

# Rat ultrasonic vocalizations demonstrate that the motivation to contextually reinstate cocaine-seeking behavior does not necessarily involve a hedonic response

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## ABSTRACT

Human self-reports often indicate that changes in mood are a major contributor to drug relapse. Still, arguments have been made that instances of drug-seeking following abstinence in animal models (i.e. relapse/reinstatement) may be outside of hedonic control. Therefore, the present study utilized ultrasonic vocalizations in the rat in order to evaluate affect during cocaine self-administration and contextual reinstatement of cocaine-seeking in a pre-clinical model of drug relapse (abstinence-reinstatement model). Results show that while subjects effectively reinstated drug-seeking (lever pressing) following 30 days of abstinence, and spontaneously recovered/reinstated drug-seeking following 60 days of abstinence, ultrasonic vocalizations did not increase over baseline levels during either reinstatement session. These results are consistent with previous results from our laboratory and current theories of addiction suggesting that cues that are weakly associated with drug consumption can motivate drug-seeking behavior that is outside of hedonic processing.

**Keywords** Affect, cocaine, dopamine, ultrasonic vocalizations.

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## INTRODUCTION

Addiction is a chronically relapsing disorder with three major characteristics: (1) compulsion to seek and use drug; (2) loss of control over drug intake (excess drug consumption); and (3) negative emotional states following the cessation of drug use (Koob & LeMoal 1997, 2008; Koob 2008). Clinical and pre-clinical models of addiction focus on understanding these facets in order to develop therapies that prevent relapse.

One set of powerful tools in human studies of addiction are retrospective self-reports (Breiter *et al.* 1997). While the accuracy of these measures is sometimes questioned (McNagny & Parker 1992), self-report data have inarguably uncovered a role for positive and negative affective states in drug relapse that is difficult to quantify using other measures (Hodgins, el-Guebaly & Armstrong 1995; Mendelson & Mello 1996; Bijlsma,

Olivier & Groenink 2010). Indeed, it seems that drug-seeking can be driven by pursuit of the positive reinforcement that users recall from early drug experiences (Bijlsma *et al.* 2010), but can also be driven by negative reinforcement as individuals seek to alleviate a negative mood or state (Baker *et al.* 2004; Witkiewitz & Marlatt 2004). It is likely that—for stimulant users—affect can shift rapidly from positive to negative both prior to drug use (Epstein *et al.* 2009) and following drug use (Breiter *et al.* 1997). Importantly, this same duality is present in pre-clinical data and theories derived from animal models of addiction (Robinson & Berridge 1993; Koob 2000; Wheeler *et al.* 2008, 2011).

Positive symptoms associated with cocaine use include euphoria, alertness and increased confidence (Levinthal 2010 pp. 90–115), while negative symptoms include dysphoria, irritability, paranoia, insomnia and depression (Mendelson & Mello 1996; Williamson *et al.*

1997). While self-reports can be used to gather indices of affective state from human drug users, analogous measures in pre-clinical models have recently gained attention (Panksepp, Knutson & Burgdorf 2002; Burgdorf *et al.* 2001; Covington & Miczek 2003; Thompson, Leonard & Brudzynski 2006; Wheeler *et al.* 2008, 2011; Ahrens *et al.* 2009; Mu *et al.* 2009; Barker *et al.* 2010; Ma *et al.* 2010, 2012; Maier *et al.* 2010; Simola, Ma & Schallert 2010; Williams & Undieh 2010; Wright, Gourdon & Clarke 2010; Meyer, Ma & Robinson 2012; Taracha *et al.* 2012; Wright, Deng & Clarke 2012; Mahler *et al.* 2013).

One technique for measuring affect in pre-clinical models is the use of ultrasonic vocalizations (USVs) in the rat. Rat USVs serve as a convenient dependent measure of affect as their bimodal frequency distribution has been reliably segmented into two frequency ranges: one from ~18 to 33 kHz ('22-kHz range') that has been repeatedly correlated with negative/aversive outcomes (Brudzynski, Ociepa, Bihari 1991; Brudzynski & Ociepa 1992; Brudzynski *et al.* 1993; Barros & Miczek 1996; Mutschler & Miczek 1998a,b; Vivian & Miczek 1999; Burgdorf, Knutson & Panksepp 2000; Knutson, Burgdorf & Panksepp 2002) and the other ranging from ~38 to 80 kHz ('50-kHz range') that has been reliably correlated with positive/appetitive outcomes (Barfield & Thomas 1986; Knutson, Burgdorf & Panksepp 1999; Burgdorf *et al.* 2000; Knutson *et al.* 2002; Brudzynski 2005, 2008; Schwarting, Jegan & Wöhr 2007; Burgdorf, Panksepp & Moskal 2011).

