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ORIGINAL INVESTIGATION

Dose-dependent differences in short ultrasonic vocalizations emitted by rats during cocaine self-administration

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Abstract
Rationale The motivational impetuses underlying self-administration of cocaine and other drugs of abuse are not fully understood. One emerging factor is affect. Both positive and negative affective states have been hypothesized to influence drug seeking and drug taking. In parallel, it has been posited that the ultrasonic vocalizations (USVs) of *Rattus norvegicus* provide insight into the animals' affective reactions. Furthermore, it has been shown that mesolimbic dopamine (DA) plays a key role in cocaine self-administration and in USV production. Thus, affective processing as measured by rodent USVs likely coincides with cocaine self-administration, but to date has not been studied.
Objective The present study examined USVs in both the negative affective (18–32.99 kHz) and positive affective (38–80 kHz) ranges of rats during self-administration of a low (0.355 mg/kg/infusion) or high (0.71 mg/kg/infusion) dose of cocaine.
Results USVs in both ranges were observed in both dose groups. Vocalizations of the low-dose animals occurred primarily in the 22-kHz range (18–32.99 kHz), but exhibited shorter durations (10–500 ms) than those traditionally observed for 22-kHz calls in aversive situations. In contrast, USVs of the high-dose group were primarily observed in the 50-kHz frequency range (38–80 kHz), typically associated with appetitive outcomes.
Conclusions These results provide evidence for the presence of USVs during cocaine self-administration. The observed dose-dependent difference in USVs provides novel support for the view that affect is one potential motivational factor influencing human drug use and relapse behaviors. Rodent USVs may provide a powerful tool for understanding the role of affect in addiction.

Keywords Ultrasonic vocalizations · Cocaine · Compulsion · Dopamine · Accumbens · Striatum · Affect · Negative reinforcement

Abbreviations

USV	Ultrasonic vocalization
S^D	Discriminative stimulus
FM	Frequency-modulated
FF	Fixed frequency
ITI	Inter-trial interval
VTA	Ventral tegmental area
VI	Variable interval
NAcc	Nucleus accumbens
DA	Dopamine

Introduction

Historically, rat ultrasonic vocalizations (USVs) have been classified into two distinct categories: "22-kHz calls" (18–32 kHz) and "50-kHz calls" (35–70 kHz; Knutson et al. 2002; Portfors 2007). Most commonly, 22-kHz call durations range between 300 and 3,400 ms (here termed "long 22-kHz

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calls"), although a subset of shorter 22-kHz calls ranging between 20 and 300 ms (termed "short 22-kHz calls") has been described (Brudzynski et al. 1991, 1993). Both types of 22-kHz calls as well as the 50-kHz call type (20–80 ms) can be further divided into either fixed frequency (FF), trills, or frequency-modulated (FM) calls based upon the pitch modulation (or lack thereof) during a single call (Ahrens et al. 2009).

It is hypothesized that USVs provide insight into the affective state of animals (Knutson et al. 2002) or the semiotic value of a given situation (Brudzynski 2005). More specifically, long 22-kHz calls occur in the presence of aversive events such as social isolation (Francis 1977), predatory odors (Blanchard et al. 1991), or anticipation of footshock (Tonoue et al. 1986) while 50-kHz calls are linked with anticipatory and appetitive circumstances such as social contact (Knutson et al. 1998; Brudzynski and Pniak 2002), amphetamine conditioned place preference (Knutson et al. 1999), or sexual behaviors (Barfield and Thomas 1986).

Pharmacological manipulations can also modulate USVs. Intracerebral glutamate infused into the anterior hypothalamic/preoptic area increases 50-kHz calls in a dose-dependent manner, with higher doses inducing USVs in animals that failed to vocalize at lower doses (Fu and Brudzynski 1994). Intracerebral glutamate produced a ceiling effect on the rate of USVs when administered in combination with systemic amphetamine, and the effects of glutamate were later attenuated following systemic administration of the dopamine (DA) antagonist, haloperidol (Wintink and Brudzynski 2001). These data implicate a dopaminergic involvement in 50-kHz vocalizations.

