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Ultrasonic Vocalizations as a Measure of Affect in Preclinical Models of Drug Abuse: A Review of Current Findings

David J. Barker[1,2,*], Steven J. Simmons[3] and Mark O. West[2]

[1]*National Institute on Drug Abuse, Neuronal Networks Section, National Institutes of Health, Baltimore, Maryland, USA;* [2]*Department of Psychology, Rutgers University, New Brunswick, New Jersey, USA;* [3]*Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA, USA*

D.J. Barker

Abstract: The present review describes ways in which ultrasonic vocalizations (USVs) have been used in studies of substance abuse. Accordingly, studies are reviewed which demonstrate roles for affective processing in response to the presentation of drug-related cues, experimenter- and self-administered drug, drug withdrawal, and during tests of relapse/reinstatement. The review focuses on data collected from studies using cocaine and amphetamine, where a large body of evidence has been collected. Data suggest that USVs capture animals' initial positive reactions to psychostimulant administration and are capable of identifying individual differences in affective responding. Moreover, USVs have been used to demonstrate that positive affect becomes sensitized to psychostimulants over acute exposure before eventually exhibiting signs of tolerance. In the drug-dependent animal, a mixture of USVs suggesting positive and negative affect is observed, illustrating mixed responses to psychostimulants. This mixture is predominantly characterized by an initial bout of positive affect followed by an opponent negative emotional state, mirroring affective responses observed in human addicts. During drug withdrawal, USVs demonstrate the presence of negative affective withdrawal symptoms. Finally, it has been shown that drug-paired cues produce a learned, positive anticipatory response during training, and that presentation of drug-paired cues following abstinence produces both positive affect and reinstatement behavior. Thus, USVs are a useful tool for obtaining an objective measurement of affective states in animal models of substance abuse and can increase the information extracted from drug administration studies. USVs enable detection of subtle differences in a behavioral response that might otherwise be missed using traditional measures.

Keywords: Addiction, affect, emotion, stimulant, ultrasonic vocalizations.

ACOUSTIC AND FUNCTIONAL CHARACTERISTICS OF RAT ULTRASONIC VOCALIZATIONS (USVs)

Rats produce vocalizations in sonic and ultrasonic frequencies that can be defined by their acoustic and functional properties, as well as their method of production. Sonic vocalizations, ranging from 0-18 kHz, are produced through slow vibrations of the vocal folds and are emitted when rats encounter a threat. Sonic vocalizations have been observed in the laboratory when rats experience pain or when they are handled by experimenters [1, 2]. Separately, rats can emit vocalizations in ultrasonic frequencies (18-80 kHz) which are appropriately termed "ultrasonic vocalizations" (USVs; [1]). Among other functions, USVs can signal alarm to conspecifics or indicate reward reception. Rats are capable of producing sonic vocalizations and USVs that can be characterized by the acoustic parameters of the emission.

USVs serve as a method of intraspecies communication in rats [3, 4]. Given that USVs are acoustically heterogeneous in nature (for examples, see [5]), one might expect that different categories of vocalizations serve somewhat different signaling functions. Researchers have categorized USVs based on the presence and number of pitch modulations during a single emission. For example, individual USVs that are maintained around a single frequency throughout the entirety of emission are referred to as "fixed-frequency calls" (FF). Individual USVs that are observed to change in frequency throughout emission (i.e. > 3-kHz shift in frequency) [6-10] are referred to as "frequency-modulated calls" (FM). Lastly, individual USVs that are observed to have more than one frequency modulation throughout emission are referred to as "trills". Frequency modulations are often described as being a "sweep" or "jump/step" from one pitch to another, and the frequency modulations that characterize trills can consist of multiple cycles of either frequency modulation type or a combination thereof [5]. The acoustic parameters of individual emissions have been used by researchers to classify USVs into unique call types.

USVs naturally dichotomize into two frequency ranges. Specifically, USVs in the 18-33 kHz frequency range, collectively referred to as "22-kHz USVs", can be long or short in duration. Long duration (300-3400 ms; [11]) 22-kHz calls are emitted during aversive stimulation such as social isolation [12], predatory odor exposure [13], foot-shock [14] or anxiogenic drug administration [15]. Furthermore, 22-kHz USVs have been shown to function as alarm cries to conspecifics [2]. Short duration 22-kHz USVs have been

*Address correspondence to this author at the National Institute on Drug Abuse, Neuronal Networks Section, 251 Bayview Boulevard, Baltimore, MD 21224; Tel: 443-740-2708; E-mail: David.Barker@nih.gov

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observed during the formalin footpad pain test [16], experimenter handling and foot-shock [17, 18], and following the injection of muscarinic agonists into the anterior hypothalamus [17, 18], which is known to be an aversive stimulus. Interestingly, studies have shown that shorter duration USVs carry more information for conspecifics and elicit the greatest defensive responses [19].

Unlike 22-kHz USVs, USVs in the 38-80 kHz frequency range, collectively termed "50-kHz USVs", are emitted during rewarding and appetitive states such as social contact [20, 21], psychostimulant conditioned place preference (CPP; [22]) and copulation [23]. Another difference from 22-kHz calls is that 50-kHz USVs do not exhibit the wide range of durations but are relatively restricted to short durations ranging from 20-80 ms [24]. Thus, given that 22-kHz USVs occur in the presence of aversive stimuli while 50-kHz USVs occur in the presence of rewarding stimuli, virtually without exception, it is generally accepted that USVs provide insight into opposing affective states of rats [24].

In the present review, we illustrate ways in which USVs emitted by rats have been used as a tool for studying substance abuse. Accordingly, we review studies of inferred affective processing in response to the presentation of drug-related cues, experimenter- and self-administered drug, drug withdrawal, and during tests of relapse/reinstatement. We will focus on data collected from studies of psychostimulants (e.g., cocaine and amphetamine), where a large body of evidence has been collected. Nevertheless, similarities and differences to other drugs are considered throughout. A summary of the data presented in the review can be found in Table **1**.

