Published in final edited form as:

Psychopharmacology (Berl). 2013 October ; 229(4): 687–700. doi:10.1007/s00213-013-3130-9.

Individual differences in the conditioned and unconditioned rat 50-kHz ultrasonic vocalizations elicited by repeated amphetamine exposure

Allison M. Ahrens,

Department of Psychology, University of Texas at Austin, Austin, TX 78712, USA; Department of Psychology, University of Michigan at Ann Arbor, Ann Arbor, MI 48109, USA

Cameron W. Nobile,

Department of Psychology, University of Texas at Austin, Austin, TX 78712, USA

Lindsay E. Page,

Department of Psychology, University of Texas at Austin, Austin, TX 78712, USA

Esther Y. Maier,

College of Pharmacy, University of Texas at Austin, Austin, TX 78712, USA

Christine L. Duvauchelle, and

College of Pharmacy, University of Texas at Austin, Austin, TX 78712, USA

Timothy Schallert

Department of Psychology, University of Texas at Austin, Austin, TX 78712, USA; Department of Psychology, University of Michigan at Ann Arbor, Ann Arbor, MI 48109, USA

Allison M. Ahrens: allison.ahrens@gmail.com

Abstract

Rationale—Adult rats often produce 50-kHz ultrasonic vocalizations (USVs), particularly the frequency-modulated varieties, in appetitive situations. These calls are thought by some to reflect positive affective states and the reinforcing value of drugs such as amphetamine and cocaine.

Objective—The objective of this study was to determine whether the number of unconditioned 50-kHz USVs elicited by amphetamine predicts the development and/or magnitude of drug-conditioned motivation.

Methods—In three experiments, we recorded USVs before and after injections of 1 mg/kg amphetamine (i.v. or i.p.) administered once per session. Rats were categorized as “high callers” or “low callers” according to individual differences in the number of 50-kHz USVs elicited by their first amphetamine injection. We examined the conditioned appetitive behavior and conditioned place preference (CPP) that emerged in high and low callers after repeated pairings of amphetamine with specific contexts. We also examined whether amphetamine-induced calling was affected by treatment within an unfamiliar (test chamber) versus familiar (home cage) context.

Results—Within an unfamiliar environment, the high callers consistently produced more amphetamine-induced 50-kHz USVs than the low callers. Compared to the low callers, high callers showed significantly greater amphetamine CPP as well as enhanced conditioned 50-kHz

USVs and locomotor activity during anticipation of amphetamine. Individual differences were stable when amphetamine was administered in test chambers, but when it was administered in home cages, low callers showed an increase in 50-kHz calling that matched the high callers.

Conclusions—These findings suggest that individual differences in drug-induced USVs can reveal environment-sensitive traits involved in drug-related appetitive motivation.

Keywords

Appetitive learning; Positive affect; 50 kHz; 22 kHz; USVs; Reward; Motivation

Introduction

Rat ultrasonic vocalizations (USVs), the high-frequency calls that rats use to communicate with conspecifics (Wöhr and Schwarting 2012; Wöhr et al. 2008), have recently gained attention as potential indices of the emotional and motivational states produced by drugs of abuse (Ma et al. 2010; Maier et al. 2010, 2012; Browning et al. 2011; Mahler et al. 2013). Calls in the 50-kHz frequency range tend to increase in response to natural and drug rewards and have therefore been postulated to reflect positive affective states associated with appetitive behavior (Knutson et al. 2002; Burgdorf and Pankepp 2006; Burgdorf et al. 2009). Calls in the 22- to 28-kHz frequency range are often thought to signal negative affect since they are associated with anxiety and/or distress occurring in situations such as social defeat, foot shock, predatory threats, and withdrawal from addictive drugs (Burgdorf et al. 2009; Covington and Miczek 2003; Brudzynski 2001). There are different subtypes of 50-kHz USVs, and it seems that frequency-modulated subtypes, particularly “trill” calls, are more closely related to positive affect than flat-frequency calls (Burgdorf et al. 2010; Wright et al. 2010, 2012); however, much of the literature refers to counts of total 50-kHz USVs without identifying subtypes.

Rats often produce spontaneous 50-kHz calls in the absence of discernible social or appetitive stimuli (Wöhr et al. 2008; Schwarting et al. 2007). However, manipulations that stimulate mesolimbic dopamine activity can dramatically increase the rate of 50-kHz calling above baseline levels. For instance, increased 50-kHz USVs are elicited by micro-injections of amphetamine into the nucleus accumbens (Thompson et al. 2006; Burgdorf et al. 2007; Brudzynski et al. 2011a) and by electrical brain stimulation (EBS) of areas known to mediate reward and support self-stimulation (Burgdorf et al. 2007). Acute systemic administration of amphetamine or cocaine elicits 50-kHz USVs (Maier et al. 2012, Barker et al. 2010; Wintink and Brudzynski 2001; Wright et al. 2010, 2012; Natusch and Schwarting 2010), and this effect is sensitized by repeated exposure to these drugs (Ahrens et al. 2009; Mu et al. 2009; Williams and Undieh 2010). Fifty-kilohertz calls are also elicited by cues that predict natural rewards, such as positive social interactions (Knutson et al. 1998) and sexual encounters (Bialy et al. 2000; Ciucci et al. 2007, 2009; McGinnis and Vakulenko 2003). Conditioned 50-kHz calls have been observed during the expression of conditioned place preference for an environment paired with cocaine, amphetamine, or morphine (Knutson et al. 1999; Meyer et al. 2012; Burgdorf et al. 2007) and during anticipation of cocaine self-administration (Ma et al. 2010; Maier et al. 2010).

A number of studies have found stable individual differences in rat vocalization. These have been reported in spontaneous USVs produced during exposure to a non-home environment (Schwarting et al. 2007) and in drug-induced USVs (Wright et al. 2010; Brudzynski et al. 2011a; Browning et al. 2011). Individual differences in vocalization have a genetic component and often appear to be linked to emotional traits (e.g., anxiety) and sensitivity to reward (Brudzynski et al. 2011a; Burgdorf et al. 2009; Mu et al. 2009; Schwarting et al. 2007; Wöhr et al. 2009). Among outbred rats, those that emit 50-kHz USVs after their first

exposure to EBS also show greater rates of EB self-stimulation compared to rats that do not vocalize after the first experience (Burgdorf et al. 2007). In addition, the number of unconditioned 50-kHz USVs produced during the first day of cocaine self-administration correlates with the speed at which rats achieve stable cocaine self-administration rates (Browning et al. 2011). Previous studies have reported individual differences in the number of 50-kHz USVs elicited by amphetamine (Wright et al. 2010, 2012; Brudzynski et al. 2011a), yet none have explicitly examined the stability of these differences across various measures of amphetamine-associated reward.

In the current study, rats were characterized as either “high callers” or “low callers” according to the number of 50-kHz USVs elicited by their first exposure to amphetamine. These individual differences in USV amphetamine responses were examined in three separate experiments and compared with behavioral measures more commonly collected in drug abuse studies. In experiment 1, USVs and locomotor activity were recorded prior to, and immediately after non-contingent delivery of intravenous (i.v.) amphetamine (1 mg/kg) within a distinctive environment. In experiment 2, we examined whether the familiarity of the drug administration environment (test chambers versus home cages) could influence the 50-kHz vocalization elicited by i.v. amphetamine. In experiment 3, we examined whether differences in intraperitoneal (i.p.) amphetamine-induced USVs were predictive of the magnitude of amphetamine-induced conditioned place preference (CPP).

Methods

Animals

A total of 69 adult male Sprague–Dawley rats (Charles River) were used. Rats were housed two per cage upon arrival to the colony and were handled 5 min daily for at least 1 week prior to the start of experiments. Home cages were standard polycarbonate cages with pine bedding, and food and water were available ad libitum. Rats were switched to single housing at least 1 week prior to the start of each experiment. All animals were maintained on a reverse 12-h light/dark cycle, with all testing taking place during the dark phase of the cycle. All procedures were approved by the University of Texas Institutional Animal Care and Use Committee.

Surgery

In experiments 1 and 2, jugular catheters were surgically implanted to enable i.v. drug delivery, and rats received post-surgical care and catheter maintenance as previously described (Maier et al. 2010, 2012). At the end of experiments, catheter patency was tested by i.v. injection of 10–20 mg/kg ketamine hydrochloride (50 mg/ml, infused at a rate of 0.03 ml/s). Data from rats failing to become ataxic within 20 s were excluded from this report.

