotyped movements (collectively termed “psychomotor” activity) have been proposed by many to reflect drug appetitive behavior and motivation [3,17,18,34]. However, relationships between behavioral activity and motivational processes are indirect at best. Yet, it is possible that a class of ultrasonic vocalizations (USVs) may be an additional and very useful marker of anticipation of drug availability, or even craving.

Rats vocalize in ultrasonic frequencies that serve a social and communicatory function and express subjective emotional states [5,26]. Long calls (>0.3 s) in the 22-kHz range are thought to reflect negative emotional states associated with anxiety and distress, since these calls are elicited by aversive stimuli such as predatory odors, footshock cues, social defeat, and aversive drugs [26,51]. Conversely, short calls (<0.3 s) in the 50-kHz range are thought to reflect a state of positive affect associated with appetitive behavior, since these calls are increased during or in anticipation of mating, social play, or food presentation and are associated with reinforcement-related dopamine activity [4,8,24,26]. For example, very large increases in 50-kHz range calling have been elicited by electrical brain stimulation in areas that support self-stimulation [9] and by systemic injections of cocaine [30] or d-amphetamine [2]. Caffeine, at doses that increase locomotion, does not evoke USVs, in contrast to amphetamine, which does [41]. 50-kHz USVs elicited by natural or pharmacological reward can be blocked or acoustically degraded by the loss of midbrain dopamine neurons or pretreatment with dopamine receptor antagonists [9,11,12]. Increases in the emission of 50-kHz calls have been linked to environments previously paired with drugs of abuse. In conditioned place preference (CPP) studies rats emit more 50-kHz calls in the drug-paired side of a CPP chamber than in the vehicle-paired side [25], and the number of 50-kHz calls elicited by an acute intracranial opioid injection predicts the subsequent development of CPP for the drug-paired side [9]. In addition, not only are 50-kHz calls in male rats emitted when odor cues of sexually receptive females are presented, these calls increase dramatically with repeated mating experience [4,12]. Recent research indicates that 50-kHz calls occur immediately and very rapidly increase with repeated exposure to cocaine or amphetamine during the periods in which the drugs are on-board [2, 30]. However, the appearance and gradual *escalation* of USV production during *pre-drug* periods as the rats learn to expect drug availability has not yet been demonstrated. Indeed, investigators examining USVs across 5 days of cocaine sessions did not observe cocaine anticipatory USVs [30]. We hypothesized that more trials might be needed for adequate conditioning to take place.

The primary goal of the present study was to examine whether, with repeated sessions of cocaine administration, 50-kHz calls will progressively increase in anticipation of impending cocaine delivery and, with extinction, decrease in anticipation of not receiving cocaine. USVs may become a useful adjunct model for specifically investigating neural changes associated with drug anticipation (“looking forward to” drug delivery). If so, this measure could become an important tool to design and confirm the effectiveness of treatment strategies aimed at reducing drug associations, wanting, craving and/or relapse.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) were housed in an animal colony in polypropylene cages (45 × 23.75 × 20 cm) under a 12:12 reversed light/dark cycle (lights off at 8 am). Prior to lever response training, rats were handled for 10 minutes a day for a period of 2 weeks, with food and water provided *ad libitum*. Animals were approximately 9–10 weeks old at the start of cocaine conditioning sessions. Sessions were conducted daily between 10 am and 2 pm, 5 days a week.

Apparatus

Lever response training, cocaine conditioning and extinction sessions were conducted in one-lever operant chambers (28 × 22 × 21 cm) housed within sound-attenuating boxes (Med-Associates, St. Albans, VT) to limit outside noise and light. During the administration sessions, the animals were intravenously connected via tubing to a syringe mounted on a motorized pump (Razel Scientific Instruments, Model A, St. Albans, VT) containing either a cocaine solution (0.75 mg/kg/injection) or sterile saline. After each lever press, cocaine or saline was administered over a 6-sec infusion time and a stimulus light above the lever blinked for the duration of the infusion. The operant chamber was also equipped with a house light and 3 sets of photocells; two located 5 cm from each sidewall and one in the middle of the chamber. The number of interruptions of the photobeams was assessed in 10-min intervals as a measure of locomotor activity. For USV recordings, an ultrasonic microphone (PCB Piezotronics, Buffalo, NY) with a flat frequency response ranging from 10-kHz to 100-kHz, was securely mounted over the center of the operant chamber. Recordings were then digitally stored and analyzed later.