It is difficult to objectively define emotional processing, and certain components of affective state changes are internalized and perhaps immeasurable. However, we are often able to detect emotions by observing their externalized manifestations. USVs in the rat are one such manifestation. Other examples might include changes in heart rate, facial expression, or skin temperature. With this in mind, we propose that rats' USVs provide an objective measure of emotional state in preclinical models. Indeed, unlike human self-reports, USVs are passively measured and avoid extraneous influences on measured emotion. Moreover, studies of individual differences in USV production suggest that animals' emotional responses provide predictive power for identifying individuals with phenotypes and genotypes which are at risk for addiction. Lastly, USVs are—in many cases—independent of traditional behavioral measures (e.g., locomotion or lever responding) and therefore provide more information to experimenters. Thus, USVs are a useful tool for inferring affective states in the rat and can be incorporated in research paradigms modeling important human conditions such as drug abuse [5-9, 25-35]; depression, fear and anxiety disorders [36-40]; or Parkinson's Disease [41-43]. Moreover, USVs are a useful tool for examining the neural substrates of reward processing [44-46].

AFFECT AND SUBSTANCE DEPENDENCE

Addiction is thought of as a chronically relapsing disorder with three major characteristics: 1) compulsion to seek and use drug, 2) loss of control over drug intake (i.e. excessive drug consumption), and 3) negative emotional states following cessation of drug use [47-49]. One goal of current clinical and preclinical models is to focus on understanding these characteristics in order to develop therapies which prevent relapse.

Affective responses from human addicts are recorded using retrospective self-reports (e.g., [50]). While the accuracy of these measures is sometimes questioned (e.g., [51]), self-report data suggest that both positive and negative affective states play a role in drug relapse [52-54]. Specifically, it has been argued that drug-seeking behavior can be driven by positive recollections of previous drug experiences [52] or by the desire to alleviate a negative affective withdrawal state [55, 56]. Moreover, it has been reported that affective states shift from positive to negative just prior to drug use [57] or just after drug administration [50], suggesting that these mood states may be potent contributors to drug-seeking behavior and ultimately to the maintenance of addiction. Notably, this same duality is present in preclinical data and theories derived from animal models of addiction [58-61].

Some of the negative symptoms self-reported during psychostimulant withdrawal include dysphoria, irritability, paranoia, insomnia, and depression [53, 62], while positive symptoms experienced following psychostimulant administration include euphoria, alertness, and increased confidence [63]. Given the relationship between psychostimulant use and both positive and negative affective states, modeling affective states in preclinical studies has recently gained attention [5-9, 25-34, 60, 61, 64-69]. These models allow for effective comparisons between data collected from animal models and human self-reports.

AFFECTIVE RESPONSES TO THE ADMINISTRATION OF PSYCHOSTIMULANTS AND OTHER DRUGS

Studies of drug administration are important for determining the reinforcing efficacy of drugs of abuse, secondary and peripheral effects induced by drugs of abuse, and the neural mechanisms which underlie these processes. In preclinical models, USVs emitted by rats are capable of extending current knowledge by providing insights into affective processing. Indeed, preclinical studies have employed various behavioral paradigms to detect preferences or to determine the rewarding or aversive properties of certain drugs. Nevertheless, while often correlated with the production of USVs, behavioral effects of drugs also may be separable from inferences about animals' affective responses [e.g., 7, 44, 64, 66, 70]. For example, our laboratory has observed USVs in 30- and 60-day cocaine reinstatement tests with no clear correspondence to drug-seeking behavior (i.e. operant lever presses; [7]). Therefore, these differences may provide insights into subtle differences between the roles of various circuits implicated in reward processing and drug-seeking motivation, which may subsequently improve our understanding of the factors that mediate relapse propensity.

Experimenter-Administered Drugs

Seminal work on animals' responses to psychostimulants performed by Burgdorf and colleagues [44] demonstrated

Table 1. Summary table of select findings examining rat ultrasonic vocalizations (USVs) in drug addiction models.

Treatment	Independent Variable(s)	Condition/Group	USV Frequency	Effect	Comparison Group	References
Anticipation						
Cocaine (i.v., S-A)	Cue incentive salience	Sign-Trackers	50-kHz	↑*	Goal-Trackers	Meyer *et al*. 2012
Cocaine (i.v., S-A)	"Caller group" (mean across 10 sessions)	High USV Caller	50-kHz[1]	↑*	Low USV Caller	Reno *et al*. 2013
Amphetamine (1.5 mg/kg, i.p.)	"Caller group"; exposure history	High USV Caller, 2 d	50-kHz[1]	↑**	Low USV Caller	Taracha *et al*. 2014
		High USV Caller, 9 d		–		
				↓*	High USV Caller, 2 d	
		High USV Caller, 10 d		–	Low USV Caller	
Ethanol (10%, oral, S-A)	Exposure history	Dependent (via chronic intermittent vapor exposure, 14 h/d)	50-kHz	–	Non-dependent	Buck *et al*. 2014[a]
Administration						
Cocaine (15 mg/kg, i.p.)	Pre-treatment (5 d)	Cocaine (following 2 d abstinence)	50-kHz	↑*	Saline pre-treated (following 2 d abstinence)	Mu *et al*. 2009
Cocaine (i.v., S-A)	Dose	Low: 0.355 mg/kg/inf	22-kHz	↑*	High-dose group	Barker *et al*. 2010
		High: 0.710 mg/kg/inf	50-kHz	↑*	Low-dose group	
Cocaine (20 mg/kg, i.p.)	Drug pre-treatment, i.p.	SCH 23390 (D_1 antagonist), 0.1 mg/kg	50-kHz	↓*	Vehicle pre-treated controls	Williams and Undieh 2010
		Raclopride (D_2 antagonist), 0.1 mg/kg		↓*		
Cocaine (i.v., S-A)	Drug availability	S-A Conditioning	50-kHz	↑	Vehicle-treated controls	Maier *et al*. 2012
		Extinction		–		
Cocaine (i.v., S-A)	Dose (satiety level)	Sub-Satiety	22-kHz	↑*	Baseline USVs (respective frequencies)	Barker *et al*. 2014
		Circa-Satiety		–		
		Supra-Satiety		–		
	Infusion Number	0[b]		↑*		
		1		↑*		
		0[b]	50-kHz	↑*		
		1		↑*		
		2		↑*		
		3		↑*		
		4		↑*		
		5		↑*		
		6		↑*		
		7		↑*		
		8+		–		

Table 1. contd….