Drugs

Rats received either 1 mg/kg D-amphetamine sulfate (expressed as the weight of the salt; Sigma-Aldrich, St. Louis, MO) dissolved in sterile 0.9 % saline at a volume of 0.5 ml/kg or an equivalent volume of 0.9 % saline. In experiments 1 and 2, amphetamine and saline were delivered i.v. (rate of 0.1 ml per 10–15 s). In experiment 3, amphetamine and saline were delivered via i.p. injection.

Experiment 1

Apparatus—In experiment 1, we used operant conditioning chambers (30.5×24.1×21 cm) housed in sound-attenuating boxes (Med-Associates, St. Albans, VT). Chambers were equipped with house lights, stimulus lights, and ultrasonic microphones with a frequency

detection range of 10–100 kHz (PCB Piezotronics, Buffalo, NY). Microphones were mounted on the side of the chambers 14.5 cm from the floor. The microphone output was digitized with an A/D card (National Instruments, Austin, TX) at a 200-kHz sampling rate with 16-bit resolution. Horizontal beam breaks were used as a measure of locomotor activity and were recorded with three pairs of photocells (two positioned 5 cm from the chamber walls and one in the center of the chamber) and MED-PC software (Med Associates). Drug and saline injections were delivered through i.v. catheters via tubing connected to single-channel swivels and syringes mounted above the chambers.

Procedure—Twenty-five drug-naïve rats (320–520 g) were used in this experiment. The experiment began with 4 days of habituation to the chambers (30 min/session) in which rats were placed in the chambers without tethering or cage illumination and baseline rates of vocalization and locomotor activity were recorded. After habituation, rats were randomly assigned to receive i.v. amphetamine ($n=16$) or saline ($n=9$) during fifteen 30-min treatment sessions occurring once every 2–3 days (i.e., Mondays, Wednesdays, and Fridays). Each session was identical and consisted of a 10-min pre-injection “anticipatory” period and then a single i.v. injection of amphetamine or saline delivered at 10 min, followed by a 20-min post-injection period. During the first 9.5 min of the anticipatory period, all lights in the chamber were off. In the last 30 s of this period, a stimulus light was illuminated to signal the impending drug or saline infusion. At the start of the i.v. injection (6-s infusion time), the stimulus light was turned off and the house light illuminated for the rest of the session. USVs and locomotor activity were recorded throughout the session (i.e., 10-min pre-injection and 20-min post-injection).

Experiment 2

Apparatus—In this experiment, USVs were recorded with CM16 ultrasonic microphones (Avisoft Bioacoustics, Berlin, Germany), and microphone output was digitized with an UltraSoundGate system (Avisoft Bioacoustics) at a sampling rate of 250 kHz and a 16-bit resolution. Test chambers were identical to the operant conditioning chambers described in experiment 1. During home cage sessions, rats' individual home cages were placed in custom-made sound-attenuating cabinets, each equipped with a house light and stimulus light. Cage tops were modified to allow USV microphone placement and passage of i.v. drug delivery tubing.

Procedure: phase I—test chambers—A separate group of 14 drug-naïve rats (330–460 g) were used in this experiment. Rats underwent four 30-min habituation sessions within the operant conditioning chambers, followed by three 30-min drug-exposure sessions (spaced every 2–3 days) in which they received 1 mg/kg i.v. amphetamine delivered 10 min into the session.

Procedure: phase II—home cages—Phase II began with two 30-min habituation sessions in the home cage recording environment (with lights off). This was followed by fifteen 30-min sessions (spaced every 2–3 days) in which 1 mg/kg i.v. amphetamine was administered after a 10-min delay to all rats in their home cage environment. A stimulus light was illuminated during the last 30 s of the anticipatory period and a house light illuminated from the start of amphetamine delivery until the end of the session. In phases I and II, USVs were recorded throughout all sessions (i.e., 10-min pre-injection and 20-min post-injection).

Experiment 3

Apparatus—CPP chambers were custom-made acrylic boxes with two side compartments (each 26.6×27.6×31 cm), one with white walls and smooth black acrylic floors and one with

black walls and textured white acrylic floors. Two removable walls and a central gray compartment separated the black and white sides. During baseline and test sessions, video cameras were mounted over the boxes and raters blind to the condition counted the number of seconds spent in each side. CM16 ultrasonic microphones were mounted in clear acrylic lids placed over the compartments, and microphone output was collected with the Avisoft UltraSoundGate system at a sampling rate of 250 kHz and a 16-bit resolution.

Procedure—A separate group of 30 drug-naïve rats (400–520 g) were used in the CPP experiment. Fifteen-minute baseline sessions were conducted to determine initial preference for the black or white side. For baseline and CPP test sessions, rats were placed in the central gray compartment with the walls raised 8 cm, allowing access to both sides of the apparatus. The time spent in either the black or white side was calculated, excluding the time spent in the central compartment. The initially non-preferred side (<50 % time) was designated as the reinforced conditioned stimulus (CS+) and the initially preferred side (>50 % time) designated as the non-reinforced conditioned stimulus (CS−). Conditioning sessions were then conducted on four consecutive days. The amphetamine group ($n=20$) had saline (i.p.) paired with the CS− side on days 1 and 3 and amphetamine (1 mg/kg i.p.) paired with the CS+ side on days 2 and 4. The control group ($n=10$, not run in parallel with the amphetamine group) had saline (i.p.) paired with both the CS− and CS+ sides. During conditioning sessions, rats were placed in the compartments immediately after injections and remained there for 30 min while USVs were recorded. The CPP test was conducted the day after the last conditioning session; USVs were recorded with two microphones at once, one over each side. At the start of test sessions, a brief tone was played; audible in the video recordings and visible in the USV spectrogram. The appearance of the tone made it possible to synchronize video and USV recordings. This allowed USV emissions to be paired with a specific side of the CPP apparatus.

USV analysis

Vocalizations were recorded with Avisoft-RECORDER software and analyzed with Avisoft-SASlab Pro software (Avisoft Bioacoustics). Spectrograms were generated with a 512 FFT length and a 75 % overlap window (100 % frame, Hamming window). All USVs that fell between 30 and 95 kHz were considered “50-kHz” calls, and all flat-frequency USVs that fell between 20 and 29 kHz were considered “22-kHz” calls (Wright et al. 2010; Brudzynski 2001). To count 50- and 22-kHz USVs, recordings were first analyzed with the “Automatic parameter measurement” feature in SASlab Pro, which automatically labeled ultrasonic sounds using a single-threshold detection method (−63-dB threshold, 23-ms hold time). Trained raters manually examined and corrected the automatically labeled elements to ensure accurate detection of USVs according to set requirements, including being visible and distinct from background noise, resembling previously identified call types (Wright et al. 2010), and having a “whistle-like” sound when played in the human detection range (i.e., 50-kHz frequency slowed to approximately 20 % of the original speed, or 11.025 kHz). This largely automatic method of USV detection produced almost identical results to entirely manual USV detection by raters ($r=0.998$; 20 recordings analyzed with both methods). For USV counts, recordings were randomly assigned to one of four raters blind to the experimental condition. Raters had high inter-rater reliability (average inter-rater correlation of 0.995), as determined from 12 randomly chosen USV files. In selected recordings, 50-kHz USVs were separated into flat or frequency-modulated (FM) categories based on the presence or absence of fluctuations in frequency (Ahrens et al. 2009; Simola et al. 2010). The FM category included all calls with varying frequencies, such as “trill” calls with rapid oscillations in frequency as well as “non-trill” calls with step and pitch-jump components (Wright et al. 2012; Mahler et al. 2013). Flat and FM classification was performed by two

raters with high inter-rater reliability (Cohen's $\kappa=0.89$) determined by categorization of the same 112 individual 50-kHz calls.

Experiment 1 used different ultrasonic recording equipment (PCB Piezotronics/National Instruments) than experiments 2 and 3 (Avisoft Bioacoustics). Therefore, in a comparison study, rats were recorded using both systems simultaneously (seven rats, two sessions each). We found that the PCB Piezotronics/National Instruments system detected $80\pm4\%$ of the total 50-kHz USVs ($88\pm5\%$ of the flat calls and $72\pm5\%$ of the FM calls) detected by the Avisoft system. Twenty-two-kilohertz USVs were not compared because none were produced during these recordings.