Recent pre-clinical studies have utilized relapse and reinstatement paradigms in concert with USVs (Browning *et al.* 2011; Mahler *et al.* 2013), but a test of cocaine contextual reinstatement that includes USVs has yet to be conducted. Theories of addiction posit that all types of cues (paraphernalia, odors and sounds, availability of cocaine, drug-use partners, contexts, stress, etc.) present during cocaine self-administration (S-A) become learned and increase the probability that relapse will occur when an abstinent user is confronted with these cues (Jaffe 1990; Ahmed & Koob 1997). Moreover, compounds of multiple cues have been shown to produce additive effects on drug responding (Liu & Weiss 2002). Thus, it is important to ascertain the contribution of various types of drug-related cues and affect to reinstatement.

The goal of the present study was to examine affective responses during a contextual reinstatement paradigm following long-access cocaine S-A (Ahmed & Koob 1999). Although reinstatement often includes an extinction phase, forced abstinence was chosen over an extinction procedure given the relevance of the abstinence-reinstatement model to drug dependence and relapse in the clinical setting (Fuchs *et al.* 2009). In the present experiment, subjects were trained in the absence of other programmed cues (i.e. CS<sup>+</sup> or S<sup>D</sup>) in order to

obtain a cue-free assessment of the contribution of affect to contextual reinstatement following 30 and 60 days of abstinence.

## MATERIALS AND METHODS

### Subjects/Surgery

Subjects ( $n = 8$ ) were catheterized and cared for as described previously (Root *et al.* 2009, 2011). Male Long-Evans rats (Charles River, Wilmington, MA, USA) were singly housed on a 12 hours : 12 hours light : dark cycle with dawn at 10:30 AM. All protocols were performed in compliance with the Guide for the Care and Use of Laboratory Animals and have been approved by the Institutional Animal Care and Use Committee, Rutgers University.

### Apparatus

All experiments were conducted in Plexiglas chambers measuring  $24 \times 34.5 \times 34.5$  cm and housed inside a larger sound-attenuating chamber (~76 cm<sup>3</sup>). Animals were attached to an intravenous fluid delivery system consisting of a syringe pump (Razel Scientific, St. Albans, VT, USA), which connected to a fluid swivel. A spring leash was connected to the bottom of the swivel and extended to the head of the animal through the top of each chamber. The intravenous catheter was contained inside a spring leash and continued through a steel cannula on the animal's head and into the right jugular vein. For S-A sessions, a glass lever ( $4.7 \times 2.5$  cm) was inserted through a hole 4 cm off the floor of the chamber and 6 cm from the door. The lever was set so that 0.049 N of force were required to activate the lever. Experimental apparatuses were controlled by a personal computer running MED-Associates hardware and software (St. Albans, VT, USA).

### Self-administration

S-A training began daily at 10:30 AM, immediately following the commencement of the light cycle. At the start of the session, a single non-retractable response lever was mounted on one wall of the operant conditioning chamber. For the first 10 infusions, drug was available during an unsignaled 120-second availability period, followed by a 40-second timeout (TO). A response during the availability period produced a 0.71 mg/kg infusion of cocaine and initiated the next TO. If no responses occurred for 2 minutes, the availability period was terminated and the next TO was initiated. The initial 40-second TO promoted shaping in early sessions and allowed subjects to rapidly load on cocaine during the remainder of training. Following the 10th infusion, for the remainder of the session, unsignaled availability periods (120

seconds) were presented after a 1–6-minute variable TO. All shaping and training sessions were long access, lasting for 6 hours or 80 response–contingent infusions, whichever occurred first. Subjects received post-session feeding in order to maintain weights of approximately 320–340 g. Water was available *ad libitum* except during S-A sessions. Animals were trained for 14 days (7 days/week) with no breaks between training sessions. Subjects were then given a series of five probe sessions (data published elsewhere) consisting of 90-minute periods of normal S-A followed by a manipulation in which animals' drug-levels were transitioned (over 30 minutes) to and subsequently maintained within a finite range for the remaining 4 hours of the session using a series of computer-controlled micro-infusions (0.0064 mg/5.33  $\mu$ l/infusion)—similar to methods reported by Root *et al.* (2011)—over the 5–10 days following normal training. For all but two of the probe sessions, levers were present (but had no programmed response). Each probe session was followed by a one session return to normal S-A contingencies. Importantly, a study by Kippin, Fuchs & See (2006) demonstrated that this type of manipulation has little effect on reinstatement responding.

#### Abstinence/Contextual reinstatement (CR) tests

Following the final S-A session, the catheter was disconnected at the level of the headstage, and animals were returned to their home cages for 60 days of abstinence. At 30 (CR1) and 60 days (CR2) of abstinence animals were returned to the S-A chamber for contextual reinstatement testing. For both tests, the lever was inserted prior to testing and the session began by placing the animals in the chamber immediately following illumination of the houselight. All reinstatement sessions lasted for 6 hours. Lever presses during these sessions were recorded, but had no consequence.