Treatment	Independent Variable(s)	Condition/Group	USV Frequency	Effect	Comparison Group	References
Administration						
Amphetamine (local infusion, NAcc, mixed doses)	Dose	0.3 µg	50-kHz	–	Vehicle-treated controls	Burgdorf *et al*. 2001[c]
		1.0 µg		↑***		
		3.0 µg		↑***		
		10.0 µg		↑***		
Amphetamine (local infusion, mixed sites, 7.0 µg)	Infusion site	NAcc (shell)	50-kHz	↑*	NAcc (core)	Thompson *et al*. 2006
	Drug pre-treatment, local infusion, NAcc	SKF-83566 (D_1 antagonist), 7.0 µg	50-kHz	↓	Vehicle pre-treated controls	
		Raclopride (D_2 antagonist), 7.0 µg		↓*		
Amphetamine (2.0 mg/kg, i.p.)	"Caller group"; exposure history	High USV Caller, 7 d	50-kHz	–	Low USV Caller	Taracha *et al*. 2012
		High USV Caller, 20 d		–		
				↑*	High USV Caller, 7 d	
		High USV Caller, 35 d		↑*	Low USV Caller	
				↑***	High USV Caller, 7 d	
Amphetamine (0.1 mg/kg, i.p.)	Drug pre-treatment, i.p./s.c.	Clonidine (α_2 agonist)	50-kHz	↓†	Vehicle pre-treated controls	Wright *et al*. 2012[d]
		Prazosin (α_1 antagonist)		↓†		
		Atipamezole (α_2 antagonist)		–		
		Propranolol (β_1/β_2 antagonist)		–		
		Betaxolol (β_1 antagonist)		–		
		ICI 118,551 (β_2 antagonist)		–		
Amphetamine (1.0 mg/kg, i.p)	Drug pre-treatment, i.p.	SCH 23390 (D_1 antagonist)	50-kHz	↓†	Vehicle pre-treated controls	Wright *et al*. 2013[d]
		SCH 39166 (D_1/D_5 antagonist)		↓†		
		Haloperidol (D_2 antagonist)		↓*		
		(-)Sulpiride (D_2/D_3 antagonist)		–		
		Raclopride (D_2 antagonist)		↓*		
		Clozapine (D_2/5-HT_{2A} antagonist)		↓*		
		Risperidone (D_1/D_5 antagonist)		↓*		
		Pimozide (D_2 antagonist)		↓*		
Caffeine (i.p.)	Dose	3.0 mg/kg	50-kHz	–	Vehicle-treated controls	Simola *et al*. 2010
		10.0 mg/kg		–		
		30.0 mg/kg		–		
		50.0 mg/kg		–		
		2.0 mg/kg (amphetamine)		↑*		
				↑*	Caffeine groups (all doses)	

Table 1. contd….

Treatment	Independent Variable(s)	Condition/Group	USV Frequency	Effect	Comparison Group	References
Administration						
Mixed drugs (i.p.)	Drug; dose	Amphetamine, 2.0 mg/kg	50-kHz	↑*	Vehicle-treated controls	Simola *et al*. 2012
		Methylphenidate, 2.5 mg/kg		–		
		Methylphenidate, 5.0 mg/kg		–		
		Methylphenidate, 10.0 mg/kg		↑*		
		MDMA (mixed doses)		–		
		Morphine (mixed doses)		–		
		Nicotine (mixed doses)		–		
Mixed drugs (i.p.)	Drug; exposure history	Amphetamine, 2.0 mg/kg, first exposure	50-kHz	↑*	Vehicle-treated controls	Simola *et al*. 2013
		MDMA, 7.5 mg/kg, first exposure		–		
		Morphine, 7.5 mg/kg, first exposure		–		
		Nicotine, 0.4 mg/kg, first exposure		–		
		Amphetamine, 2.0 mg/kg, fifth exposure		–	First exposure, respective groups	
		MDMA, 7.5 mg/kg, fifth exposure		–		
		Morphine, 7.5 mg/kg, fifth exposure		↑*		
		Nicotine, 0.4 mg/kg, fifth exposure		–		
Withdrawal						
Cocaine (oral, S-A, 30 d)	Post-cessation time point	1 d	22-kHz	–	Vehicle-treated controls	Barros and Miczek 1996
		3 d		↑		
		7 d		–		
		28 d		–		
Cocaine (i.v., S-A, 12 h binge)	Post-cessation time point	6 h	22-kHz	↑*	Vehicle-treated controls	Mutschler and Miczek 1998a
		24 h (1 d)		↑*		
		72 h (3 d)		–		
Cocaine (i.v., S-A, 16 h binge)	Administration control	Active S-A, 24 h post-binge	22-kHz	↑*	Vehicle-treated controls	Mutschler and Miczek 1998b; Mutschler *et al*. 2000
		Yoked, 24 h post-binge		↑*	Active S-A	
		Yoked, 3 d post-binge		–	Active S-A	
Cocaine (i.v., S-A, 16 h binge[s])	Number of binge episodes	First binge	22-kHz	↑*	Vehicle-treated controls	Mutschler *et al*. 2001
				–	Second binge, 10-d interval	
				–	Third binge, 10-d interval	
				–	Fourth binge, 1-d interval	
Morphine (s.c., pellets, 72 h)	Post-cessation time point	6 h	22-kHz	↑*	Vehicle-treated controls	Vivian and Miczek 1991
		24 h (1 d)		↑*		
		96 h (4 d)		–		

Table 1. contd….