Self-Administration and Yoked Groups

After the handling phase, the animals were divided into Self-Administration (SA) and Yoked (Y) groups. In the SA groups, rats were trained for lever response (see below) and later allowed to self-administer cocaine or saline. In the Y groups, rats were matched with a member of the SA group and passively received the same amount of treatment (food pellets, cocaine or saline infusions) as its self-administering counterpart.

Lever Response Training

Food-restricted animals in the SA group learned to lever press for sucrose pellets (45 mg) on a fixed ratio-1 (FR-1) schedule of reinforcement. All rats had 12 days of 10-min lever response training sessions on the FR-1 schedule in which each lever response resulted in illumination of a stimulus light (1 sec) above the lever and delivery of a sucrose pellet. Food-restricted yoked animals were placed into the operant chamber under the same conditions, in the absence of the lever, and received the same daily schedule of sucrose pellet delivery as their lever-pressing counterparts. After the 12 days of lever response training, all animals underwent jugular catheterization surgery.

Jugular Catheterization Surgery

All animals were surgically implanted with a Silastic catheter under anesthesia composed 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 through a gas delivery system (VetEquip, Inc, Pleasanton, CA). Catheters were constructed 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 right jugular vein and the cannula endpiece was passed subcutaneously to an incision on the head. The catheter was anchored to the top of the head with four stainless steel screws and dental acrylic cement. Just prior to the closing suture, gentamicin sulfate (50 mg/ml) was applied (3 drops) into the jugular incision to prevent infection. The anti-inflammatory agent, carprofen (5 mg/kg, s.c.) was given as a prophylactic for post-surgical pain. Rats were allowed to recover from the surgery for one week prior to starting the cocaine conditioning sessions. During this time, catheters were flushed daily with 0.1 ml of a solution containing an antibiotic (Timentin; 100 mg/1.5 ml) dissolved in heparinized saline. Animals continued to receive the same solution daily without the antibiotic component through the duration of the experiment to maintain catheter patency.

Conditioning and Extinction Sessions

At the start of each Conditioning session, the animals were placed into the chambers and connected to the drug delivery tubing. The chamber remained dark for a 10-min interval prior to the start of cocaine or saline availability. During this time, the lever was withdrawn from the chamber to ensure that no opportunities for lever responding were possible. At the end of this interval, a house light within the chamber illuminated, the lever extended into the chamber, and SA rats were able to perform lever presses for cocaine or saline over a period of 60 minutes. Each lever press resulted in a cocaine or saline infusion to themselves and to their yoked counterpart. Extinction sessions commenced the day after the 20th Conditioning session (e.g., Day 21). During Extinction, the house light and lever were activated exactly as in Conditioning sessions, but all lever responses by the SA rats resulted in saline infusions (0.1 ml) for themselves and their yoked counterparts. Conditioning and Extinction sessions were conducted 5 days per week, for approximately 8 weeks (e.g., 20 Conditioning and 19 Extinction sessions).

Data Collection

USV and locomotor activity (photobeam interruptions) data were collected during each 10-min (dark) interval prior to cocaine availability or extinction sessions. Locomotor activity was recorded by MED-PC software (Med Associates, St. Albans, VT). USV data were captured by ultrasonic microphones (detection range = 10 – 100 kHz), recorded by RECORDER multi-channel recording software and analyzed by SASLab Pro analyzing software (Avisoft Bioacoustics, Berlin, Germany).

Drugs

Cocaine HCl (0.75 mg/kg/injection, RTI International, Triangle Park, NY) was diluted in sterile 0.9% sodium chloride daily and adjusted according to each individual rats' weight so that each cocaine-reinforced lever response resulted in a 0.1 ml delivery of cocaine solution at the 0.75 mg/kg dosage.