The involvement of mesolimbic DA in USV production has been evidenced by Burgdorf et al. (2000), who demonstrated that the rate of 50-kHz USVs increases when rats anticipate the delivery of electrical brain stimulation to the ventral tegmental area (VTA). These results extend behavioral findings, which have shown that the anticipation of natural rewards (social interaction, food, etc.) also increases the number of 50-kHz USVs (Knutson et al. 1998). Importantly, since increases in USVs during VTA stimulation were dissociated from locomotor activity and general arousal, increases in these measures cannot account for rates of USV production. Furthermore, intracranial injections of amphetamine into the nucleus accumbens (NAcc) core and shell caused increases in the rate of 50-kHz USVs compared to injections localized in the dorsal striatum, with the most robust increases occurring in response to injections localized to the NAcc shell (Burgdorf et al. 2001). These results were confirmed by a more recent study, which noted increases in 50-kHz USVs following amphetamine injections to the NAcc shell as well as the ventral core (Thompson et al. 2006).

Studies of rats' USVs during acute systemic cocaine withdrawal have demonstrated increased rates of long 22-kHz USVs in response to a diffuse air puff relative to controls for both oral (Barros and Miczek 1996) and intravenous cocaine (Mutschler and Miczek 1998a) as well as opiates (Covington and Miczek 2003). This increase in aversive long 22-kHz USVs is exacerbated when subjects receive non-contingent infusions of cocaine as compared to self-administering controls (Mutschler and Miczek 1998b). Interestingly, recent evidence has indicated that 50-kHz USVs can be sensitized much like locomotor activity (Ahrens et al. 2009), suggesting that these calls are intimately tied with the neural circuitry affected by long-term administration of psychostimulants (Mu et al. 2009). Finally, a recent study by Williams and Undieh (2010) demonstrated that the increase in 50-kHz USVs observed during cocaine sensitization is likely mediated by brain-derived neurotrophic factor (BDNF) function within the striatum, and that BDNF may ultimately play a role in long-term synaptic changes that lead to drug abuse.

At present, no studies have examined USVs during intravenous cocaine self-administration. The present study therefore examined ultrasonic calls emitted by rats during a 6-h self-administration session at two doses of cocaine: 0.355 or 0.71 mg/kg/infusion using a discriminative stimulus (S^D) to signal cocaine availability on a variable interval (VI) 3–6-min schedule. The roles of DA in cocaine self-administration (Koob et al. 1994) and as a modulating factor in USV production (Williams and Undieh 2010; Wintink and Brudzynski 2001) suggest that USVs might provide important insight into the affective processes underlying cocaine-seeking behaviors.

Materials and methods

Subjects and Surgery Male Long-Evans (N=17) rats from multiple litters (Charles River, Wilmington, MA) were singly housed on a 12:12-h light–dark cycle with dawn at 1130 hours. Subjects were selected and run in a low-dose (n=10; 0.355 mg/kg) condition between July and April, while subjects in the high-dose (n=7; 0.71 mg/kg) group were run immediately subsequent between June and November. Prior to surgery, subjects were given sufficient food to maintain a pre-operative weight of 325–335 g. Once subjects reached a stable weight, they were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and given 10 mg/kg of atropine methyl nitrate (i.p.) as well as a 0.25 mg i.m. dose of penicillin (300,000 U/ml). Anesthesia was maintained throughout the remainder of the surgery using intermittent doses of ketamine hydrochloride (60 mg/kg i.p.) and sodium pentobarbital (5–10 mg/kg i.p.). Animals were implanted with an indwelling catheter

in the right jugular vein, which exited through a j-shaped stainless steel cannula attached to the skull using dental cement and three jeweler's screws. Following surgery, animals were housed in the self-administration chamber for the remainder of the experiment. While living in this chamber, a 200-μL infusion of heparinized saline was delivered by a syringe pump to subjects every 25 min around the clock, controlled by a mechanical timer, in order to preserve catheter patency. On days in which animals self-administered cocaine, these infusions were delivered during the 18-h period in which the experimental contingencies were not in effect. This regime continued over the course of the entire experiment. Protocols were performed in compliance with the Guide for the Care and Use of Laboratory Animals (NIH, Publications 865–23) and were approved by the Institutional Animal Care and Use Committee, Rutgers University.