Treatment	Independent Variable(s)	Condition/Group	USV Frequency	Effect	Comparison Group	References
Withdrawal						
Morphine (s.c., osmotic minipump, 12 d)	Post-cessation time point (spontaneous)	3 h	22-kHz	↓***	Vehicle-treated controls	Kalinchev and Holtzman 2003[e]
		6 h		–		
		24 h (1 d)		–		
	Naltrexone dose (precipitated)	0.01 mg/kg, s.c.		–		
		0.10 mg/kg, s.c.		↓***		
		1.00 mg/kg, s.c.		↓***		
Heroin (s.c., pulsatile osmotic minipump, 14 h)	Dose (6-10 h post-cessation)	0.75 mg	22-kHz	–	Vehicle-treated controls	Williams *et al*. 2012
		1.50 mg		↑***		
		3.00 mg		↑***		
Diazepam (i.p., 2x/d, 5 d)	Dose (24 h post-cessation)	2.5 mg/kg/inj	22-kHz	↑*	Vehicle-treated controls	Miczek and Vivian 1993
		5.0 mg/kg/inj		↑*		
		7.5 mg/kg/inj		↑*		
Ethanol (oral/intragastric)	Administration method (6-12 h post-cessation)	Oral (14 d, 7% ethanol[‡])	22-kHz	↑**	Vehicle-treated controls	Knapp *et al*. 1998
		Intragastric (4 d, 15% ethanol[‡])		↑**		
Ethanol (oral, 14 d)	Drug (6-8 h post-cessation)	7% ethanol[‡]	22-kHz	↑*	Vehicle-treated controls	Moy *et al*. 2000
Cues, Relapse, and Reinstatement						
Cocaine (i.v.)	Context re-exposure group (mean across 19 extinction trials)	Active S-A	50-kHz	–	Vehicle-treated controls	Ma *et al*. 2010
		Yoked		↑*		
Cocaine (i.v., S-A)	Abstinence period	0 d	50-kHz	↑**	Vehicle-treated controls	Maier *et al*. 2010
		2 d		↑**	Vehicle-treated controls	
				↑**	0 d abstinence group	
Cocaine (i.v., S-A)	Reinstatement method (following ≤14 extinction sessions); time in reinstatement session	Cue, 5 min	50-kHz	↑*	Last extinction trial for respective group	Browning *et al*. 2011
		Cue, 10 min		–		
		Cue, 15 min		–		
		Cue, 20 min		–		
		Cocaine, 5 min		↑*		
		Cocaine, 10 min		↑*		
		Cocaine, 15 min		↑*		
		Cocaine, 20 min		↑		
Cocaine (i.v., S-A)	Context reinstatement time	30 d	22-kHz	↑*	Baseline USVs	Barker *et al*. 2013
			50-kHz	↑		
		60 d	22-kHz	–		
			50-kHz	–		

Table 1. contd….

Treatment	Independent Variable(s)	Condition/Group	USV Frequency	Effect	Comparison Group	References
Cues, Relapse, and Reinstatement						
Amphetamine (i.v.)	Trial number (exposure time)	Trial 1 (day 1)	50-kHz[1]	↑*	Vehicle-treated controls	Ahrens *et al*. 2009
		Trial 2 (day 3)		↑**	Trial 1 USVs	
		Trial 3 (day 5)		↑*		
		Challenge (day 19)		↑*		
Methamphetamine (i.v., S-A)	Reinstatement method (following ≤9 extinction sessions)	Cue	50-kHz[1]	↑	Last extinction trial for respective group	Mahler *et al*. 2013
		Methamphetamine		↑**		
		Compound (cue and methamphetamine)		↑**		
		Stress (yohimbine, 2.5 mg/kg, i.p.)		↑		

*$p < 0.05$
**$p < 0.01$
***$p < 0.001$
[†]Significant dose-dependent effects were observed.
[‡]Dose-titration used to achieve final doses indicated under "Condition/Group".
[1]In the summaries for Ahrens *et al*. 2009, Mahler *et al*. 2013, Reno *et al*. 2013, and Taracha *et al*. 2014, differences in frequency-modulated (FM) 50-kHz USVs are reported.
[a]While the number of anticipatory USVs were not different between ethanol-dependent and non-dependent animals in Buck *et al*. 2014, a positive correlation was found between anticipatory USVs and subsequent operant responses in dependent animals only ($p < 0.05$).
[b,c]"0" infusion USVs occurred prior to first infusion of cocaine and can be considered anticipatory, "1" infusion USVs occurred after first infusion of cocaine, etc.
[c]In a post-hoc sub-region analysis, Burgdorf *et al*. 2001 observed more 50-kHz USVs when amphetamine was locally infused into NAcc (shell) relative to NAcc (core).
[d]In reports by Wright and colleagues (2012, 2013), multiple pharmacological compounds were used and only primary mechanisms of action are described under the "Condition/Group" column in this summary table.
[e]Kalinchev and Holtzman (2003) used % of animals vocalizing as dependent USV measure.

that 50-kHz USVs can be evoked *via* intracranial injections of amphetamine into the nucleus accumbens (NAcc) core and shell subregions. In line with previous studies on the effects of stimulants, the rate of elicited vocalizations followed a canonical inverted-u dose-response function. Moreover, intracranial injections into the caudate-putamen (i.e., dorsal striatum) failed to increase rates of USV production [44], consistent with the suggestion that mesostriatal and nigrostriatal circuitry process motivation/emotion and sensorimotor information, respectively (but see [71]). These effects were later replicated by Thompson and colleagues [27], and it was further demonstrated that microinjections into the NAcc shell were more effective at eliciting 50-kHz USVs than injections localized to the NAcc core. Thus, it has been suggested that 50-kHz USVs are mediated by dopaminergic signaling in the ventral striatum.