Statistical Analyses

USVs and locomotor activity were recorded during the 10-min periods prior to each Conditioning and Extinction session. 50-kHz USV data and locomotor activity were analyzed using two-way repeated measures ANOVA (e.g., Group $\times$ Days), with "Days" as a within-subjects variable and "Group" (SA Cocaine, Yoked Cocaine, SA Saline and Yoked Saline) as a between-subjects variable. Due to equipment or computer tabulation error, 9 out of 1,833

USV data points and 12 out of 1,833 locomotor activity intervals were lost. In these cases, group means were inserted so that collected data from each animal could be included in overall analyses. Significant interaction effects were further analyzed using post hoc tests (Fishers LSD) with significant comparisons reported as $p < 0.05$ or $p < 0.01$.

RESULTS

Acquisition

50-kHz USVs—There was a significant increase in the number of 50-kHz USVs elicited in the cocaine-paired context (during the pre-drug interval) over repeated days of drug exposure (see Fig 1). Two-way repeated measures ANOVA comparing all treatment groups during the interval prior to cocaine availability showed significant Group [$F(3,21)=3.92$; $p=0.023$], Day [$F(19,399)=5.296$; $p<0.001$] and marginal Group $\times$ Day interaction effects [$F(57,399)=1.337$; $p=0.060$]. Post hoc analyses revealed that both cocaine-receiving groups (SA and Yoked Cocaine) elicited significantly more USVs prior to cocaine Conditioning sessions than both control groups (SA and Yoked Saline) prior to saline availability (see Fig 1). Sample USVs sonograms and slowed-down calls can be viewed and heard at the following web sites: <http://www.utexas.edu/pharmacy/divisions/pharmtox/faculty/duvauchelle2.html> and www.schallertlab.org (Media).

Locomotor Activity—In the 10-min interval prior to cocaine availability, overall locomotor activity was higher in rats about to receive cocaine compared to Controls (see Fig 2). Significant Group [$F(3,21)=8.647$; $p=0.001$] and Group $\times$ Day interaction [$F(57,399)=1.727$; $p=0.002$], but no significant Day effects [$F(19,399)=1.147$; n.s.] were observed. Post hoc analyses revealed SA Cocaine rats had the highest activity levels overall and were significantly greater than both Saline groups ($p < 0.01$). Locomotor activity in the Yoked Cocaine group was significantly higher than the Yoked Saline group ($p < 0.01$) and (marginally) significantly greater compared to the SA Saline group ($p = 0.065$; see Fig 2).

Extinction

50-kHz USVs—During the 10-min intervals just prior to Extinction sessions, the cocaine anticipatory USVs significantly decreased once cocaine was no longer available during Extinction sessions (see Fig 3). A two-way repeated measures ANOVA revealed significant Group [$F(3,19)=4.54$; $p=0.015$], Day [$F(18,342)=5.69$; $p<0.001$] and Group $\times$ Day interaction effects [$F(54, 342)=1.71$; $p=0.002$]. Post hoc analyses indicated that across extinction sessions, USVs were significantly greater in the Yoked Cocaine (but not SA Cocaine) group than either Saline group. Fig 3 shows cocaine anticipatory USVs in the SA Cocaine group dropping to control levels before USVs in the Yoked Cocaine group (see Fig 3).

Locomotor Activity—In the 10-min intervals prior to the Extinction sessions, cocaine-conditioned locomotor activity in the SA Cocaine group decreased across sessions but remained significantly greater overall than the Yoked Saline group ($p < 0.01$) (see Fig 4). Two-way repeated measures ANOVA showed significant Day [$F(18,342)=3.586$; $p<0.001$], marginal Group [$F(3,19)=2.954$; $p=0.059$], but no significant Groups $\times$ Day interaction effects [$F(54,342)=0.785$; n.s.].