High callers and low callers

Rats often make spontaneous calls for the first ~ 5 min after initial placement in a testing environment (Schwartz et al. 2007; Wöhr et al. 2008; Knutson et al. 1998). Therefore, in experiments 1 and 2, USVs produced in the session 1 pre-amphetamine interval (first 10 min) were designated as baseline values and compared with session 1 post-amphetamine calls (minutes 11–30). From these data, two values were calculated: the difference in absolute number of USVs during pre- and post-amphetamine intervals and the factor increase from baseline (note that if pre-injection USVs=0, factor increase was calculated as USV=1). Rats were categorized as “high callers” or “low callers” based on whether or not their first exposure to amphetamine elicited an increase in 50-kHz USVs compared to their baseline rates of calling. Our criteria for high callers were that they showed an amphetamine-induced increase from baseline ≥ 50 calls or $5\times$ baseline levels. Rats that did not meet these criteria were categorized as low callers.

High and low callers in experiment 3 were determined using 50-kHz USVs per 30-min values of the first amphetamine session minus the first saline session. Rats with difference scores of ≥ 300 calls were designated as high callers and those with ≤ 1 call were low callers. Remaining rats (difference scores ranged from 2 to 94 calls) were assigned to high and low caller groups by a difference score median split. In all three experiments, 22-kHz USVs were also taken into consideration when determining groups. A requirement of high callers to produce only 50-kHz USVs during the first amphetamine exposure resulted in the exclusion of two rats from experiment 2 due to several emitted 22-kHz USVs in addition to a high number of 50-kHz USVs.

Data analyses

In experiments 1 and 2, data were analyzed separately for pre- and post-injection periods with two-way session $\times$ group (high callers, low callers, and saline-treated controls) repeated-measures (RM) ANOVAs. Unless otherwise indicated, ANOVAs included 16 sessions: baseline (i.e., averaged habituation sessions) plus 15 amphetamine sessions. Tukey's post hoc tests were used to determine group differences in data averaged across all sessions included in the ANOVAs. In some cases, separate RM ANOVAs were used to examine interactions between high callers versus controls and low callers versus controls; significant interactions were followed by within-subjects contrasts comparing the first session included in the ANOVA to all other sessions. Two-tailed Pearson's correlations and t tests were also used in some analyses.

All USV data sets displayed unequal variances, so data transformations were performed prior to statistical analyses. Square root transformations were used in experiments 1 and 2; however, in experiment 3, the unequal variance required a logarithmic transformation. If the assumption of sphericity was violated after data transformations, Huynh–Feldt-corrected p values were reported in the ANOVA results. Data lost due to equipment error (1.3 % of

USV data and 2.8 % of locomotor activity data) were replaced using a “linear trend at point” method that predicted missing values based on existing data from the animal. Statistical procedures were conducted with SPSS for Windows (version 19), and all results are presented as the mean $\pm$ SEM.

Results

Experiment 1: 50-kHz USVs

The amphetamine-treated group consisted of eight high callers and eight low callers. USVs were highly variable between rats, but stable within subjects across repeated days of testing. For instance, habituation USVs ranged from 0 to 476 calls per 10 min, yet correlations between habituation sessions (minutes 0–10 of four sessions) were all significant ($r=0.538$ – 0.785 , $p<0.01$ – 0.001 , $n=25$). Amphetamine-induced calling varied from 0 to 920 calls per 10 min, but was also individually stable; individual USVs across amphetamine sessions (minutes 11–20 of sessions 1, 5, 10, and 15) were all significantly correlated ($r=0.552$ – 0.921 , $p<0.01$ – 0.001 , $n=16$). Individual baseline USVs did not predict amphetamine-induced USVs as no significant relationships were revealed between USVs elicited during habituation versus amphetamine sessions (four sessions of each; $r=0.313$ – 0.407 , all n.s., $n=16$).

Anticipatory period (minutes 0–10 of sessions)—In the high-caller group, the number of anticipatory 50-kHz USVs increased across sessions. A 3×16 RM ANOVA of anticipatory calls showed significant main and interaction effects [session: $F(15,330)=2.58$, $p<0.01$; group: $F(2,22)=5.24$, $p<0.05$; session $\times$ group: $F(30,330)=3.09$, $p<0.001$]. Post hoc comparisons revealed that high callers made more 50-kHz USVs than both low callers and controls ($p<0.05$ for all) and that low callers did not differ from controls. There was a significant interaction between the high callers and controls with a separate 2×16 RM ANOVA [$F(15,225)=4.79$, $p<0.001$], and within-subjects contrasts were significant when sessions 7, 8, and 11–15 were compared to baseline ($p<0.05$ – 0.01). There was no significant interaction between the low callers and controls [$F(15,225)=0.98$, n.s.] (Fig. 1a).

First 10 min of the post-injection period (minutes 11–20 of sessions)—A 3×16 RM ANOVA showed significant main and interaction effects [session: $F(15,330)=5.12$, $p<0.001$; group: $F(2,22)=9.43$, $p<0.001$; session $\times$ group: $F(30,330)=3.73$, $p<0.001$]. Post hoc tests showed that high callers made significantly more post-injection 50-kHz USVs than low callers and controls ($p<0.01$ for all) and that low callers did not differ from controls (Fig. 1b).

Sessions 1, 5, 10, and 15—The time course of USV production was examined in detail for sessions 1, 5, 10, and 15 (Fig. 2). In these four sessions, anticipatory USVs were counted in the last 30 s of the pre-injection period (minutes 9.5–10 of sessions), during illumination of the stimulus light that signaled impending infusions. A 3×4 RM ANOVA of the last 30 s showed significant main and interaction effects [session: $F(3,66)=2.95$, $p<0.05$; group: $F(2,22)=7.09$, $p<0.01$; session $\times$ group: $F(6,66)=5.31$, $p<0.001$]. Separate 2×4 RM ANOVAs showed that there was no difference between low callers and controls [$F(3,45)=2.75$, n.s.], but that there was a significant interaction between high callers and controls [$F(3,45)=12.06$, $p<0.001$], with high callers producing more anticipatory calls in later sessions compared to the first session (see Fig. 2).

A 3×4 RM ANOVA including only the calls produced in the first 5 min post-injection (minutes 11–15 of sessions 1, 5, 10, and 15) showed significant main and interaction effects [session: $F(3,66)=3.27$, $p<0.05$; group: $F(2,22)=9.04$, $p<0.01$; session $\times$ group: $F(6,66)=2.65$,

$p < 0.05$]. Separate 2×4 RM ANOVAs showed a significant interaction between high callers and controls [$F(3,45) = 4.86$, $p < 0.05$], but not between low callers and controls [$F(3,45) = 2.72$, n.s.]. Within-subjects contrasts showed that the amphetamine-induced increase in USVs seen in high callers relative to controls was enhanced by repeated drug exposure (see Fig. 2).

Experiment 1: FM and flat 50-kHz USV subtypes

To examine FM and flat subtypes, we took short samples of recordings from sessions 1, 5, 10, and 15 and categorized all 50-kHz USVs produced during the sampled time periods as FM or flat (see Fig. 3). Within-group comparisons of FM versus flat calls were performed with 2×4 RM ANOVAs, with both session and call type as within-subjects factors.

Anticipatory calls (30 s cue light)—High callers made slightly more FM calls than flat calls during the last 30 s of the anticipatory period, but the difference was not significant across the four sampled sessions [session: $F(3,21) = 8.39$, $p < 0.001$; call type: $F(1,7) = 1.03$, n.s.; session $\times$ call type: $F(3,21) = 1.37$, n.s.] or when session 15 was examined separately [$t(7) = 1.83$, n.s.] (Fig. 3a).

Amphetamine-induced calls (minutes 11–15 of sessions)—Within the high-caller group, amphetamine elicited more FM calls than flat calls [session: $F(3,21) = 3.07$, $p < 0.05$; call type: $F(1,7) = 7.17$, $p < 0.05$] (Fig. 3d). No significant effects were seen in the low callers or controls during the anticipatory or post-injection periods.

Experiment 1: locomotor activity

Scores from one saline control rat were lost due to equipment failure; statistical tests were performed with $n = 8$ in this group.