Consistent with these effects and with the more general hypothesis that 50-kHz USVs are mediated by dopamine transmission, multiple studies have demonstrated that dopaminergic antagonists can affect psychostimulant-induced USVs. Accordingly, D_1 receptor antagonists, such as SCH23390, SKF-83566 and SCH39166, inhibit psychostimulant-induced 50-kHz USVs (i.e., amphetamine and cocaine [27, 66, 70]). Similarly, D_2 receptor antagonists, such as raclopride, haloperidol, and pimozide, have been shown to inhibit 50-kHz USVs in animals treated with psychomotor stimulants [27, 66, 70]. Interestingly, Wright and colleagues [70] demonstrated that D_1 or D_2 antagonists attenuate 50-kHz USVs primarily by reducing the number of emitted FM USVs while having little effect on FF USVs, demonstrating that only the FM positive affective USVs are dopamine-dependent.

In apparent contrast, attempts to reproduce psychostimulant-elicited 50-kHz USVs using D_1 receptor-like or D_2 receptor-like agonists have proven unsuccessful [66]. Also, neither the dopamine transporter (DAT) inhibitor GBR 12909 nor the norepinephrine transporter (NET) inhibitor nisoxetine were shown to mimic the effects of amphetamine when examining USV emission [5]. Lastly, atypical antipsychotics have been shown to have mixed effects on psychostimulant-induced 50-kHz USV production. Specifically, pre-treatment with clozapine or risperidone was shown to inhibit 50-kHz USVs in both saline- and amphetamine-treated animals while sulpiride failed to have any effect on 50-kHz USV emissions [70]. Nevertheless, the combined D_1/D_2 receptor agonist apomorphine has been shown to elicit 50-kHz USVs at rates similar to those observed following cocaine administration [66]. Moreover, the combined D_2/D_3 agonist quinpirole has also been shown to increase 50-kHz USVs when injected intracranially into the NAcc [144]. Thus, it appears that both D_1- and D_2-like receptors may be necessary for eliciting 50-kHz USVs following psychostimulant administration, but clarifying the precise role of dopamine in the production of USVs requires further study and is likely to be circuit-specific.

Dopamine depleting lesions cause changes in the acoustic features of USVs (i.e. reduce the number and quality of FM USVs) but do not eliminate their emission (e.g., [43]). Therefore, other systems have been evaluated for their role in the production of 50-kHz USVs. For example, Wright and colleagues [32] demonstrated that multiple noradrenegeric drugs affect amphetamine-elicited 50-kHz USVs. Namely, the α_1 antagonist prazosin and the α_2 agonist clonidine were shown to dose-dependently reduce amphetamine-elicited 50-kHz USVs. Similar to dopaminergic manipulations, it was shown that these agents predominantly affected FM USVs and trills. On the other hand, the α_2 antagonist atipamezole and the β_1/β_2 blocker propranolol failed to affect amphetamine-elicited rates of 50-kHz USVs. Propranolol did, however, qualitatively change the profile of the observed USVs such that more FF calls were observed. Concordant with this observation, multiple other β_1 or β_2 antagonists and mixed β_1/β_2 blockers failed to have any effect on amphetamine-elicited 50-kHz USVs (i.e. betaxolol, ICI 118,551, and nadolol). Still, it was shown that the combination of a β_1 antagonist (Betaxolol) and β_2 antagonist (ICI 118,551) was able to affect the qualitative properties of the observed USVs (i.e., the complexity or number of observed modulations in frequency; [32]). Finally, it has also been shown that neurotrophic factors may play a role in modulating USV production. Specifically, blockade of the Trk-B brain-derived neurotrophic factor (BDNF) receptor with intracerebroventricular injections of K252A reduced the rate of cocaine-elicited 50-kHz USVs while causing no change in the cocaine-elicited locomotor response [66]. Thus, multiple systems may be affected by psychostimulants and combine to modulate animals' emotional response.

Repeated drug exposure is known to produce changes in animals' behavioral responses to further administration of the drug (e.g., [72]) in conjunction with neuroanatomical changes (e.g., [73]). Concordant with these findings, rates of USVs sensitize across repeated cocaine or amphetamine exposure [28, 64]. Notably, Ahrens and colleagues [28] demonstrated that increases in rates of FM USVs and trills predominantly account for the increase in USV emissions across repeated amphetamine injections. Moreover, it was shown that USVs remain sensitized for at least two weeks following amphetamine exposure despite a period of abstinence [28]. Finally, the time course of sensitization for USVs (i.e. the number of sessions) roughly corresponds to the canonical sensitization of locomotor activity [64]. However, USV emissions within early sessions were transient, while locomotor activity remained elevated throughout the testing period, suggesting that these two behaviors are dissociable [64]. Nevertheless, this dissociation disappeared across protracted treatment, illustrating that changes in behavioral responses occur as drug use continues.

Interestingly, Mu and colleagues [64] also demonstrated that individual differences exist in the degree of sensitization based on animals' baseline USV rates. Indeed, USV rates are known to show high inter-individual variability but low intra-individual variability [34]. Most importantly, individual differences seem to correspond to a variety of behavioral attributes that may prove important for targeting individuals at risk for addiction. Studies of individual differences have shown that animals with high baseline rates of USVs show more 50-kHz USVs in anticipation of drug and develop a stronger place preference for drug [74, 75]. Also, animals with high baseline USVs show a greater escalation of stimulant-induced 50-kHz USVs than animals with low baseline USVs [75]. Moreover, subjects that emit the greatest numbers of 50-kHz USVs also demonstrate a greater-than-average latency to show a response in a hot-plate test for pain, and also spend more time in the open arms of an elevated plus maze, suggesting a decrease in anxiety [34]. Finally, when examining Long-Evans rats selectively bred for high or low rates of USV emission in response to 'tickling' by experimenters [76], it was shown that high-vocalizing animals exhibit more robust locomotor responses to amphetamine than animals bred for low rates of vocalization (although both groups exhibit drug-induced increases in locomotion). Thus, inherent differences in emotionality may also relate to individual differences in animals' behavioral responses to abused drugs as well as susceptibility for abuse.