Shaping During training, at 1130 hours (immediately following the commencement of the light cycle), a single non-retractable response lever was mounted on the wall of the operant conditioning chamber. To facilitate the acquisition of cocaine self-administration, subjects were initially shaped to press the lever in the presence of a continually sounding S^D tone. Responding on the lever during shaping terminated the tone, produced either a 3.75-s (0.355 mg/kg/inf) or 7.5-s (0.71 mg/kg/inf) cocaine infusion (depending on group assignment), and initiated a 30-s time out. Following each timeout period, the S^D tone was again presented. Once subjects had accumulated a sum of 10 self-administered cocaine infusions during a single daily session, they immediately transitioned to the training contingencies described below. If subjects did not accumulate 10 infusions in one session, the shaping procedure was enacted during the subsequent session. All subjects successfully completed shaping within two or three daily sessions.

Self-administration Following a 3–6 min inter-trial interval (ITI), subjects were presented with an audible tone (3.5 kHz, 70 dB) S^D. Responses on the lever in the presence of the S^D terminated the audible stimulus, produced either a 0.355-mg/kg or 0.71 mg/kg intravenous infusion of cocaine, and began the subsequent ITI. If no response occurred in the presence of the tone within 2 min, the tone was terminated by the computer and a new 3–6 min ITI began. Although groups differed by dose, due to the 3–6 min time out schedule of reinforcement, low-dose animals were unable to attain drug levels above 4.5 mg/kg while high-dose animals were capable of attaining cocaine levels of 10 mg/kg. All sessions lasted for 6 h or 80 response-contingent infusions, whichever occurred first. Subjects received post-session feeding in order to maintain a weight of approximately 320–340 g. Water was available ad libitum except during self-administration sessions. All animals were run daily for 21 days (7 days per week) with no breaks between training sessions.

USV recording A condenser microphone (UltraSoundGate 116, Avisoft Bioacoustics, Berlin, Germany) was inserted into the sound-attenuating chamber 15–30 min before the commencement of the self-administration session of well-trained subjects. The microphone was suspended 2.5 cm from a set of small holes in the top of the self-administration chamber above the quadrant proximal to the response manipulandum. On average, USV recordings took place on day 17.0±0.73 of training. It was verified that all subjects had reached asymptotic levels of drug intake (mg/kg) on the day of USV recordings. Following the start of the session, a recording window triggered by each S^D tone consisted of a 5-s period prior to the onset of the S^D, the entire duration of the S^D tone, and a 2-min portion of the subsequent ITI. Data from each recording window throughout the entire 6-h session was stored as a file for offline analysis. Sonorous activity was recorded at a 192-kHz, 16-bit sampling frequency using Avisoft Recorder software (Avisoft Bioacoustics, Berlin, Germany) and subsequently analyzed using Avisoft SASLab Pro (Avisoft Bioacoustics, Berlin, Germany), which enabled the creation of a spectrogram indicating the frequency and duration of individual calls.

Characterization of USVs Each file created by Avisoft Recorder software during self-administration was opened and visually scanned for patterns resembling USVs. For analysis, the fast fourier transform (FFT) length was set to 256, and a flat-top window with a 50% overlap was used. Once a putative, individual USV had been identified; it was transposed to a lower frequency (5% of its normal speed) within the audible range of humans for a separate auditory confirmation of its "whistle-like" characteristic (Ciucci et al. 2007; Brudzynski and Holland 2005; Fu and Brudzynski 1994). Calls that were temporally proximal to each other were designated as separate calls only if a period of silence greater than 50 ms intervened and no continuity of pitch was observed. USVs were then designated as FF (Fig. 1a, b) if they maintained a stable median pitch (kHz) or FM calls based on a 3-kHz or greater modulation of the call's median frequency. The median frequency was calculated for each USV by averaging the minimum and maximum observed frequency values. Calls for which this median frequency modulated by 3-kHz or more were then designated as either "FM" (Fig. 1c) calls or "trills" (Fig. 1d) based on the number of pitch modulations within the call. FM calls were designated as those with only a single pitch modulation, usually characterized by a "sweep-