Anticipatory period—When locomotor activity was counted during the last 30 s of the anticipatory period (corresponding with the illuminated cue light), a 3×15 RM ANOVA (not including a baseline session) showed significant group and interaction effects [session: $F(14,294) = 1.10$, n.s.; group: $F(2,21) = 3.96$, $p < 0.05$; session $\times$ group: $F(28,294) = 1.53$, $p < 0.05$]. Post hoc comparisons showed that this conditioned locomotor activity was significantly greater in high callers compared to controls ($p < 0.05$), but that there were no significant differences between high callers and low callers or between low callers and controls. With separate 2×15 RM ANOVAs, there was a significant interaction between high callers and controls [$F(14,196) = 1.96$, $p < 0.05$] and within-subjects contrasts were significant for all sessions, except 2, 5, and 10 ($p < 0.05$ – 0.01); however, there was no significant interaction between low callers and controls [$F(14,196) = 1.25$, n.s.] (Fig. 4a). In contrast to the last 30 s, locomotor activity counted during the entire 10-min anticipatory period (minutes 0–10 of sessions) did not differ significantly between groups [session: $F(15,315) = 1.30$, n.s.; group: $F(2,21) = 0.33$, n.s.; session $\times$ group: $F(30,315) = 1.44$, n.s.] (Fig. 4b).

Post-injection period (minutes 11–30 of sessions)—After amphetamine injections, both high and low callers showed significantly greater locomotor activity than controls (Fig. 4c). There were significant main and interaction effects [group: $F(2,21) = 14.75$, $p < 0.001$; session: $F(15,315) = 3.46$, $p < 0.001$; session $\times$ group: $F(30,315) = 3.17$, $p < 0.001$] as well as significant post hoc differences between high callers and controls ($p < 0.001$) and between low callers and controls ($p < 0.001$), but not between high and low callers.

Experiment 2: phase I—50-kHz USVs in conditioning chambers

In experiment 2, amphetamine-treated rats consisted of six high and six low callers. When habituation calls were averaged across the four sessions, there were no significant group differences during minutes 0–10 of habituation [$t(10)=1.51$, n.s.] or minutes 11–20 of habituation [$t(10)=2.21$, n.s.].

Anticipatory period (minutes 0–10 of sessions)—A 2×4 RM ANOVA (baseline plus three amphetamine sessions) showed significant group [$F(1,10)=8.84$, $p<0.05$] but not session [$F(3,30)=2.55$, n.s.] or interaction effects [$F(3,30)=2.12$, n.s.]. Group effects were examined with independent t tests (Bonferroni-corrected) comparing high and low callers in each of the three amphetamine sessions. Significant differences were observed in session 2 ($p<0.05$) and session 3 ($p<0.01$; Fig. 5a).

First 10 min of the post-injection period (minutes 11–20 of sessions)—Within the conditioning chambers, high callers made more post-injection 50-kHz USVs than low callers. A 2×4 RM ANOVA showed significant main effects [group: $F(1,10)=27.77$, $p<0.001$; session: $F(3,30)=6.32$, $p<0.01$], but not interaction [$F(3,30)=2.84$, n.s.]. Independent t tests (Bonferroni-corrected) comparing high and low callers in the three amphetamine sessions were significant in sessions 1 ($p<0.05$), 2 and 3 (both $p<0.01$; Fig. 5b).

Experiment 2: phase II—50-kHz USVs in home cages

Anticipatory period (minutes 0–10 of sessions)—In phase II, all rats were switched to home cage drug administration, and both high and low callers showed an increase in anticipatory 50-kHz USVs across sessions (Fig. 5c). A 2×16 RM ANOVA showed significant session effects [$F(15,150)=6.60$, $p<0.001$], but no significant group or interaction effects [group: $F(1,10)=1.18$, n.s.; session $\times$ group: $F(15,150)=1.00$, n.s.]. Linear trends (assessed with 1×15 between-subjects ANOVAs) were significant for both high callers [$F(1,89)=16.38$, $p<0.001$] and low callers [$F(1,89)=12.74$, $p<0.001$]. Paired t tests (uncorrected) comparing each amphetamine session to baseline showed high callers with significant USV increases ($p<0.05$) in sessions 6, 8, 12, 14, and 15 and low callers with significant increases ($p<0.05$ – 0.001) in sessions 8–15.

First 10 min of the post-injection period (minutes 11–20 of sessions)—When amphetamine was delivered in home cages, both groups showed an increase in 50-kHz calling compared to baseline (Fig. 5d). A 2×16 RM ANOVA showed significant session effects [$F(15,150)=2.93$, $p<0.001$], but no significant group or interaction effects [group: $F(1,10)=1.62$, n.s.; session $\times$ group: $F(15,150)=0.72$, n.s.]. During home cage session 1, five of six low callers met the criteria for a high-caller response, showing post-injection increases of 160–1,360 calls. When the 15 amphetamine sessions were each compared to baseline with paired t tests (uncorrected), high callers showed significant increases ($p<0.05$) in all sessions except 5, 6, 9, and 15 and low callers showed significant increases ($p<0.05$ – 0.01) in all sessions except 4 and 7.

FM and flat calls—FM and flat calls were examined in the first 5 min of the post-injection period and during the last 30 s of the anticipatory period in home cage sessions 1, 5, 10, and 15. The results followed the same patterns as experiment 1, with amphetamine eliciting predominately FM calls and anticipation of amphetamine eliciting equal numbers of flat and FM calls (data not shown). An overall positive correlation between amphetamine-induced USVs and anticipatory USVs is shown in Fig. 6.

Experiment 3: amphetamine-induced CPP

50-kHz USVs—The 50-kHz USVs produced during amphetamine and saline pairings are shown for high and low callers (Fig. 7a, b). High callers made significantly more 50-kHz USVs per 30 min than low callers in the first amphetamine session [715.6 ± 270.5 and 4.6 ± 1.7 , respectively; $t(18)=7.04$, $p<0.001$] and the second amphetamine session [883.2 ± 253.9 and 47.1 ± 28.8 , respectively; $t(18)=4.90$, $p<0.001$]. Controls produced few calls during the four saline sessions (i.e., highest number of USV/session= 14.4 ± 6.2). During the CPP test session, high callers showed an increase in 50-kHz USVs, but these calls were not specifically associated with the amphetamine-paired side. Compared to the other groups, high callers made more 50-kHz calls per minute while occupying the CS+ side (high= 14.3 ± 6.6 , low= 4.4 ± 3.0 , control= 3.7 ± 2.2) as well as the CS− side (high= 11.7 ± 4.2 , low= 5.5 ± 3.2 , control= 4.6 ± 2.9). Differences between high and low callers were significant for both the CS+ side [$t(18)=2.44$, $p<0.05$] and the CS− side [$t(18)=2.48$, $p<0.05$].

Conditioned place preference—High callers spent significantly more time in the CS+ side of the chamber than low callers and controls. Separate 2×2 RM ANOVAs were used to compare percent of time spent in the CS+ side during baseline and test sessions. There were significant interactions between high callers and controls [$F(1,18)=10.56$, $p<0.01$] and between high and low callers [$F(1,18)=4.94$, $p<0.05$], but no significant interaction between the low callers and controls [$F(1,18)=1.43$, n.s.] (Fig. 7c). Among amphetamine-treated rats, there was a significant positive correlation between the 50-kHz USVs produced during the first 30-min amphetamine session and the time spent in the CS+ side ($r=0.454$, $p<0.05$, $n=20$; Fig. 7d). Only one rat (a low caller) produced 22-kHz USVs during amphetamine conditioning. The same rat also showed a reduction in time spent in the amphetamine-paired side during CPP testing compared to baseline.

22-kHz USVs

In experiments 1 and 2, 22-kHz USVs were observed in 10 % of all USV files. These were produced by approximately one third of the amphetamine-treated rats, but were not seen in the saline control rats. The rats that made 22-kHz USVs did so with varying consistency across sessions, and counts of 22-kHz USVs did not follow any clear patterns at the group level. With the exception of the first amphetamine session (when rats making amphetamine-induced 22-kHz USVs were excluded from the high-caller group), there were no discernible differences in the 22-kHz USVs produced by the high callers versus low callers. There were also no detectable differences in 22-kHz calling during early versus late amphetamine sessions. For rats that emitted 22-kHz USVs, correlational analyses between 50- and 22-kHz calls are shown in Fig. 8. In general, we did not differentiate short (<300 ms) and long (>300 ms) 22-kHz USVs; however, in a random subset of 14 recordings, we found that 84.2 ± 5.7 % of 22-kHz USVs were of the long variety.