#### USV recordings

A condenser microphone (CM16/CMPA, Avisoft Bioacoustics, Berlin, Germany) was inserted into the sound-attenuating chamber 15–30 minutes before recording sessions. The microphone was suspended ~2.5 cm from a set of small holes in the top of the S-A chamber inside of a Plexiglas tube that increased the signal:noise ratio of the recordings. Following the start of the session, recordings were triggered by a +5V transistor-transistor logic (TTL) pulse sent from MED-PC IV to the recording hardware (Ultrasound Gate 116H, Avisoft Bioacoustics). Sonorous activity was recorded at a 250-kHz sampling frequency (16 bits) using Avisoft Recorder software (Avisoft Bioacoustics) and stored for offline analysis. Recorded '.wav' files were then analyzed using Avisoft SASLab Pro (Avisoft Bioacoustics).

#### Recording sessions

##### Baseline recordings

Prior to surgery, subjects were allowed to live in the S-A chamber for 3 days. On the fourth day, USVs were recorded for all subjects during a 6-hour baseline period. These recordings occurred from 10:30 AM to 4:30 PM.

##### S-A USV recordings

USVs were sampled at two timepoints across weeks of S-A training: days 5 and 10. Day 5 was used as an early sample after the initial 1–3-day shaping period. Day 10 was chosen as an equally spaced sample that occurred late in training. The USV recording was triggered at the start (time zero) of the session and continued until the end of the session.

##### Contextual reinstatement

For both the 30- and 60-day reinstatement tests, recordings were triggered immediately after animals were placed in the chamber, and were recorded continuously until the end of the session.

#### Characterization of USVs

Avisoft SASLab Pro (Avisoft Bioacoustics) was used for *post hoc* analysis. Each '.wav' file was opened as a spectrogram with a fast Fourier transform length of 512 samples and a flat-top window with 50% overlap. Spectrograms were visually scanned for patterns resembling USVs. Once a putative, individual USV had been identified, the mean frequency was calculated by averaging the minimum and maximum frequencies of the call. While USVs can be differentiated into several call-types based on the presence or lack of frequency modulation (Schwartzing *et al.* 2007; Wright *et al.* 2010), no trends related to call type were observed in the present data. Moreover, the inclusion of call type in the present data problematically increased zero inflation of the data for statistical analysis. Thus, only call frequency was included for statistical analysis.

#### Analysis

S-A behavior was analyzed across training sessions for the first 2 weeks of training. Behavioral data were analyzed using a repeated measures analysis of variance (ANOVA, SAS PROC GLIMMIX, SAS Institute Inc., Cary, NC, USA) with Sidak adjustments for *post hoc* comparisons. Pairwise comparisons were made between session 1 and each subsequent session of training (2–14). Lastly, a Sidak-adjusted 'plateau contrast' was designed to test whether behavioral measures fit an asymptotic learning curve [contrast coefficients: (–6, –2.25, –1, 0.01, 0.5,

0.75, 1, 1, 1, 1, 1, 1, 1, 1)]. Lever presses during reinstatement conditions were analyzed across all 6 hours to test for extinction and subsequently, spontaneous recovery.

USVs were analyzed using three-way repeated measures ANOVAs examining Hour  $\times$  Call Frequency  $\times$  Condition. Hour consisted of six, 1-hour bins for each session. Call Frequency consisted of the 22- and 50-kHz ranges as well as a '33-kHz' range [33–37.99 kHz; inserted as a buffer between the 22-kHz (18–32.99 kHz) and 50-kHz (38–80 kHz) ranges]. This buffer is necessary, as graphical analyses have shown that the 50- and 22-kHz frequency distributions overlap in this region and become uninterpretable (e.g. Barker *et al.* 2010). The inclusion of this middle range allows all USVs to be included in the model, but assigns no affective state to USVs in this ambiguous range. Condition consisted of each session during which USVs were recorded. *Post hoc* comparisons focused on the first hour of each experimental condition. This allowed reinstatement sessions to be directly compared with both S-A and baseline rates of calling, but reduced the influence of extinction learning on USVs during reinstatement sessions, which has been shown to cause a shift from appetitive to aversive calling (Burgdorf *et al.* 2000). To match reinstatement, the first hour was always operationally defined as starting at the moment of lever insertion and ending sixty minutes after the insertion of the lever.

## RESULTS

### Self-administration

Behavioral measures included the number of lever presses, number of infusions earned, percent hit [calculated as: (earned infusions/total number of cocaine availability periods)  $\times$  100; colloquially defined as the percentage of opportunity periods during which animals responded, thereby administering an infusion], total drug consumption (mg/kg), peak drug level (mg/kg) and bodyweight (grams). Behavioral data for S-A training are shown in Fig. 1.