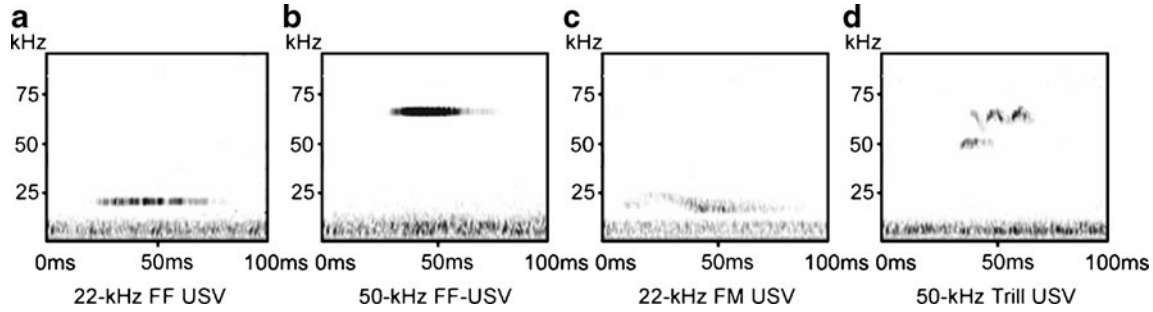

Fig. 1 Examples of ultrasonic vocalizations during cocaine self-administration. The *x-axis* is time (ms) and the *y-axis* is frequency (kHz). The visible noise band in the lower portion of each figure is the audible white noise present during all sessions. **a** A short FF USV in the frequency range of 22 kHz. **b** A FF USV in the range of 50 kHz. **c** A frequency modulated USV in the range of 22 kHz. **d** A USV with multiple pitch modulations (trill) in the range of 50 kHz

up" or a "sweep-down" (Fu and Brudzynski 1994; Brudzynski and Holland 2005; Knutson et al. 2002), while trills were designated as those calls exhibiting two or more pitch modulations. For the sake of data analysis, FM call and trills were denoted as a single median value calculated using the absolute minimum and maximum frequency values for each call.

Statistical analysis For purposes of the analyses, the observed USVs were placed into three bins: 18 to 32.99 kHz (22-kHz range), 33 to 37.99 kHz (middle range), and 38 to 80 kHz (50-kHz range). Instances of long 22-kHz calls were too few in number for independent statistical analysis (0.002% of all calls). Therefore, the long 22-kHz calls—none of which were longer than 1,000 ms—were combined with short 22-kHz USVs in the 18–32.99-kHz frequency bin. The dependent variable was the number of USVs (counts) observed at each possible combination for two levels of dose, three levels of frequency bin, and three levels of call type, i.e., FF, FM, and trill. The main statistical model consisted of a nonlinear mixed ANOVA (SAS PROC GLIMMIX, SAS Institute Inc., 2005, Cary, NC) in which the observed counts of USV calls were modeled as a function of dose, USV category, and type of call ($2\times3\times3$). Because graphical pilot analyses determined that the distribution of USV counts was highly skewed, a gamma distribution with a log link was specified for the USV counts in the mixed ANOVA. The distribution of the USV counts was transformed slightly by adding a constant value of 0.001 in order to make it compatible with the gamma distribution. Subjects were specified as a random effect. Maximum pseudo-likelihood based on an expansion locus of the mean of the random effects was used to estimate the final model. The absolute estimate convergence criterion for the estimation procedure was set to 1×10^{-7}. Bias corrected residual-based robust standard errors for the mixed model were specified using the first order residual empirical (sandwich) estimators. Post-hoc comparisons were run using a Tukey–Kramer test. All reported p values for post-hoc comparisons are Tukey–Kramer adjusted.