It has been demonstrated that a number of behavioral variables can affect USV emission. For example, behavioral manipulations can affect the production of 50-kHz USVs and further modulate the effects produced by psychomotor stimulants. Namely, Wright and colleagues [5] demonstrated that social interaction further increased the number of observed 50-kHz USVs produced by amphetamine administration when compared to singly tested animals. Furthermore, Natusch & Schwarting [77] demonstrated that animals emit greater numbers of amphetamine-induced 50-kHz USVs when testing in cages with bedding material as opposed to traditional testing chambers without bedding, and that animals exhibit a place preference for a bedding-covered floor. Overall, these results suggest that 1) environmental familiarity or social contact facilitates the production of 50-kHz USVs, 2) negative emotional states (e.g., those produced by a novel environment) may sum with positive affect produced by drugs of abuse to mediate the animal's net emotional output, and 3) animals' ongoing behaviors may *contribute* to rates of USV emission, although multiple sources demonstrate that these behaviors do not directly *produce* USVs.

Overall, results from multiple studies suggest that psychostimulant administration increases rates of 50-kHz USVs. Both cocaine and amphetamine are capable of producing such an increase, with amphetamine producing a slightly greater effect than cocaine [32]. While USV analysis has proven fruitful for studies of psychomotor stimulants, it is clear that these results are not consistent across all abused drugs. For example, experimenter-administered caffeine (an 'atypical' stimulant) fails to increase rates of 50-kHz USVs over saline controls but does produce differences in the qualitative parameters of individual vocalizations [29]. Along these same lines, morphine administration has been shown to either suppress 50-kHz USVs [32] in experimental subjects or produce no difference when compared saline controls [9, 30, 78]. Nevertheless, morphine produces elevations in locomotor activity and induces a CPP ([32]), both of which are also observed for psychomotor stimulants. Finally, MDMA [145] and nicotine administration did not

elicit 50-kHz USVs but returning animals to the drug paired environment in the days following drug exposure did evoke 50-kHz USVs in drug-treated animals [30]. Thus, administration of different drugs of abuse cause different reward profiles as characterized by USVs and supplemental behavioral tasks (e.g., CPP). Overall, this suggests that the pharmacological effects of the drug may differ from the behavioral or emotional response when anticipating drug or in response to drug paired cues.

Self-Administered Drugs

There are only a few studies of USVs during self-administration. Such studies are important as they capture the influences of both learning and pharmacology on the development of drug addiction. Models of psychostimulant self-administration provide robust face validity when measuring affective responses in anticipation of impending drug availability, in response to the presentation of drug-related cues, or when measuring differences in affective responses between short- and long-access paradigms or short- and long-term drug exposure. More importantly, USVs also provide predictive and construct validity, as it has been shown that the emotional response to drug relates to an individual's propensity for consumption and USVs provide a passive measure of emotion which is free from the extraneous influences described above.

In the first study to examine USVs during cocaine self-administration, Barker and colleagues [6] trained animals to self-administer cocaine under a variable-interval schedule in a long-access self-administration paradigm. This schedule was specifically chosen, as it can be used to manipulate rates of responding and drive animals to respond perseveratively. Specifically, low doses of cocaine on a variable-interval schedule cause high rates of responding and prevent animals from attaining drug 'satiety' [8, 79-80], whereas higher doses or fixed ratio 1 schedules produce more steady rates of responding by allowing animals to achieve satiety. When comparing animals receiving either high (~0.71 mg/kg/infusion) or low (~0.355 mg/kg/infusion) doses of cocaine under this schedule, it was observed that animals in the high dose group emitted predominantly 50-kHz USVs, while animals in the low-dose group emitted predominantly short 22-kHz USVs [6]. Thus, while not directly tested in the experiment, these results suggest that high doses of cocaine produce positive affect. Moreover, sub-satiety doses produce a negative affective state that is observed in concordance with craving, as suggested by high levels of operant responding.

That initial study of USVs during cocaine self-administration produced a number of subsequent questions. Barker and colleagues designed an experiment to explicitly test whether or not USVs differed as a function of the animal's cocaine level (calculated according to first-order pharmacokinetics; [8]). After initial load-up, calculated cocaine levels were manipulated using a series of drug 'clamps' wherein cocaine levels were held constant below, at, or above each animal's self-determined satiety threshold *via* computer-controlled micro-infusions (.0018-0.021 mg/kg/infusion). Consistent with previous work from our laboratory, animals whose drug level was clamped below satiety exhibited robust increases in responding when compared to their normal self-administration contingencies. On the other hand, responding was attenuated in subjects whose drug levels were held at or above their individual satiety thresholds. Consistent with our hypothesis, it was shown that subjects emitted high rates of 22-kHz USVs when levels of cocaine were held below satiety threshold. Interestingly, it was also observed that 50-kHz USVs were emitted almost exclusively during animals' first self-administered infusions. Following the drug-loading period, rates of 50-kHz USVs decayed to near zero under all conditions tested: during continued maintenance of preferred drug level, or during any of the clamp conditions. This result is similar to observations from intraperitoneal (i.p.) drug administration studies, which have shown that USVs subside prior to the decay of other stimulant-induced behaviors (e.g., increases in locomotor activity; [64]) and suggest that positive affect is only acutely experienced during the transitory state from sobriety to intoxication. Notably, when levels of cocaine were held at or above satiety threshold, few USVs of either 22- or 50-kHz were observed. However, during normal maintenance, the rate of 22-kHz calls increased as a function of how far drug level fell below satiety threshold during the inter-infusion interval. The highest rates of 22-kHz emissions were observed during the sub-satiety clamp, which corresponded to holding drug levels at approximately half the animal's preferred level. Overall, these results suggest that cocaine self-administration produces an initial positive affective response followed by an opposing negative affective state whenever drug level falls below satiety threshold. Accordingly, the negative relationships between calculated level of cocaine and both rate of responding and negative affective USVs, plus the paucity of positive affective USVs during maintenance, suggests that responding during the maintenance phase of self-administration is perhaps more reliably driven by the motivation to escape declining, sub-satiety levels of the drug (i.e., negative reinforcement) rather than motivation to seek further bouts of euphoria. Subjects initiating a drug binge may anticipate the initial positive affective response and transiently experience positive reinforcement. An opponent process may follow, in which the subject is effectively "trapped" in a binge by the aversive experience whenever drug level begins to fall. Each episode of sub-satiety drug level may be experienced similarly to the onset of withdrawal symptoms, the difference during maintenance being the continued self-administration of drug, enabling avoidance or escape from the aversive state of sub-satiety.