DISCUSSION

Daily cocaine experience led to a delayed and gradual increase in anticipatory 50-kHz vocalizations. After 4 weeks cocaine was discontinued, and over the course of extinction, USVs decreased as the animals learned to no longer expect drug delivery despite unchanging contextual cues. Together, the acquisition data can be reasonably attributed to the development

of cue-related anticipation of cocaine availability, and the extinction data to dampening drug expectation.

Previous reports of USV sensitization with repeated exposure to amphetamine or cocaine were limited to the drug exposure periods [2,30]. This is the first demonstration that these calls also increase *progressively* in response to situational cues (e.g., placement in the drug-delivery environment) that predict impending drug exposure. 50-kHz calls are associated with positive affect in a variety of non-drug situations [7,26]. As noted previously, with sexual experience, 50-kHz calls increase dramatically in response to cues that predict the opportunity to mate [4,11,12]. These reports are consistent with the possibility that this class of calls may reflect learning-enhanced anticipation of, attention to, or perhaps craving for cocaine. Burgdorf and associates [6] reported that rats that make more 50-kHz range calls in anticipation of 10-min of rewarding brain stimulation are more likely to engage in self-stimulation behavior than rats that do not emit anticipatory USVs. Thus, increases in the number of USVs may reflect enhanced incentive salience. Because drug craving/wanting is an important component in the development and persistence of drug dependence [10,35], USVs may be a highly useful method for estimating the extent of drug wanting in animals [31].

Other work indicates that the mode of administration of drugs of abuse (e.g., self-administered versus yoked delivery) can have different influences on drug-induced behavior [27] and neurochemical events in mesolimbic pathways of the brain [21,27,29]. In the present study, USV recordings revealed comparable escalation of 50-kHz USV in anticipation of cocaine administration between rats contingently or non-contingently receiving cocaine. Indeed, our findings suggest that the cocaine-receiving environment serves as a Pavlovian contextual cue for either 1) both the SA and Yoked Cocaine groups, or 2) just the Yoked rats [20], while the SA Cocaine group associates the cocaine environment as an occasion-setter for operant responding [22]. In either case, since both groups showed anticipatory positive USVs in the cocaine context, it is clear that these USVs were not elicited only in anticipation of reinforced operant responding. However, since USVs in the 50-kHz range are associated with positive affect [8,26], these findings may appear at odds with a recent study reporting that yoked cocaine delivery can be aversive and inhibit future cocaine-seeking behavior [44]. Yet, it remains possible that drug anticipation/seeking may have underlying neural substrates that differ from that occurring during actual drug delivery. In the present study, no long-duration 22-kHz USV calls, which are thought to reflect aversive affect [5], were detected during the reported 10-min drug-free intervals. In any event, the progressive increase in anticipatory USVs is consistent with studies that have demonstrated long-lasting CPP with chronic psychostimulant exposure [39,45]. Knutson and associates [25] have argued that 50-kHz USVs may be more sensitive than CPP in estimating the degree of drug preference (especially for psychostimulants that are less potent than cocaine in the central nervous system). It is likely that CPP and USVs, both of which may assess drug cue salience without the drug being on-board, together may provide more useful information than either alone. In addition, the reduction in USV calls during extinction supports the notion that this measure directly reflects both learned anticipation of forthcoming drug and unrewarded events. These data further suggest that USVs may be a reliable measure of the presence or absence of cocaine anticipation. It should be noted that during the Extinction phase, the Yoked Cocaine group received non-contingent saline injections and did not show as rapid a decline in anticipatory USVs compared to the SA Cocaine group. These findings suggest that USVs conditioned by Yoked delivery of cocaine is resistant to extinction, or that Yoked delivery of saline in a cocaine-associated environment does not suppress conditioned USVs as efficiently as does non-reinforced operant responding.

As expected, we found that locomotor activity during the 10-min periods prior to drug access was significantly greater in animals with cocaine experience. Others report enhanced behavioral activity in a cocaine-associated environment [1,3,28]. Cocaine-induced motor

activity may be influenced, at least in part, by the activation of neural circuits known to support rewarding brain stimulation and drug reinforcement [9], though it is unclear whether anticipatory locomotor activity is mediated by similar anatomical substrates [40]. In the present study, locomotor activity was electronically tabulated as the number of detected photobeam interruptions within the operant chamber, rather than by direct observation of behavior activity. Future research should address the possibility that qualitative analysis of cue-related motor activity may reveal, as with USV production, a more specific form of anticipatory behavioral activity that reliably increases incrementally with drug exposure.