Subjects acquired the task rapidly, with lever presses increasing from  $37.13 \pm 13.12$  [mean  $\pm$  standard error of the mean (SEM)] in session one to  $225.38 \pm 28.79$  in session 14 [ $F(7, 91) = 5.01, P < 0.001$ ], the number of earned infusions increasing from  $16.25 \pm 2.31$  in the first session to  $53.50 \pm 2.44$  in the 14th session [ $F(7, 91) = 51.73, P < 0.001$ ] and total drug consumption increasing from  $12.96 \pm 1.99$  to  $38.91 \pm 1.82$  mg/kg [ $F(7, 91) = 13.92, P < 0.001$ ]. Subjects also became more efficient in the task, increasing the percentage of opportunities where subjects received an infusion from  $13.41 \pm 1.71\%$  in session one to  $61.07 \pm 2.89\%$  in

session 14 (earned infusions/number of opportunities) [ $F(7, 91) = 27.03, P < 0.001$ ]. This demonstrates that subjects increased their drug consumption over sessions, but also missed opportunities to administer drug on nearly 40% of trials. Results from our laboratory (Root *et al.* 2011) would suggest that these misses correspond to periods when the animal's drug level is at or above drug-satiety.

Peak drug levels increased from  $2.97 \pm 0.38$  mg/kg in session one, to nearly double that ( $5.61 \pm 0.30$  mg/kg) by the end of training [ $F(7, 91) = 16.97, P < 0.001$ ]. As has been reported previously (Bozarth & Wise 1985), repeated S-A training also corresponded with a significant decrease in bodyweight over days [ $332.75 \pm 6.42$  to  $319.14 \pm 2.76$  g;  $F(7, 91) = 13.80, P < 0.001$ ].

Sidak *post hoc* comparisons indicated that the number of lever presses was significantly greater in sessions 3–14 compared with session one [all  $|t(91)| \geq 3.97, P \leq 0.01$ ]. Similarly, drug consumption, peak drug level, earned infusions and percent hit were significantly greater than session one in all subsequent sessions [Sessions 2–14, all  $|t(91)| \geq 3.24, P \leq 0.01$ ]. All behavioral measures except bodyweight fit an asymptotic curve, as evidenced by a significant plateau contrast [all  $|t(91)| \geq 3.03, P \leq 0.01$ ]. This demonstrates that animals increased task efficiency over early training sessions and reached stable behavioral performance and drug consumption by the end of training.