The earliest USV recording for any subject occurred on training day 11. Therefore, measures of self-administration were compared for all subjects from sessions 1 to 11. The results of self-administration data were calculated for dose, self-administration session, and the dose × session interaction using a mixed ANOVA similar to that described above, but specifying a normal distribution. This analysis was conducted separately for three dependent measures of self-administration: total daily drug intake (mg/kg), missed reward opportunities (defined as a tone presentation that did not result in a response-contingent infusion), and total number of daily responses.

Results

Self-administration All animals acquired stable cocaine self-administration prior to the commencement of USV recordings (Fig. 2). Subjects in the low-dose group administered 11.78±0.80 (Mean±S.E.M.) mg/kg on their first day of self-administration, escalating to 28.10±0.37 mg/kg on day 11, while high-dose subjects increased drug intake from 15.80±0.96 to 48.77±1.21 mg/kg. The total number of daily responses also increased in both groups. Low-dose animals increased responding from 292.30±36.73 responses on day 1 to 2,112.30±194.97 responses on day 11. High-dose animals emitted 198.75±38.69 responses on day 1 and increased to 638.13±60.84 responses on day 11. Finally, the number of missed opportunities to self-administer decreased between days 1 and 11 for both low- (37.50±2.18 to 3.3±0.69) and high-dose (11.88±1.0 to 7.63±0.94) subjects.

The mixed ANOVA revealed a significant main effect of self-administration session and dose, as well as a significant dose × session interaction for all three measures of training proficiency: drug intake (mg/kg) [dose: $F(1,160)=26.32$,

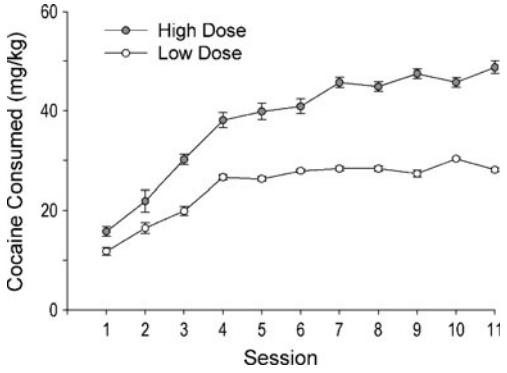

Fig. 2 Cumulative daily drug intake for both the high-dose (*dark circles*) and low-dose (*open circles*) cocaine self-administration groups. The *x-axis* shows all sessions from the start of training until session 11, when the earliest USV recordings occurred. The average amount (mg/kg) of cocaine consumed daily±SEM is shown on the *y-axis*

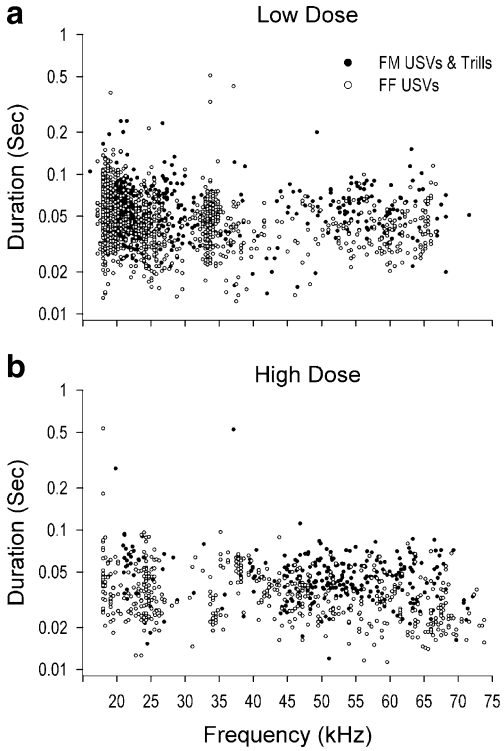

Fig. 3 Distribution of ultrasonic vocalizations as a function of duration for both the low- (**a**) and high-dose (**b**) groups. Median frequency (kHz) is represented on the *x-axis*, USV duration is shown on the *y-axis* (nonlinear scale reduces overlap of data points). *Open circles* represent FF calls while the *dark circles* represent both frequency modulated (FM) calls and trills

$p<.001$; session: $F(10,160)=24.83$, $p<.001$; dose × session: $F(10,160)=4.10$, $p<.001$] missed opportunities [dose: $F(1,160)=11.25$, $p=.001$; session: $F(10,160)=25.02$, $p<.001$; dose × session: $F(10,160)=14.68$, $p<.001$] and total presses [dose: $F(1,160)=32.44$, $p<.001$; session: $F(10,160)=7.33$, $p<.001$; dose × session: $F(10,160)=4.42$, $p<.001$].