Escalating motivation for and cognitive obsession about a drug over time are considered key features of addiction [13]. It is possible that with more extensive experience, 50-kHz USVs in rodents, along with sign-tracking [19,46], CPP [33,38] motivation to overcome obstacles or to work hard to gain access to a drug [13,47] and related assessments [42], may become a major indicator of the presence and extent of drug incentive motivation, hedonic state, drug anticipation and/or wanting. If so, then USVs may serve as pre-clinical model of craving that will help to develop treatments to block impulsive drug taking, relapse and obsessive attention to cues associated with drugs of abuse.

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Figure 1. Anticipatory USV Acquisition

50-kHz USVs (mean $\pm$ SEM) of SA (self-administering) and Yoked rats recorded during daily (Days 1–20) 10-min intervals prior to Conditioning sessions for SA Cocaine (filled circle, $n = 7$), Yoked Cocaine (filled trident, $n = 6$), SA Saline (open circle, $n = 6$) and Yoked Saline (open trident, $n = 5$) groups. Mean USVs across all Conditioning sessions were significantly greater in both Cocaine groups compared to either Saline group. Posthoc comparisons (LSD) revealed differences between groups on specific days. *, ** = significantly more USVs elicited than either or both Saline conditions; $p < 0.05$ or $p < 0.01$, respectively. No long-duration 22-kHz calls were detected in any animals during this interval.

Figure 2. Conditioned Locomotor Activity During Acquisition

Locomotor activity (photobeam interruptions) recorded during 10-min intervals prior to Conditioning sessions. Data presented as the mean ($\pm$ SEM) daily intervals for SA Cocaine (filled circle) Yoked Cocaine (filled trident), SA Saline (open circle) and Yoked Saline (open trident) groups (same animals as in Fig 1). Mean locomotor activity across all pre-Conditioning intervals was significantly greater in the SA Cocaine group than either Saline group, and in the Yoked Cocaine group compared to the Yoked Saline group ($p < 0.01$). Posthoc comparisons (LSD) revealed group differences on specific days. *, ** = significantly greater daily mean than either or both Saline conditions; $p < 0.05$ or $p < 0.01$, respectively. ^ = significantly greater than Yoked Cocaine group; $p < 0.05$.

Figure 3. Anticipatory USVs During Extinction

50-kHz USVs (mean $\pm$ SEM) of SA (self-administering) and Yoked rats recorded during daily (Days 21–39) 10-min intervals prior to Extinction sessions for SA Cocaine (filled circle, $n = 6$) Yoked Cocaine (filled trident, $n = 6$), SA Saline (open circle, $n = 6$) and Yoked Saline (open trident, $n = 5$) groups. Mean USVs across Extinction sessions were significantly greater in the Yoked Cocaine (but not SA Cocaine) group than either Saline group. Posthoc tests (LSD) revealed that USVs in the Yoked Cocaine group (but not the SA Cocaine group) remained significantly higher than controls over several days. *, ** = significantly greater daily mean than either or both Saline conditions; $p < 0.05$ or $p < 0.01$, respectively. No long-duration 22-kHz calls were detected in any animals prior to Extinction sessions.

Figure 4. Conditioned Locomotor Activity During Extinction

Locomotor activity (photobeam interruptions) recorded during 10-min intervals prior to Extinction sessions. Locomotor activity data presented as the mean ($\pm$ SEM) daily intervals for SA Cocaine (filled circle) Yoked Cocaine (filled trident), SA Saline (open circle) and Yoked Saline (open trident) groups (same animals as in Fig 3). Over the course of Extinction sessions, locomotor activity in the SA Cocaine group decreased, but was significantly greater overall compared to the Yoked Saline group ($p < 0.01$).