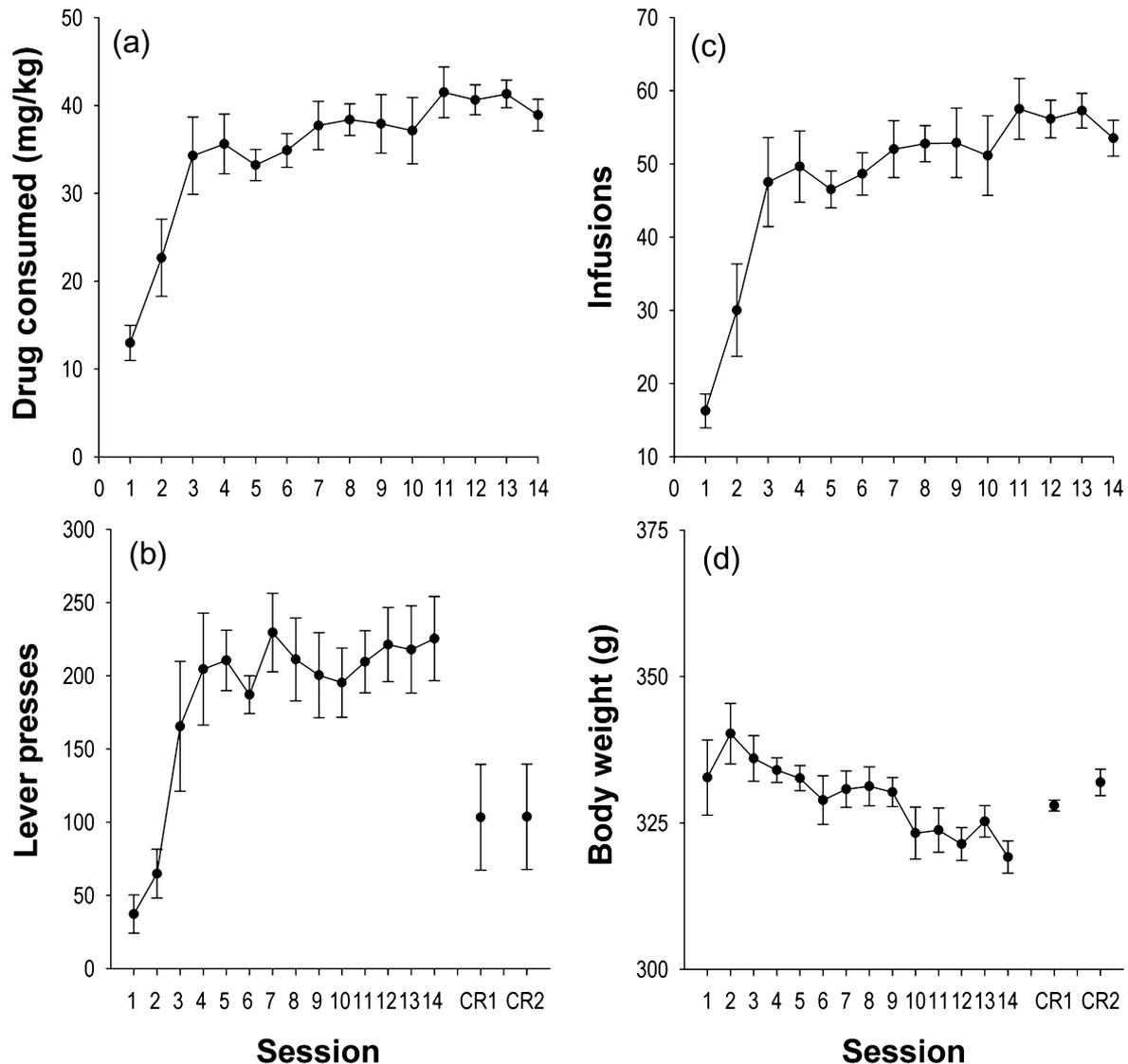
### Contextual reinstatement

The highest number of lever presses occurred during the initial hours of re-exposure to the cocaine-associated context (Fig. 2). The number of responses decreased in CR1 from  $32.63 \pm 13.48$  lever presses in hour 1, to  $3.00 \pm 1.94$  in hour 6. Indeed, animals extinguished responding within the reinstatement session, then exhibited spontaneous recovery of responding between hour 6 of CR1 and the first hour of CR2 [ $|t(7)| = 2.97, P < 0.05$ ] (Fig. 2). Similar to CR1, responding in CR2 decreased from  $46.375 \pm 14.12$  lever presses in hour 1 to  $9.63 \pm 4.47$  in hour 6.

When lever responding was examined using a  $2 \times 6$  ANOVA for Condition  $\times$  Hour, a significant interaction was observed [ $F(5, 35) = 2.882, P < 0.05$ ] as well as a significant main effect of hour [ $F(1.18, 8.25) = 7.087, P < 0.05$ ] but no significant effect of condition. Although more responses were emitted during hour 1 of CR2 than hour 1 of CR1, a planned comparison between these hours showed the increase to be non-significant.

### USVs

USVs were analyzed using an omnibus three-way repeated measures ANOVA with five levels of condition



**Figure 1** Mean ( $\pm$  standard error of the mean) (a) daily drug consumption; (b) number of lever presses; (c) number of earned infusions; and (d) body weight (y-axes) across sessions (x-axes). Subjects escalated drug intake over sessions (a and c) and became more task proficient (b) across sessions. As is typical of chronic drug administration (Bozarth & Wise 1985), subjects' bodyweight decreased across sessions

[Baseline, week 1 (WK1) of S-A, week 2 (WK2) of S-A, the 30-day Contextual reinstatement test (CR1), and the 60-day Contextual reinstatement test (CR2)], three levels of frequency, and six levels of hour. The average number of USVs in each hour and condition (mean  $\pm$  SEM) can be found in Table 1.

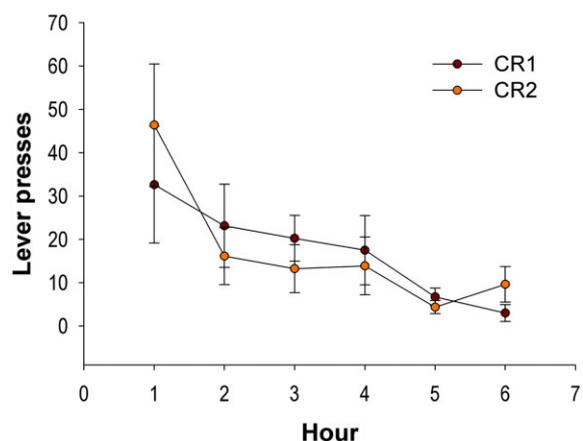
The repeated measures ANOVA revealed a significant three-way interaction [ $F(7, 623) = 8.37, P < 0.001$ ], significant two-way interactions for Frequency  $\times$  Hour [ $F(7, 623) = 143.40, P < 0.001$ ], Condition  $\times$  Hour [ $F(7, 623) = 13.17, P < 0.001$ ], and Condition  $\times$  Frequency [ $F(7, 623) = 81.67, P < 0.001$ ], and significant main effects for Frequency [ $F(2, 623) = 16.68, P < 0.001$ ] and Hour [ $F(5, 623) = 90.93, P < 0.001$ ], but not Condition.