A short-access (1 h) self-administration study by Maier and colleagues [35] suggests that the decline in 50-kHz USVs during load-up and subsequent shift towards 22-kHz USVs may occur as a result of repeated stimulant exposure. Specifically, Maier and colleagues [35] demonstrated that 50-kHz USVs briefly escalate early in self-administration training, suggesting sensitization to the drug. Continued exposure resulted in a decline in 50-kHz USVs late in training, suggesting the development of tolerance and perhaps dependence on the drug. This notion is further supported by the observation that lever responding continued to increase despite the observed decline in 50-kHz USVs. Thus, it might be suggested that animals become tolerant to

the affective effects of psychostimulants over repeated exposure, causing a shift in animals' initial affective responses to the drug. Maier and colleagues [35] also observed an increase in 50-kHz USVs following a two-day abstinence period. Given the available evidence, one might suggest that the observed increase results after 1) development of an opponent, negative process following repeated drug experience and 2) attenuation of negative affect following a brief period of abstinence/withdrawal.

A mixture of 50- and 22-kHz USVs were also observed in animals trained to self-administer methamphetamine [68]. In this study, 22-kHz USVs were most pronounced during the first day of self-administration, although the number of observed 50-kHz USVs was always greater than the number of 22-kHz USVs. Similar to other self-administration studies, the number of USVs emitted during self-administration decayed across repeated training, and very few long 22-kHz USVs were observed. Indeed, the absence of long 22-kHz USVs may be related to observations that abused drugs can cause changes in the quality of emitted USVs [9, 32, 33, 70] or observations that stimulants can reduce the duration of USVs [9].

Similar to studies involving experimenter-administered cocaine, evidence from self-administration data suggests that individual differences exist in an animal's emotional response to abused psychostimulants. Specifically, Reno and colleagues [81] observed that high- and low-calling animals exhibit differences in USVs in anticipation of the opportunity to self-administer cocaine. In addition, high calling animals exhibit greater escalations in cocaine-induced positive affective USVs across training when compared to low-calling animals. On the other hand, high- and low-calling animals exhibit no differences in the amount of cocaine they self-administer, nor in their psychostimulant-induced locomotor activity.

DRUG WITHDRAWAL AND AFFECTIVE DISTRESS: EVIDENCE FROM USVS

Addicts experiencing psychostimulant withdrawal report symptoms of depressed mood, fatigue, anhedonia, craving and anxiety [82, 83]. Withdrawal-induced psychosomatic symptoms have been modeled in rodents (e.g., [84]), and these models can be used to better understand the precipitation and alleviation of withdrawal symptoms. Concordantly, pharmacotherapy development for cocaine addiction has focused on reducing withdrawal-induced affective distress [85]. Rat USVs provide a non-invasive means of characterizing affective states in preclinical models of withdrawal [25, 26]. Indeed, evidence has shown that rats emit 22-kHz USVs when experiencing withdrawal from cocaine [86-89], opiates [90, 91] and ethanol [91-93]. The emergence and cessation of affective distress can better inform our understanding of drug withdrawal states, and USVs can serve as a tool to accomplish an improved understanding.

Affective distress is experienced during withdrawal from orally self-administered cocaine. In a seminal report characterizing USVs in cocaine-withdrawn rats, Barros and Miczek [86] observed startle-induced 22-kHz USVs when rats were withdrawn from orally self-administered cocaine at 72- but not at 24-hours post-cessation. The presence of 22-kHz USVs was interpreted as reflecting affective distress and/or anxiogenesis during cocaine withdrawal. The presence of 22-kHz USVs at 72- but not 24-hours post-cessation suggests that the emergence of affective distress may follow that of anhedonia. Rats withdrawn from chronic cocaine show signs of anhedonia within 24-hours post-cessation as evaluated by intracranial self-stimulation (ICSS; [84]), which is often used to measure hedonic state *via* changes in the relationship between responding and stimulation current. Moreover, Barros and Miczek [86] observed 22-kHz USVs from rats that had either continuous (24 hours/day for 30 days) or intermittent (4 hours/day for 30 days) access to cocaine, which supports earlier studies showing withdrawal symptom induction from different dosing regimens [84, 94-96]. Irrespective of dosing regimen, all animals emitted fewer 22-kHz USVs by 7-days post-cessation (as compared to observations at 72 hours) and returned to baseline calling rates by 4-weeks post-cessation [86]. Thus, some investigators have concluded that affective distress is experienced during drug withdrawal and suggested that a similar withdrawal state both in intensity and duration is experienced irrespective of drug dosing regimen.