Discussion

In this study, rats were classified as high callers and low callers based on initial 50-kHz USV responses to amphetamine. In a non-home environment, high and low callers maintained stable individual differences in amphetamine-induced 50-kHz USVs over repeated exposures. In addition, the high callers, but not the low callers, emitted 50-kHz USVs during anticipation of amphetamine and demonstrated amphetamine-induced CPP. Fifty-kilohertz USVs have been associated with dopamine-mediated appetitive behavior and incentive motivation (Ma et al. 2010; Maier et al. 2010, 2012; Ciucci et al. 2007; Burgdorf et al. 2007) and have been suggested to reflect positive emotional states (Knutson et al. 2002; Burgdorf and Pankepp 2006). Therefore, the present findings suggest that 50-kHz

USVs can identify rats most susceptible to both the acute and conditioned rewarding effects of amphetamine.

Several varieties of 50-kHz USVs have been identified (Wright et al. 2010), and evidence indicates that FM USVs may be more closely linked to positive emotional states than flat USVs (Burgdorf et al. 2008, 2010; Wöhr et al. 2008). Consistent with previous studies examining stimulant-induced 50-kHz USVs (Ahrens et al. 2009; Wright et al. 2010, 2012; Brudzynski et al. 2011a; Maier et al. 2012; Burgdorf et al. 2007; Meyer et al. 2012; Simola et al. 2010), experiment 1 showed that high callers made more FM calls than flat calls after amphetamine administration. We also expected anticipatory calls to be predominately FM based on previous research showing more FM than flat calls prior to mating (Burgdorf et al. 2008). However, we found that high callers made similar numbers of FM and flat calls during anticipation of amphetamine. There is evidence that rapidly oscillating “trill” types may be more responsive to stimulants than other FM calls (Wright et al. 2010, 2012), and we cannot rule out the possibility that more extensive classification of FM subtypes would have yielded different results in the present study.

Only half of the amphetamine-treated rats in experiment 1 showed an increase in 50-kHz calling, a finding seemingly at odds with the literature showing robust increases in 50-kHz USVs after amphetamine (Ahrens et al. 2009; Wintink and Brudzynski 2001; Simola et al. 2010; Natusch and Schwarting 2010). Therefore, experiment 2 examined the role of the drug administration environment in amphetamine-induced 50-kHz USVs. Stressful environments can elicit aversive reactions to the same activity in the nucleus accumbens that provokes appetitive behaviors within a familiar home environment (Reynolds and Berridge 2008). The idea that USVs can be enhanced by the comfort of familiar surroundings has also been suggested by our previous work (Ahrens et al. 2009) reporting significant amphetamine-induced USVs in home cages, as well as by a study showing that a cage with bedding (versus no bedding) can increase amphetamine-induced calling (Natusch and Schwarting 2010). The findings from experiment 2 showed that low callers emitted more 50-kHz USVs, both before and after amphetamine injections, when sessions were conducted in home cages rather than conditioning chambers. This experiment did not include a saline control group, however, so we cannot rule out the potentially confounding influence of time on changes in vocalization.

Conditioned place preference has been used for decades to measure motivation and attraction toward contextual cues paired with a drug of abuse (Tzschentke 2007; Cunningham et al. 2006). In experiment 3, we examined amphetamine-induced USVs in a CPP paradigm. USVs elicited within the CPP apparatus (a relatively novel environment) formed the basis of high and low caller categorization. During CPP testing, high callers spent more time in the amphetamine-paired side than low callers. These findings are consistent with previous studies of cocaine- and opioid-induced CPP which found that rats that produced high numbers of 50-kHz USVs during drug exposure also showed stronger CPP than rats that did not vocalize in response to the drug (Burgdorf et al. 2007; Meyer et al. 2012). High callers also emitted more 50-kHz USVs than low callers during the CPP test session, but these conditioned USVs were not localized to the CS+ side of the apparatus, contrary to previous findings with amphetamine (Knutson et al. 1999) and cocaine (Meyer et al. 2012). However, these previous studies included four pairings of the drug with the CS+ side, whereas the current study included only two. Our findings suggest that although CPP was observed, the expression of context-specific conditioned USVs requires additional drug/context pairings. One interpretation of these results is that amphetamine is more rewarding in high callers than low callers; however, another possible interpretation is that high callers tend to habituate to the non-preferred side more rapidly than low callers. Rats in the saline

control group were never exposed to amphetamine and could not be classified as high or low callers; therefore, we cannot exclude this alternative interpretation based on the present data.

In contrast to USV findings, amphetamine-stimulated locomotor activity did not differ substantially between high and low callers. These findings are consistent with a number of studies showing that stimulant-induced locomotion follows different patterns of expression than stimulant-induced 50-kHz USVs (Browning et al. 2011; Maier et al. 2012; Mu et al. 2009; Natusch and Schwarting 2010; Burgdorf et al. 2001; Brudzynski et al. 2011a, b). These data suggest that USVs are modulated in part by different mechanisms than those responsible for non-affective behavioral responses to drugs. Though, in experiment 1, high but not low callers were more active than controls during a 30-s illumination of the cue light just prior to amphetamine delivery. This finding was likely a sign of appetitive behavior since conditioned increases in activity are frequently observed in amphetamine- or cocaine-paired environments (Ma et al. 2010; Anagnostaras et al. 2002; Fraioli et al. 1999) and escalate toward the end of anticipatory periods (Barbano and Cador 2005). In the post-injection period, both high and low callers showed comparable levels of amphetamine-induced locomotor hyperactivity that remained fairly stable across all sessions. However, behavioral sensitization, an outcome predicted by existing literature (Mu et al. 2009; Robinson and Berridge 1993; Vanderschuren and Kalivas 2000), was not observed. The discrepancy may be the result of equipment limitations as our photobeam array captured horizontal activity to a greater degree than rearing or focused, stereotypic movements, behaviors often manifested during psychomotor sensitization (Flagel and Robinson 2007; Ferrario et al. 2005).

An unexpected finding of the current report was that 22-kHz USVs were produced by a subset of amphetamine-treated rats. Long 22-kHz USVs are typically associated with anxiety, negative affect, and drug aversion (Knutson et al. 2002; Borta et al. 2006; Brudzynski 2001; Mutschler and Miczek 1998). Fifty- and 22-kHz USVs are generally thought to reflect oppositely valenced emotional states, and typically, rats that make high numbers of 22-kHz USVs during testing also show few 50-kHz USVs (Burgdorf et al. 2009; Knutson et al. 2002). Yet, there are reports of rats producing both 22- and 50-kHz USVs during experimenter tickling (Mällo et al. 2007; Schwarting et al. 2007) as well as during self-administration of cocaine (Barker et al. 2010) and methamphetamine (Mahler et al. 2013). In the present study, high callers were those making only 50-kHz USVs and no 22-kHz USVs during the first exposure to amphetamine. However, a few high callers subsequently emitted 22-kHz USVs in addition to 50-kHz USVs, pre- and post-amphetamine, suggesting that anticipation and exposure to the drug had both positive and negative features.

A potential limitation of the present study is that only one dose of amphetamine was used. A 1-mg/kg intravenous dose is substantially higher than the typical unit dose used for i.v. self-administration of amphetamine (Bardo et al. 1999; Carroll and Lac 1997). Thus, it is possible that amphetamine at this dose does not have optimal reinforcement value since it does not readily support self-administration. However, the same amphetamine dose produces robust conditioned place preference, whether administered i.p. or i.v. (Bardo et al. 1995, 1999). In addition, i.v. amphetamine has been shown to elicit the same number of 50-kHz USVs whether administered at 0.5 or 1 mg/kg (Wright et al. 2012), and similar patterns of individual differences in 50-kHz USV subtypes have been observed after a variety of i.p. amphetamine dosages (i.e., 0.25, 0.5, 1, and 2 mg/kg; Wright et al. 2010).

The link between 50-kHz USVs and positive affect suggests that high callers may experience amphetamine as pleasurable, whereas low callers may be more sensitive to anxiogenic drug effects. Psychostimulants are highly addictive drugs known to possess

pleasurable and euphoric properties as well as aversive effects (Twining et al. 2009; Blanchard and Blanchard 1999; Mutschler and Miczek 1998; Hunt and Amit 1987). There is evidence that sensitivity to the aversive effects of stimulants can keep some rats from self-administering the drugs as readily as others (Rademacher et al. 2000; Kunin et al. 2001) and that, for example, pretreatment with anxiolytics can increase the voluntary consumption of cocaine (Ettenberg and Geist 1991; Maier et al. 2008). Also, rats with low baseline anxiety levels show faster acquisition of cocaine self-administration than rats with high baseline anxiety (Schramm-Sapota et al. 2011). Since individual differences in non-drug 50-kHz USVs have been associated with anxiety and emotional predispositions (Burgdorf et al. 2009; Schwarting et al. 2007; Wöhr et al. 2009), it is possible that low callers were more prone to anxiety in a novel testing environment than high callers and therefore less likely to respond positively to amphetamine, though additional research will be needed to confirm this speculation.