Tukey–Kramer post-hoc comparisons revealed that the total number of presses was stable by training day 3 [days 3–11 (all): $|t(160)|<2.70$, $p>0.21$], while drug intake (mg/kg) and missed opportunities were stable by day 4 of training for both groups [drug intake days 4–11 (all): $|t(160)|<2.70$, $p>0.20$; missed opportunities days 4–11 (all): $|t(160)|<1.53$, $p>0.91$]. These data demonstrate that animals were reliably self-administering cocaine well before their respective recording days. Finally, post-hoc comparisons of the dose × session interaction for milligram/kilogram drug intake revealed that subjects in the high-dose group (day 11=48.77±3.41) administered more cocaine than the low-dose group (day 11=28.10±1.18) on days 7 through 11 [Days 7–11 (all): $|t(160)|>4.84$, $p<0.001$] of training. This demonstrates that following acquisition of the self-administration task, subjects in the high-dose group escalated their intake over that of the low-dose group.

Ultrasonic vocalizations Individual USVs incorporated into the mixed model are shown in Fig. 3 for both the low- (Fig. 3a) and high-dose (Fig. 3b) groups. The mixed ANOVA revealed a significant three-way interaction of dose × frequency bin × type of call [$F(4,831)=22.66$, $p<0.001$]. Significant two-way interactions were observed for frequency bin × type of call [$F(4,831)=22.75$, $p<0.001$], dose × type of call [$F(2, 831)=4.36$, $p<0.05$], and dose × frequency bin [$F(2, 831)=92.54$, $p<0.001$]. The main effects of call type [$F(2, 831)=98.31$, $p<0.001$] and frequency bin [$F(2, 831)=76.62$, $p<0.001$] were significant. The main effect of dose [$F(1,831)=3.65$, $p=0.06$] was marginally significant.

Tukey–Kramer post-hoc tests revealed that during the 6-h self-administration session, subjects in the high-dose group made significantly fewer short FF calls [26.57±8.27; $|t(831)|=5.91$, $p<0.001$], FM calls [4.57±1.54; $|t(831)|=6.32$, $p<0.001$], and trills [1.14±0.26; $|t(831)|=12.15$, $p<0.001$] in the 22-kHz range than subjects in the low-dose group [FF=77.3±22.58; FM=23.7±8.88; trill=15.7±7.25]. On the other hand, subjects in the high-dose group made significantly more FF calls [41.29±15.06; $|t(831)|=3.69$, $p<0.05$] and FM calls [23.57±11.03; $|t(831)|=6.10$, $p<0.001$] in the 50-kHz range than subjects in the low-dose group [FF=19.0±4.34; FM=6.9±2.11]. The number of 50-kHz trills between the low- [5.6±1.83] and high-dose [12.0±3.96] did not differ [$|t(831)|=2.51$, $p=0.52$, NS]. Furthermore, no differences were observed for the 33–38-kHz range, which separates the 22- and 50-kHz distributions [FF: $|t(831)|=0.93$, $p=1.00$, NS; FM: $|t(831)|=0.11$, $p=1.00$, NS; and trill: $|t(831)|=0.65$, $p=1.00$, NS]. The observed group differences between the 22 and 50-kHz ranges are shown in Fig. 4 with all call types combined.