#### Post hoc tests

Post hoc comparisons were planned specifically for analyzing USVs during the first hour, for which mean  $\pm$  SEM are shown in Fig. 3. No significant differences were found in the 33-kHz range and thus, the data are not reported. Post hoc comparisons between baseline and hour 1 of CR1 and CR2 revealed that neither 22- nor 50-kHz USVs were greater during reinstatement sessions when compared with baseline. Furthermore, neither 22- nor 50-kHz USVs correlated with rates of responding during the first hour of CR1 or CR2 [22-kHz CR1,  $r(8) = 0.17, N.S.$ ; 50-kHz CR1,  $r(8) = 0.20, N.S.$ ; 22-kHz CR2,  $r(8) = 0.31, N.S.$ ; 50-kHz CR2,  $r(8) = 0.08, N.S.$ ]. There were, however, fewer 50-kHz USVs in hour 1 of CR1, CR2, as

well as baseline when compared with the first hour of WK1 and WK2 of self-administration [all  $|t(623)| \geq 2.63$ ,  $P < 0.01$ ]. No differences were observed in the 22-kHz range between S-A and baseline or S-A and reinstatement, although there were more 22-kHz USVs during CR1 than CR2 [ $t(623) = 3.32$ ,  $P < 0.001$ ]. Thus, contextual reinstatement was not sufficient to increase 50- or 22-kHz USVs above baseline levels even though it was sufficient to produce rates of responding comparable with those seen during an average hour of S-A.

Comparisons of 22- and 50-kHz USVs within the first hour of each condition revealed a tendency for more



**Figure 2** The mean ( $\pm$  standard error of the mean) number of lever presses ( $y$ -axis) across hours ( $x$ -axis) for contextual reinstatement tests 1 (CR1) and 2 (CR2). Extinction of lever presses occurred across hours for both conditions. Subjects spontaneously recovered lever pressing as evidenced by the increase in responding during the first hour of CR2 as compared with extinguished responding in the last hour of CR1

22-kHz USVs than 50-kHz USVs during baseline [ $t(623) = 5.19$ ,  $P < 0.001$ ]. This difference is likely attributable to the near absence of 50-kHz USVs during the baseline condition (Table 1). Similarly, subjects emitted significantly greater numbers of 22-kHz USVs than 50-kHz USVs during the first hour of CR1 [ $t(623) = 2.02$ ,  $P < 0.05$ ], but neither was different from baseline. Moreover, no difference between 50- and 22-kHz USVs was observed for CR2. In contrast, there were more 50-kHz USVs than 22-kHz USVs during the first hour of both WK1 and WK2 of S-A [all  $|t(623)| \geq 2.41$ ,  $P < 0.05$ ]. Importantly, the robust increase in 50-kHz USVs over 22-kHz USVs observed during S-A sessions was not observed during either baseline or reinstatement recordings.

## DISCUSSION

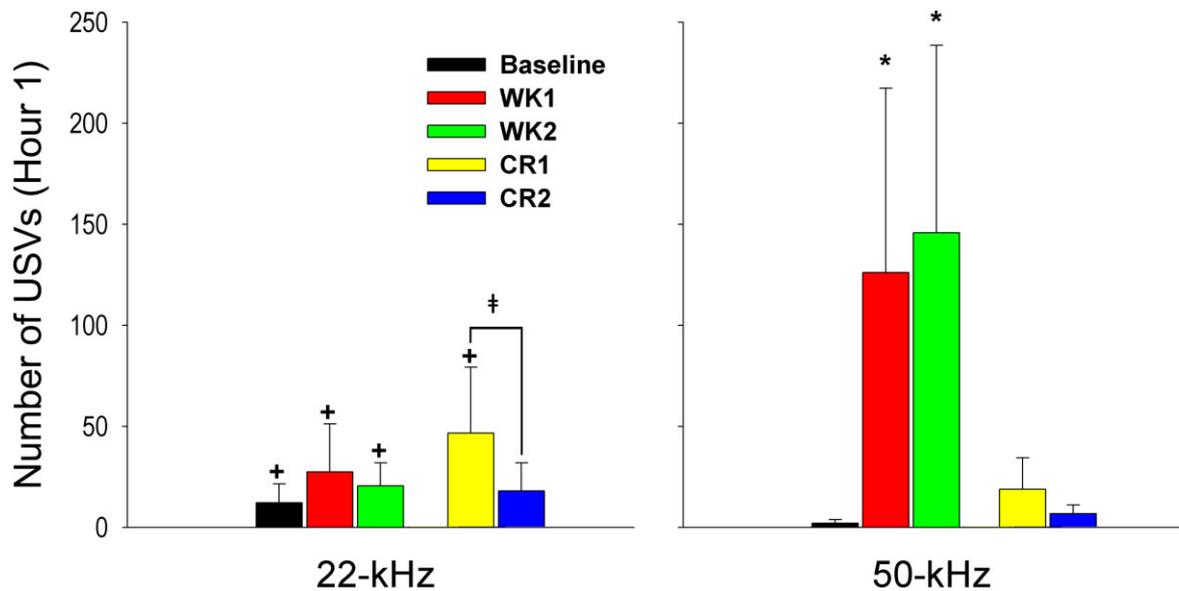
Similar to previous reports from our laboratory (Root et al. 2009; Barker et al. 2010), animals rapidly acquired S-A and stabilized behavior following 3–5 days of training. Cocaine dependence in humans is characterized by weight loss, tolerance and withdrawal symptoms (Mendelson & Mello 1996). Thus, behavioral results from the current experiment are consistent with characteristics of cocaine dependence, as (1) animals showed weight loss over the course of drug administration and (2) the acquisition of S-A also corresponded to an escalation of drug intake over sessions. The escalation of drug intake—defined as an increase in drug consumption across daily sessions—is thought to serve as a pre-clinical model of drug tolerance and compulsive drug-seeking (Ahmed & Koob 1999) and occurs only