Fifty-kilohertz USVs are often considered a sign of positive affect, yet it is difficult to draw firm conclusions regarding the relationship between USV response magnitudes and underlying emotional states. One interpretation is that, compared to low callers, high callers experience greater pleasure when anticipating amphetamine and after drug administration. This interpretation is supported by recent claims that FM 50-kHz USVs are specifically associated with hedonic states (Burgdorf et al. 2010, 2011). However, we cannot rule out the possibility that the observed 50-kHz USVs were an expression of behavioral activation independent of affect. The clear link between 50-kHz USVs and mesolimbic dopamine activity (Thompson et al. 2006; Burgdorf et al. 2007; Brudzynski et al. 2011a; Wintink and Brudzynski 2001) makes it possible to suggest that high callers experience greater amphetamine-induced dopamine release and behavioral sensitization than low callers. Though increased dopamine can facilitate reinforcement (Piazza and Le Moal 1996), it may not necessarily indicate positive hedonic or emotional responses to the drug (Berridge and Robinson 1998). Also, cued anticipation of a drug may elicit emotional states that are not entirely positive, such as frustration while waiting for a reward (Amsel 1958).

This study shows that USVs can reveal individual differences in amphetamine responses that are stable across repeated testing, but can also be influenced by environmental factors. Therefore, it is likely that these individual differences are an expression of some underlying trait (e.g., anxiety, impulsivity, emotionality) that interacts with the environment to alter the subjective drug experience. Aspects of this study support the idea that 50-kHz USVs are a sign of drug reward and positive affect, but these findings also highlight the need for further investigation into the relationship between USVs and specific emotional states. Notably, the co-occurrence of FM 50-kHz USVs with 22kHz USVs suggests that USVs are more complex than a simple dichotomy between positive and negative states. Nevertheless, the potential to uncover drug-induced emotional reactions could make USVs an important tool for understanding individual susceptibility to drug addiction.

Acknowledgments

This study was supported by The University of Texas Waggoner Center for Alcohol and Addiction Research Bruce-Jones Graduate Fellowship (E.Y.M., A.M.A.), NIH grants AA007471 (A.M.A.), DA014640, and DA014640-05S1 (C.L.D.), the Davis Phinney Foundation, and grant NS019608 (T.S.).

References

- Ahrens AM, Ma ST, Maier EY, Duvauchelle CM, Schallert T. Repeated intravenous amphetamine exposure: rapid and persistent sensitization of 50-kHz ultrasonic trill calls in rats. *Behav Brain Res*. 2009; 197:205–209. [PubMed: 18809437]

- Amsel A. The role of frustrative nonreward in noncontinuous reward situations. *Psychol Bull.* 1958; 55(2):102–119. [PubMed: 13527595]
- Anagnostaras SG, Schallert T, Robinson TE. Memory processes governing amphetamine-induced psychomotor sensitization. *Neuropsychopharmacology.* 2002; 26(6):703–715. [PubMed: 12007741]
- Barbano MF, Cador M. Various aspects of feeding behavior can be partially dissociated in the rat by the incentive properties of food and the physiological state. *Behav Neurosci.* 2005; 119:1244–1253. [PubMed: 16300432]
- Bardo MT, Rowlett JK, Harris MJ. Conditioned place preference using opiate and stimulant drugs: a meta-analysis. *Neurosci Biobehav Rev.* 1995; 19:39–51. [PubMed: 7770196]
- Bardo MT, Valone JM, Bevins RA. Locomotion and conditioned place preference produced by acute intravenous amphetamine: role of dopamine receptors and individual differences in amphetamine self-administration. *Psychopharmacology.* 1999; 143:39–46. [PubMed: 10227078]
- Barker DJ, Root DH, Ma S, Jha S, Megehee L, Pawlak AP, West MO. Dose-dependent differences in short ultrasonic vocalizations emitted by rats during cocaine self-administration. *Psychopharmacology.* 2010; 211:435–442. [PubMed: 20571780]
- Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Rev.* 1998; 28:309–369. [PubMed: 9858756]
- Bialy M, Rydz M, Kaczmarek L. Precontact 50-kHz vocalizations in male rats during acquisition of sexual experience. *Behav Neurosci.* 2000; 114:983–990. [PubMed: 11085613]
- Blanchard DC, Blanchard RJ. Cocaine potentiates defensive behaviors related to fear and anxiety. *Neurosci Biobehav Rev.* 1999; 23:981–991. [PubMed: 10580312]
- Borta A, Wöhr M, Schwarting RKW. Rat ultrasonic vocalization in aversively motivated situations and the role of individual differences in anxiety-related behavior. *Behav Brain Res.* 2006; 166:271–280. [PubMed: 16213033]
- Browning JR, Browning DA, Maxwell AO, Dong Y, Jansen H, Panksepp J, Sorg BA. Positive affective vocalizations during cocaine and sucrose self-administration: a model for spontaneous drug desire in rats. *Neuropharmacology.* 2011; 61:268–275. [PubMed: 21530553]
- Brudzynski SM. Pharmacological and behavioral characteristics of 22 kHz alarm calls in rats. *Neurosci Biobehav Rev.* 2001; 25:611–617. [PubMed: 11801286]
- Brudzynski SM, Silkstone M, Komadoski M, Scullion K, Duffus S, Burgdorf J, Kroes RA, Moskal JR, Panksepp J. Effects of intraaccumbens amphetamine on production of 50 kHz vocalizations in three lines of selectively bred Long–Evans rats. *Behav Brain Res.* 2011a; 217:32–40. [PubMed: 20937325]
- Brudzynski SM, Gibson B, Silkstone M, Burgdorf J, Kroes RA, Moskal JR, Panksepp J. Motor and locomotor responses to systemic amphetamine in three lines of selectively bred Long–Evans rats. *Pharmacol Biochem Behav.* 2011b; 100:119–124. [PubMed: 21867727]
- Burgdorf J, Panksepp J. The neurobiology of positive emotions. *Neurosci Biobehav Rev.* 2006; 30:173–187. [PubMed: 16099508]
- Burgdorf J, Panksepp J, Brudzynski SM, Beinfeld MC, Cromwell HC, Kroes RA, Moskal JR. The effects of selective breeding for differential rates of 50-kHz ultrasonic vocalizations on emotional behavior in rats. *Dev Psychobiol.* 2009; 51(1):34–46. [PubMed: 18819097]
- Burgdorf J, Wood PL, Kroes RA, Moskal JR, Panksepp J. Neurobiology of 50-kHz ultrasonic vocalizations in rats: electrode mapping, lesion, and pharmacology studies. *Behav Brain Res.* 2007; 182:274–283. [PubMed: 17449117]
- Burgdorf J, Knutson B, Panksepp J, Ikemoto S. Nucleus accumbens amphetamine microinjections unconditionally elicit 50 kHz ultrasonic vocalizations in rats. *Behav Neurosci.* 2001; 115:940–944. [PubMed: 11508733]
- Burgdorf J, Kroes RA, Moskal JR, Pfaus JG, Brudzynski SM, Panksepp J. Ultrasonic vocalizations of rats (*Rattus norvegicus*) during mating, play, and aggression: behavioral concomitants, relationship to reward, and self-administration of playback. *J Comp Psychol.* 2008; 122:357–367. [PubMed: 19014259]
- Burgdorf J, Panksepp J, Moskal JR. Frequency-modulated 50 kHz ultrasonic vocalizations a tool for uncovering the molecular substrates of positive affect. *Neurosci Biobehav Rev.* 2010; 35(9):1831–1836. [PubMed: 21144859]