Subsequent analyses of USVs within the high-dose group revealed significantly more 50-kHz FF calls

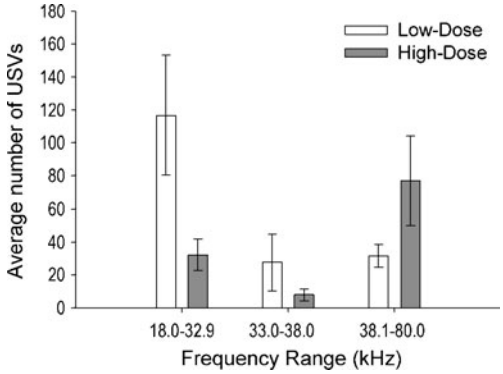

Fig. 4 The average number of USVs±SEM for both the low- (*open bars*) and high-dose (*dark bars*) groups during a 6-h cocaine self-administration binge. Frequencies (kHz) on the *x-axis* are separated into the 22-kHz range (18.0–32.9 kHz), the 50-kHz range (38.1–80.0 kHz), and the range between the 22-kHz and 50-kHz distributions (33.0–38.0 kHz). The total number (counts) of USVs is shown on the *y-axis*. All call types (FF, FM, Trill) are combined

[$|t(831)|=7.93$, $p<.001$], FM calls [$|t(831)|=7.69$, $p<.001$], and trills [$|t(831)|=7.48$, $p<.001$] relative to short 22-kHz FF calls, FM calls, and trills, respectively. In contrast, within the low-dose group, the number of short 22-kHz calls greatly outweighed the number of 50-kHz calls for all three call types [FF: $|t(831)|=17.19$, $p<.001$; FM: $|t(831)|=12.58$, $p<.001$; trills: $|t(831)|=9.76$, $p<.001$].

Discussion

This is the first demonstration, to our knowledge, of USVs exhibited by rats during cocaine self-administration. USVs emitted by high-dose subjects were consistent with those observed in subjects receiving different doses of systemic cocaine (Mu et al. 2009), or intravenous (Ahrens et al. 2009) or systemic amphetamine (Wintink and Brudzynski 2001), all of which have shown that 50-kHz USVs are produced in response to drug administration. On the other hand, the substantial number of short 22-kHz calls observed for both the low- and high-dose groups in the present study is an important difference from the results of the aforementioned studies. This difference might be accounted for by selective sampling of the 50-kHz range (Mu et al. 2009), by operational definitions which exclude USVs less than 300-ms duration, by experimenter-administered versus self-administered psychostimulants (Ahrens et al. 2009), or by the fact that the present animals likely self-administered blood drug levels below those reached via single experimenter-administered bolus injections greater than 15 mg/kg (Nicolaysen et al. 1988; Pettit and Justice 1991; Weiss et al. 1992; Stuber et al. 2005).

Whereas USVs in the 22-kHz range typically exhibit durations greater than 300 ms in sober or drug-naïve subjects (Portfors 2007; Covington and Miczek 2003; Barros and Miczek 1996; Mutschler and Miczek 1998a, b), most 22-kHz USVs observed during the cocaine binge in both low- and high-dose groups were short, lasting an average of 50 ms. Less is known about short 22-kHz calls than their longer-duration counterparts. However, short and long 22-kHz calls have been observed in experimentally naïve subjects in response to handling (Brudzynski et al. 1993) as well as in response to footshock and intracerebral injections of carbachol into the anteromedial hypothalamic/preoptic area (Brudzynski et al. 1991). In the absence of evidence to the contrary, these data suggest that much like their longer counterparts, short 22-kHz calls are produced in response to states of aversion.

The present experiments demonstrate a dose-dependent, crossover interaction of short 22- and 50-kHz USVs that remained stable between call types (FF, FM, trills). That is, animals that maintained self-administered drug levels below 4.2 mg/kg (the maximum drug level achieved by any animal in the low-dose group) exhibited predominantly short 22-kHz USVs and fewer 50-kHz USVs. In contrast, animals that maintained self-administered drug levels as high as 6.98 mg/kg (the maximum drug level achieved by any animal in the high-dose group) exhibited predominantly 50-kHz USVs and fewer short 22-kHz USVs. Furthermore, the peak drug level of high-dose subjects (all subjects >5.1 mg/kg) was in every case above the peak level observed for low-dose subjects (4.2 mg/kg or below).