**Table 1** Number of USVs: Condition  $\times$  call frequency  $\times$  session time (mean  $\pm$  SEM).

	Baseline	WK1	WK2	CR1	CR2
Hour	22-kHz USVs				
1	12.25 $\pm$ 9.31	27.50 $\pm$ 23.72	20.63 $\pm$ 11.34	46.63 $\pm$ 32.78	18.13 $\pm$ 13.81
2	5.38 $\pm$ 4.96	51.50 $\pm$ 50.22	16.75 $\pm$ 15.49	19.00 $\pm$ 13.97	27.00 $\pm$ 24.87
3	5.88 $\pm$ 4.90	27.50 $\pm$ 24.11	16.25 $\pm$ 14.02	21.13 $\pm$ 11.54	40.50 $\pm$ 22.02
4	5.38 $\pm$ 2.95	18.25 $\pm$ 17.12	15.63 $\pm$ 13.72	15.25 $\pm$ 10.75	16.50 $\pm$ 10.32
5	1.00 $\pm$ 0.63	18.63 $\pm$ 17.10	8.00 $\pm$ 5.38	13.00 $\pm$ 12.29	55.75 $\pm$ 41.68
6	4.88 $\pm$ 4.19	14.13 $\pm$ 12.85	5.13 $\pm$ 3.88	0.38 $\pm$ 0.26	2.25 $\pm$ 2.11
	50-kHz USVs				
1	2.13 $\pm$ 1.86	126.13 $\pm$ 91.22	145.88 $\pm$ 92.73	19.00 $\pm$ 15.47	6.88 $\pm$ 4.21
2	0.13 $\pm$ 0.13	35.50 $\pm$ 35.36	0.75 $\pm$ 0.41	0.25 $\pm$ 0.16	1.25 $\pm$ 0.25
3	1.00 $\pm$ 0.38	6.63 $\pm$ 6.48	0.75 $\pm$ 0.62	1.25 $\pm$ 0.45	1.25 $\pm$ 0.73
4	0.88 $\pm$ 0.30	1.88 $\pm$ 1.88	0.13 $\pm$ 0.13	0.63 $\pm$ 0.38	0.88 $\pm$ 0.52
5	1.00 $\pm$ 0.46	0.38 $\pm$ 0.38	0.13 $\pm$ 0.13	0.38 $\pm$ 0.26	0.50 $\pm$ 0.19
6	0.25 $\pm$ 0.16	1.25 $\pm$ 1.25	0.00 $\pm$ 0.00	0.63 $\pm$ 0.50	0.50 $\pm$ 0.27

The mean ( $\pm$  SEM) number of USVs emitted in the 22- and 50-kHz frequency ranges during each hour of the five recording conditions.

CR1 = first contextual reinstatement test (30 days abstinence); CR2 = second contextual reinstatement test (60 days abstinence); SEM = standard error of the mean; USVs = ultrasonic vocalizations; WK1 = week 1 of self-administration; WK2 = week 2 of self-administration.



**Figure 3** The mean  $\pm$  standard error of the mean number of 22-kHz and 50-kHz ultrasonic vocalizations (USVs) emitted during the first hour of each recording condition. WK1—week 1 of self-administration; WK2—week 2 of self-administration; CR1—first contextual reinstatement test (30 days abstinence); CR2—second contextual reinstatement test (60 days abstinence). \* $P < 0.05$  when compared with baseline. + $P < 0.05$  when compared within-condition to the 50-kHz range. † $P < 0.05$

following long-access training (Ahmed & Cador 2006; Zernig *et al.* 2007; Quadros & Miczek 2009). Furthermore, while animals in the present study were not tested for withdrawal, work by Miczek and colleagues recording USVs demonstrated that rats experience an aversive withdrawal state following long-access sessions (Mutschler & Miczek 1998a,b; Covington & Miczek 2003), which has been replicated in our laboratory (unpublished observations).

An examination of lever responding during 30- and 60-day contextual reinstatement tests (CR1 and CR2, respectively) revealed that, during the first hour of each reinstatement test, subjects responded at rates comparable with those observed in S-A. Moreover, subjects extinguished responding across hours within each reinstatement session, but spontaneously recovered responding in CR2, following extinction during CR1.