- Burgdorf J, Kroes RA, Weiss C, Oh MM, Disterhoft JF, Brudzynski SM, Panksepp J, Moskal JR. Positive emotional learning is regulated in the medial prefrontal cortex by GluN2B-containing NMDA receptors. *Neuroscience*. 2011; 192:515–523. [PubMed: 21645591]
- Carroll ME, Lac ST. Acquisition of i.v. amphetamine and cocaine self-administration in rats as a function of dose. *Psychopharmacology*. 1997; 129:206–214. [PubMed: 9084058]
- Ciucci MR, Ma ST, Fox C, Kane JR, Ramig LO, Schallert T. Qualitative changes in ultrasonic vocalization in rats after unilateral dopamine depletion or haloperidol: a preliminary study. *Behav Brain Res*. 2007; 82:284–289. [PubMed: 17397940]
- Ciucci MR, Ahrens AM, Ma ST, Kane JR, Windham EB, Woodlee MT, Schallert T. Reduction of dopamine synaptic activity: degradation of 50-kHz ultrasonic vocalization in rats. *Behav Neurosci*. 2009; 123:328–336. [PubMed: 19331456]
- Covington HE, Miczek KA. Vocalizations during withdrawal from opiates and cocaine: possible expressions of affective distress. *Eur J Pharmacol*. 2003; 467:1–13. [PubMed: 12706449]
- Cunningham CL, Gremel CM, Groblewski PA. Drug-induced conditioned place preference and aversion in mice. *Nat Protoc*. 2006; 1(4):1662–1670. [PubMed: 17487149]
- Ettenberg A, Geist TD. Animal model for investigating the anxiogenic effects of self-administered cocaine. *Psychopharmacology*. 1991; 103:455–461. [PubMed: 2062985]
- Ferrario CR, Gorny G, Crombag H, Li Y, Kolb B, Robinson TE. Neural and behavioral plasticity associated with the transition from controlled to escalated cocaine use. *Biol Psychiatry*. 2005; 58(9):751–759. [PubMed: 16098484]
- Flagel SB, Robinson TE. Quantifying the psychomotor activating effects of cocaine in the rat. *Behav Pharmacol*. 2007; 18:297–302. [PubMed: 17551322]
- Fraioli S, Crombag HS, Badiani A, Robinson TE. Susceptibility to amphetamine-induced locomotor sensitization is modulated by environmental stimuli. *Neuropsychopharmacology*. 1999; 20:533–541. [PubMed: 10327423]
- Hunt T, Amit Z. Conditioned taste aversion induced by self-administered drugs: paradox revisited. *Neurosci Biobehav Rev*. 1987; 11:107–130. [PubMed: 3554039]
- Knutson B, Burgdorf J, Panksepp J. Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats. *J Comp Psychol*. 1998; 112:65–73. [PubMed: 9528115]
- Knutson B, Burgdorf J, Panksepp J. High-frequency ultrasonic vocalization index conditioned pharmacological reward. *Physiol Behav*. 1999; 66:639–643. [PubMed: 10386908]
- Knutson B, Burgdorf J, Panksepp J. Ultrasonic vocalizations as indices of affective state in rats. *Psychol Bul*. 2002; 128:961–977.
- Kunin D, Gaskin S, Borjas MB, Smith BR, Amit Z. Differences in locomotor response to an inescapable novel environment predict sensitivity to aversive effects of amphetamine. *Behav Pharmacol*. 2001; 12:61–67. [PubMed: 11270513]
- Ma ST, Maier EY, Ahrens AM, Schallert T, Duvauchelle CL. Repeated intravenous cocaine experience: development and escalation of pre-drug anticipatory 50-kHz ultrasonic vocalizations in rats. *Behav Brain Res*. 2010; 212:109–114. [PubMed: 20382187]
- Mahler SV, Moorman DE, Feltenstein MW, Cox BM, Ogburn KB, Bachar M, McGonigal JT, Ghee SM, See RE. A rodent “self-report” measure of methamphetamine craving? Rat ultrasonic vocalizations during methamphetamine self-administration, extinction, and reinstatement. *Behav Brain Res*. 2013; 236:78–89. [PubMed: 22940018]
- Maier EY, Ledesma RT, Seiwel AP, Duvauchelle CL. Diazepam alters cocaine self administration, but not cocaine-stimulated locomotion or nucleus accumbens dopamine. *Pharmacol Biochem Behav*. 2008; 91:202–207. [PubMed: 18691612]
- Maier EY, Ahrens AM, Ma ST, Schallert T, Duvauchelle CL. Cocaine deprivation effect: cue abstinence over weekends boosts anticipatory 50-kHz ultrasonic vocalizations in rats. *Behav Brain Res*. 2010; 214:75–79. [PubMed: 20470830]
- Maier EY, Abdalla M, Ahrens AM, Schallert T, Duvauchelle CL. The missing variable: ultrasonic vocalizations reveal hidden sensitization and tolerance-like effects during long-term cocaine administration. *Psychopharmacology*. 2012; 219(4):1141–1152. [PubMed: 21870038]

- Mällo T, Matrov D, Herm L, Kõiv K, Eller M, Rinken A, Harro J. Tickling-induced 50-kHz ultrasonic vocalization is individually stable and predicts behaviour in tests of anxiety and depression in rats. *Behav Brain Res.* 2007; 184(1):57–71. [PubMed: 17675169]
- McGinnis MY, Vakulenko M. Characterization of 50-kHz ultrasonic vocalizations in male and female rats. *Physiol Behav.* 2003; 80:81–88. [PubMed: 14568311]
- Meyer P, Ma ST, Robinson TE. A cocaine cue is more preferred and evokes more frequency-modulated 50-kHz ultrasonic vocalizations in rats prone to attribute incentive salience to a food cue. *Psychopharmacology.* 2012; 219(4):999–1009. [PubMed: 21833503]
- Mu P, Fuchs T, Saal DB, Sorg BA, Dong Y, Panksepp J. Repeated cocaine exposure induces sensitization of ultrasonic vocalization in rats. *Neurosci Lett.* 2009; 453(1):31–35. [PubMed: 19429010]
- Mutschler NH, Miczek KA. Withdrawal from a self-administered or non-contingent cocaine binge: differences in ultrasonic distress vocalizations in rats. *Psychopharmacology.* 1998; 136:402–408. [PubMed: 9600587]
- Natusch C, Schwarting RK. Using bedding in a test environment critically affects 50-kHz ultrasonic vocalizations in laboratory rats. *Pharmacol Biochem Behav.* 2010; 96:251–259. [PubMed: 20493210]
- Piazza PV, Le Moal ML. Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. *Annu Rev Pharmacol Toxicol.* 1996; 36:359–378. [PubMed: 8725394]
- Rademacher DJ, Anders KA, Thompson KJ, Steinpreis RE. The failure of some rats to acquire intravenous cocaine self-administration is attributable to conditioned place aversion. *Behav Brain Res.* 2000; 117:13–19. [PubMed: 11099753]
- Reynolds SM, Berridge KC. Emotional environments retune the valence of appetitive versus fearful functions in nucleus accumbens. *Nat Neurosci.* 2008; 11:423–425. [PubMed: 18344996]
- Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Rev.* 1993; 18:247–291. [PubMed: 8401595]
- Schramm-Sapota N, Cauley M, Stangl D, Glowacz S, Stepp KA, Levin E, Kuhn C. Role of individual and developmental differences in voluntary cocaine intake in rats. *Psychopharmacology.* 2011; 215:493–504. [PubMed: 21347641]
- Schwarting RKW, Jegan N, Wöhr M. Situational factors, conditions and individual variables which can determine ultrasonic vocalizations in male adult Wistar rats. *Behav Brain Res.* 2007; 182:208–222. [PubMed: 17367876]
- Simola N, Ma ST, Schallert T. Influence of caffeine on 50 kHz ultrasonic vocalizations in male adult rats and relevance to caffeine-mediated psychopharmacological effects. *Int J Neuropsychopharm.* 2010; 13:123–132.
- Thompson B, Leonard KC, Brudzynski SM. Amphetamine induced 50 kHz calls from rat nucleus accumbens: a quantitative mapping study and acoustic analysis. *Behav Brain Res.* 2006; 168:64–73. [PubMed: 16343652]
- Twining R, Bolan M, Grigson P. Yoked delivery of cocaine is aversive and protects against the motivation for drug in rats. *Behav Neurosci.* 2009; 123:913–925. [PubMed: 19634952]
- Tzschentke T. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict Biol.* 2007; 12:227–462. [PubMed: 17678505]
- Vanderschuren LJMJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology.* 2000; 151:99–120. [PubMed: 10972458]
- Williams SN, Undieh AS. Brain-derived neurotrophic factor signaling modulates cocaine induction of reward-associated ultrasonic vocalization in rats. *J Pharmacol Exp Ther.* 2010; 332:463–468. [PubMed: 19843976]
- Wintink AJ, Brudzynski SM. The related roles of dopamine and glutamate in the initiation of 50-kHz ultrasonic calls in adult rats. *Pharmacol Biochem Behav.* 2001; 70:317–323. [PubMed: 11701203]
- Wöhr M, Houx B, Schwarting RK, Spruijt B. Effects of experience and context on 50-kHz vocalizations in rats. *Physiol Behav.* 2008; 93(4–5):766–776. [PubMed: 18191963]