An important factor that may have influenced the predominance of short 22-kHz vocalizations in the low-dose group is that the 3–6-min VI schedule of reinforcement during the present self-administration paradigm precluded rats from attaining 'satiety' at this dose (Ghitza et al. 2003; Root et al. 2009). Previous studies would suggest that changes in USVs are directly tied to rising and falling levels of extracellular DA within the mesolimbic DA system (Burgdorf et al. 2000, 2001; Thompson et al. 2006; Wintink and Brudzynski 2001), which are critical for self-administration (Koob et al. 1994; Ritz et al. 1987). Therefore, a subject's transient drug levels, which are modulated by both dose and temporal opportunities for self-administration, would play a critical role in the modulation of USVs during cocaine self-administration.

While it is known that drug level and drug-seeking behavior are intimately linked (Yokel and Pickens 1974; Norman and Tsibulsky 2006), the role that USVs and affect play in drug seeking remains unclear. It is widely accepted that 22- and 50-kHz USVs are correlated with aversive and appetitive outcomes, and therefore likely represent negative and positive affective states, respectively. Still, it is possible that the observed dose-dependent interaction represents a

cocaine-induced change in neurochemistry that causes the observed shift from 22- to 50-kHz calls without influencing drug-seeking behavior.

One theory of USV production suggests that 22-kHz USVs are modulated by cholinergic projections from the laterodorsal tegmental nucleus, while 50-kHz USVs are controlled by dopaminergic projections from the VTA (Brudzynski 2008). Given the anatomical overlap between these two systems (e.g. within the NAcc and other mesolimbic regions), and their concomitant (but differential) activation during yoked or self-administered cocaine (You et al. 2008; Mark et al. 1999), it stands to reason that different doses of cocaine alter the cholinergic–dopaminergic interaction in such a way as to modulate USV production irrespective of motivational state.

On the other hand, the observed dose-dependent interaction may reflect a feedback loop such that cocaine seeking causes changes in cocaine intake, which modulate levels of acetylcholine and DA, thereby modulating motivational state and hence USV production, as well as subsequent cocaine-seeking behavior. Assuming that both short and long 22-kHz USVs are emitted during aversive states (Brudzynski et al. 1991, 1993; Blanchard et al. 1991; Tonoue et al. 1986), the abundance of short 22-kHz USVs in the low-dose group suggests that negative reinforcement is a driving force behind "compulsive" (e.g. sub-satiety) cocaine self-administration (Norman and Tsibulsky 2006), while 50-kHz USVs occur when animals are allowed to more liberally titrate blood concentrations of cocaine to a preferred level.

Negative reinforcement has long been considered a possible driving factor for drug use (Wikler 1948). For example, one hypothesis of returned drug use posits that negative affect is universal to all drugs of abuse, and that the prepotent factor of self-administration is negative reinforcement (Baker et al. 2004). Recent evidence has supported this hypothesis. First, cocaine users with negative affective biases exhibit greater fMRI activation in the amygdala/ventral pallidum in response to briefly presented (33 ms) cocaine-related cues than cocaine users with positive affective biases (Childress et al. 2008), suggesting a susceptibility to drug use. Second, these brain regions are hypothesized to play an important role in the addicted user by inducing negative emotional states that lead to drug craving and use (Koob and Moal 2005; Koob and Volkow 2010). Third, taste cues paired with the opportunity to self-administer cocaine in rats selectively produced "aversive" orofacial reactions that predicted self-administration behavior (Wheeler et al. 2008). Finally, cocaine users report difficulties regulating their emotions (Fox et al. 2007) and retrospectively report a significant increase in "unpleasant affect" just prior to drug use (McKay et al. 1995).

Numerous questions remain regarding the factors that motivate cocaine self-administration. Further investigations focusing on changes in USVs tied to changes in plasma concentrations of cocaine or in combination with region specific measures of extracellular DA, and acetylcholine in vivo would provide necessary information regarding the progenitors of USVs during cocaine seeking. Nevertheless, the present results demonstrate that USVs provide a powerful tool for understanding one of the potentials underlying motivational factors influencing repeated drug use and relapse behaviors—affect.

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