The literature suggests that reinstatement of drug responding could involve positive affect, negative affect, or both: 1) a positive anticipation has been shown upon being returned to the drug-paired environment (Ahrens *et al.* 2009; Ma *et al.* 2010; Browning *et al.* 2011) 2) drug responding could represent a means of relieving conditioned negative affect evoked by drug cues (e.g. Siegel 1983) or 3) negative affect could also represent a reaction to reward omission (Burgdorf *et al.* 2000; Coffey *et al.* 2013). Thus, the most surprising result of the present study was the lack of correspondence between USVs and reinstated responding. While animals did emit more 22-kHz USVs than 50-kHz USVs during CR1, indicating a predominance of negative affect, no correlation

between numbers of responses and USVs was observed for either CR1 or CR2. Furthermore, neither 22- nor 50-kHz USVs were greater during CR1 or CR2 when compared to baseline. Finally, USVs actually decreased when comparing the first hour of CR1 to that of CR2, while drug-seeking (lever presses) tended to increase from CR1 to CR2.

Nevertheless, this result is in line with results suggesting that compulsive drug-seeking occurs following long-access self-administration (Ahmed & Cador 2006; Zernig *et al.* 2007; Quadros & Miczek 2009), and that the types of compulsive drug-seeking that occur following long-term drug use can be affected by drug 'wanting' (motivation to seek drug) without being affected by—or even in the absence of—hedonic responses ('liking'; Berridge & Robinson 1995; Robinson & Berridge 2001). That is, the motivation to seek drug may be able to function outside of hedonic control.

Interestingly, another study examining USVs during reinstatement has shown that USVs are prevalent at reinstatement testing (Browning *et al.* 2011). Browning and colleagues allowed animals 2 hours of daily access to cocaine under an FR-5 schedule followed by 1–2 weeks of extinction training before being tested for cue- and cocaine-primed reinstatement. It was shown that USVs increased across S-A training (to ~700 USVs/hour) before decreasing during extinction to levels comparable with the earliest S-A recordings (~250 USVs/hour). USVs showed a transient increase from extinction during cue-induced reinstatement testing and a robust increase following cocaine-primed reinstatement.

A number of differences between Browning *et al.* (2011) and the present data might explain the differences between cue-induced and contextual reinstatement. First, the USVs observed by Browning *et al.* (2011) sensitized across multiple short-access sessions, while attenuation in USVs was observed across long-access sessions in the present study. Thus, the session length, quantity of daily drug consumption, and other inherent differences between long- and short-access S-A protocols may contribute to different hedonic reactions at reinstatement. Proposed theories of addiction suggest that long-access drug use might cause certain protective allostatic changes that could be reflected in animals' affective responses at reinstatement (Koob & LeMoal 2001).

Second, it should not be overlooked that different types of cues become instilled with different motivational properties across training (Everitt & Robbins 2005). Differences in the imbued properties of a CS<sup>+</sup>, which shares a proximal relationship with reward delivery, may produce a more pronounced hedonic response than contextual cues, which do not share this same proximal relationship. Indeed, subjects in the present study lived in the chamber for approximately 3 weeks during recovery from surgery and S-A training, making the context a poor predictor of reward delivery. Also, the affective response to the CS<sup>+</sup> used by Browning and colleagues was small and transient, suggesting that the perhaps lesser salience of contextual cues was insufficient to produce an affective response. Nonetheless, despite failing in this regard, context was sufficient to produce reliable drug-seeking behavior (i.e. lever pressing) during the present reinstatement tests.

Finally, research has shown that different neural circuits are recruited during reinstatement that is preceded by extinction than by forced abstinence. Indeed, Fuchs, Branham & See (2006) demonstrated that brain regions involved in habit formation (e.g. dorsolateral striatum), but not goal-directed responding (e.g. basolateral amygdala and dorsomedial prefrontal cortex) are involved in contextual reinstatement following abstinence, while only those involved in goal-directed responding are involved in extinction-reinstatement paradigms. Thus, differences during the posttraining period may contribute to the observed differences in hedonic response at test. It is certainly possible that reinstatement to contextual cues following forced abstinence from long-access training might have been habitual, whereas CS<sup>+</sup>-induced responding following short-access training might have remained goal directed.

Given the aforementioned evidence, it might be suggested that motivated responding and affect can work in concert or independently during reinstatement/relapse. Indeed, a drug-paired CS<sup>+</sup> in a short-access extinction-reinstatement paradigm was able to produce

both a hedonic and behavioral response during reinstatement (Browning *et al.* 2011). On the other hand, contextual cues following long-access training in an abstinence-reinstatement paradigm are imbued with similar incentive value in that they can motivate responding, but do not produce the same hedonic response (present data). Accordingly, the present findings lead to the conclusion that a hedonic reaction does not appear to be necessary for reinstatement/relapse behavior in this animal model. Moreover, the presence or absence of such a hedonic response may indicate differences in neural physiology as individuals transition from drug use to drug dependence, differences in cue learning, or differences in subjects' levels of response automaticity. These insights may be important for developing successful behavioral and pharmacological treatments, suggesting that the motivational and hedonic components of relapse might necessitate specific (and perhaps different) therapies.

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#### Authors Contributions

DJB, DHR and MOW were responsible for the concept and design of the study. DJB, DB, LCS and SJS were involved in data collection and analysis, while SM and APP were involved in data analysis and statistical design. DJB and DB were responsible for writing the manuscript and all authors contributed to preparation/editing of the document.

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