- Wöhr M, Kehl M, Borta A, Schanzer A, Schwarting RK, Hoglinger GU. New insights into the relationship of neurogenesis and affect: tickling induces hippocampal cell proliferation in rats emitting appetitive 50-kHz ultrasonic vocalizations. *Neuroscience*. 2009; 163:1024–1030. [PubMed: 19638303]
- Wöhr M, Schwarting RKW. Testing social acoustic memory in rats: effects of stimulus configuration and long-term memory on the induction of social approach behavior by appetitive 50-kHz ultrasonic vocalizations. *Neurobiol Learn Mem*. 2012; 98:154–164. [PubMed: 22677211]
- Wright JM, Gourdon JC, Clarke PBS. Identification of multiple call categories within the rich repertoire of adult rat 50-kHz ultrasonic vocalizations: effects of amphetamine and social context. *Psychopharmacology*. 2010; 211:1–13. [PubMed: 20443111]
- Wright JM, Dobosiewicz MRS, Clarke PBS. Alpha- and beta-adrenergic receptors differentially modulate the emission of spontaneous and amphetamine-induced 50-kHz ultrasonic vocalizations in adult rats. *Neuropsychopharmacology*. 2012; 37:808–821. [PubMed: 22030713]

Fig. 1.

Fifty-kilohertz USVs in high callers ($n=8$), low callers ($n=8$), and the saline-treated control group ($n=9$) in experiment 1. Figure shows 50-kHz USVs per 10 min (mean+SEM) produced during habituation/baseline sessions ($h1-h4$) and amphetamine sessions ($1-15$). **a** During the anticipatory period (minutes 0–10 of sessions), the high callers made significantly more conditioned 50-kHz USVs than the low callers and controls. **b** In the first 10 min of the post-injection period (minutes 11–20 of sessions), high callers produced more amphetamine-induced 50-kHz USVs than the low callers and controls in all 15 amphetamine sessions. There were no significant differences between the low callers and controls at any point

Fig. 2.

Individual differences in the number of 50-kHz USVs elicited by amphetamine in experiment 1. Figures show calls counted in 30-s bins (mean+SEM) during sessions 1, 5, 10, and 15. All sessions began with a waiting period in the conditioning chambers, with i.v. infusions of amphetamine (or saline in the control group, $n=9$) delivered at 10 min. **a** Amphetamine-treated rats were categorized as high callers ($n=8$) or low callers ($n=8$) based on the number of post-amphetamine 50-kHz USVs made in session 1. **b–d** In the first 5 min post-injection (minutes 11–15 of sessions), the high callers showed a greater increase in 50-kHz USVs relative to controls in sessions 5, 10, and 15 compared to session 1. $*p<0.05$ (within-subjects contrasts). The high callers also showed an increase in anticipatory 50-kHz USVs during the 30-s illumination of the cue light that preceded infusions (indicated by the shaded bar), and this effect was greater in sessions 5, 10, and 15 compared to session 1. ++ $p<0.01$; +++ $p<0.001$ (within-subjects contrasts)

Fig. 3.

FM and flat 50-kHz USVs (mean+SEM) produced by high callers ($n=8$), low callers ($n=8$), and saline control rats ($n=9$) in experiment 1. Samples of 50-kHz USVs were taken from sessions 1, 5, 10, and 15. **a–c** *Top panels*, Anticipatory 50-kHz USVs produced during the 30-s illumination of the cue light. There were no significant differences between FM and flat calls in any group during this period. **d–f** *Bottom panels*, 50-kHz USVs in the first 5 min post-injection (minutes 11–15 of sessions). In the high callers, amphetamine elicited more FM calls than flat calls. When later sessions were compared to the first session, the high callers showed a significant increase in FM calls, but not flat calls. * $p<0.05$ (paired t tests)

Fig. 4.

Locomotor activity in high callers ($n=8$), low callers ($n=8$), and saline control rats ($n=8$) in experiment 1. Figure shows counts of photobeam breakages (mean+SEM) across habituation/baseline sessions ($h1-h4$) and amphetamine sessions ($1-15$). **a** In the last 30 s of the anticipatory period, conditioned locomotor activity was significantly greater in high callers than saline-treated rats. **b** When locomotor activity was counted for the entire anticipatory period (minutes 0–10 of sessions), there were no significant group differences. **c** During the post-injection period (minutes 11–30 of sessions), both high- and low-caller groups showed significant amphetamine-induced increases in activity compared to saline-treated rats

Fig. 5.

Fifty-kilohertz USVs per 10 min (mean+SEM) produced by high callers ($n=6$) and low callers ($n=6$) in experiment 2. *Left panels*, Calls made in habituation/baseline sessions ($h1-h4$) and amphetamine sessions ($1-3$) that took place in conditioning chambers. **a** Anticipatory calls made in the pre-injection period (minutes 0–10 of sessions). **b** Calls made in the post-injection period (minutes 11–20 of sessions), when high callers made significantly more 50-kHz USVs than low callers. *Right panels*, Calls produced during habituation/baseline sessions ($h1-h2$) and amphetamine sessions ($1-15$) within the home cages. **c** In the pre-injection period, both high and low callers showed a significant increase in anticipatory calls across sessions. **d** In the post-injection period, both high and low callers made significantly more 50-kHz USVs in amphetamine sessions than baseline sessions

Fig. 6.

Fifty-kilohertz USVs per 10 min (square-root-transformed values) made by individual high and low callers in experiment 1 (*Exp1 High* and *Exp1 Low*) and experiment 2 (*Exp2 High* and *Exp2 Low*). Post-amphetamine USVs were counted in minutes 11–20 of session 1; anticipatory USVs were counted in minutes 0–10 of session 15. In rats from experiment 2, this refers to home cage sessions 1 and 15, in which low callers vocalized at rates similar to high callers. Overall, there was a significant positive correlation between amphetamine-induced calls and anticipatory calls ($r=0.622$, $p<0.001$, $n=28$). Correlations were also significant when each experiment was considered separately (experiment 1: $r=0.503$, $p<0.05$, $n=16$; experiment 2: $r=0.669$, $p<0.05$, $n=12$)

Fig. 7.

In the test for amphetamine conditioned place preference (experiment 3), high callers ($n=10$) spent more time in the CS+ side of the chamber than low callers ($n=10$) and saline-treated controls ($n=10$). *Top graphs* show 50-kHz USVs made by high callers (**a**) and low callers (**b**) counted in 5-min bins (mean+SEM) during the two saline sessions (S1 and S2) and two amphetamine sessions (A1 and A2). **c** When baseline sessions were compared to test sessions, high callers showed a significant increase in the percent of time spent in the CS+ side compared to saline controls (** $p < 0.01$), whereas low callers did not differ significantly from controls. **d** There was a significant correlation between the number of 50-kHz USVs per 30 min produced in the first amphetamine session (log-transformed values) and the percent of time spent in the amphetamine-paired side during CPP testing ($p < 0.05$).

Fig. 8.

Correlations between 22- and 50-kHz USVs associated with amphetamine. *Points* represent individual amphetamine-treated high and low callers from experiment 1 (*Exp1 High* and *Exp1 Low*) and experiment 2 (*Exp2 High* and *Exp2 Low*) that made 22-kHz USVs in at least one session. Values represent counts of 50- and 22-kHz USVs per 10 min (square-root-transformed) averaged across all amphetamine sessions in which 22-kHz USVs were produced. **a** During the anticipatory period (minutes 0–10 of sessions), there was a significant positive correlation between 50- and 22-kHz calls ($r=0.746$, $p<0.01$, $n=11$). **b** During the first 10 min of the post-injection period (minutes 11–20 of sessions), there was a significant negative correlation between 50- and 22-kHz calls ($r=-0.698$, $p<0.05$, $n=10$).