Affective distress is transiently experienced following intravenously self-administered cocaine binges. Addicts often consume cocaine in episodic binges [97]. The duration of cocaine binges varies depending on route of administration, but average binge lengths range from 7 to 17 hours and have been reported to last as long as 40 hours [97]. Moreover, the short-term effects of cocaine withdrawal following a binge have been collectively termed as a "crash", and this state has been characterized by intense craving and depression (i.e. negative affect; [82]). Preclinical studies aimed at modeling human drug bingeing behavior have shown that rats withdrawn from either a 12- or 48-hour intravenously self-administered cocaine binge emitted more startle-induced 22-kHz USVs at 6- and 24-hours post-binge relative to drug-naïve control rats [87]. Interestingly, 22-kHz USVs returned to control levels by 72-hours post-binge. Although the cessation of 22-kHz USVs at 72-hours post-binge appears to contradict previous observations [86], the relatively short duration of affective distress following a cocaine binge supports clinical reports (for review, see [98]) and highlights the importance of implementing preclinical models that best match the parameters of human cocaine addiction. Moreover, these studies indicate that affective distress emerges more quickly following withdrawal from a high-dose of intraveneously self-administered cocaine binge relative to a longer-access, low-dose, orally self-administered cocaine dosing regimen.

Rats withdrawn from both actively self-administered and passively-administered cocaine experience affective distress. Mutschler and Miczek [88] found that rats emitted significantly more startle-induced 22-kHz USVs at 24 hours following a 16-hour passively-administered cocaine binge relative to cocaine self-administering animals. Moreover, both groups of cocaine-withdrawn rats emitted significantly more 22-kHz USVs compared to saline-treated control

animals. This finding extends earlier work showing that rats experience greater aversion and toxicity when cocaine is passively-administered [99] but nonetheless demonstrates that both passively- and self-administering rats experience affective distress when withdrawn from cocaine.

Prior cocaine use has been suggested to mediate the intensity of cocaine withdrawal symptomatology relative to the initial withdrawal state. For example, repeated access to psychostimulants has been previously shown to alter patterns of acquisition, maintenance and reinstatement relative to initial experience with the drug [100-103]; for review, see [59]). In support, locomotor behavior following amphetamine administration has been shown to be positively associated with subsequent cocaine self-administration behavior but was not found to be predictive of relapse propensity [103]. Previous studies have also reported that tolerance can develop to the reinforcing properties of cocaine (for review, see [72]) but that prior experience with cocaine did not alter the intensity of subsequent withdrawal states [89]. Mutschler and colleagues [89] found that the rate of 22-kHz USVs was relatively stable between rats experiencing withdrawal from either one, two or three 16-hour cocaine binge(s), which failed to support the hypothesis that prior drug use would reduce affective distress experienced during subsequent episodes of cocaine withdrawal. Indeed, the 10-day drug-free interval between cocaine binges may have reduced the tolerance-like effects established from prior use, as withdrawal-induced 22-kHz USVs have been shown to be less prevalent at 7-days post-cessation relative to earlier time points [87-88, 104]. Thus, although prior cocaine use has been suggested to modulate the reinforcing properties of subsequent cocaine use, prior use does not appear to alter the intensity of cocaine withdrawal states following subsequent uses.

Cocaine withdrawal leads to long-lasting changes in immediate early gene expression as well as in dopamine and opiate receptor-mediated neural circuits, and these changes underlie symptoms of acute and prolonged withdrawal states. For example, animals withdrawn from a 16-hour self-administered cocaine binge showed mRNA downregulation of the immediate early gene *zif268* in hippocampal but not mesolimbic brain regions at 24-hours post-cessation [104]. *Zif268* is critical for learning and synaptic plasticity [105] and is involved in aversive memory maintenance [106]. Importantly, cognitive dysfunction has been observed during cocaine withdrawal in human addicts [107]. Thus, downregulation of *zif268* in the hippocampus may contribute to impaired cognition during cocaine withdrawal but does not appear to underlie affective distress at 24 hours post-cessation. Mutschler and colleagues [104] further observed downregulation in *zif268* mRNA at 14-days post-binge in the hippocampus, nucleus accumbens and the basolateral amygdala, which corroborates other studies observing long-lasting, withdrawal-induced changes in extracellular dopamine levels in mesolimbic circuits [108-111]. Moreover, a recent study in rats has shown that κ opioid receptors (KORs), which have been previously shown to mediate motivational states [112], become dysregulated within amygdalar sub-regions during cocaine withdrawal and may underlie short-term affective distress post-cessation [113]. Combined, these studies reveal that rats emit 22-kHz USVs when withdrawn from cocaine binges, and that short- and long-term changes in immediate early genes and neurotransmitter systems regulating aversive memory formation and affect are observed during withdrawal from cocaine self-administration. While studies have yet to elucidate a predictive role of changes in immediate early gene expression with induction of affective distress, it is clear that drug withdrawal is characterized by both and thus a causal relationship may exist between the two.

Results obtained from psychostimulant withdrawal studies have been supported by findings from animal models of opiate and ethanol withdrawal. Much like psychostimulant withdrawal, animals withdrawn from morphine or heroin emit more 22-kHz USVs than control animals [90, 91]. Furthermore, systemic administration of naltrexone, an opiate receptor antagonist, was found to dose-dependently reduce 22-kHz USVs in animals withdrawn from chronic morphine [114], suggesting that activated opiate circuits underlie the emergence of affective distress during opiate withdrawal. It is noteworthy that cocaine withdrawal was recently shown to lead to long-term changes in KOR-modulated limbic circuits [113], which suggests a ubiquitous role of opiate systems in mediating affective distress during withdrawal from multiple drugs of abuse. Separate lines of research have found that withdrawal from drugs of abuse that act on GABAergic transmission, such as ethanol, leads to increased rates of 22-kHz USVs relative to saline-treated control animals [92, 115-117]. Moreover, administration of a KOR antagonist, nor-binaltorphimine (nor-BNI), attenuated the increase in 22-kHz USVs during acute ethanol withdrawal, which suggests involvement of KOR circuits in underlying this opposing process [93]. Thus, evidence from animal models of opiate and ethanol withdrawal corroborate results obtained from psychostimulant withdrawal studies to indicate common underlying neural substrates mediating affective distress during withdrawal from drugs of abuse (for review, see [118]).

Withdrawal from drugs of abuse produces affective distress, and multiple factors mediate the presence or relative intensity of withdrawal states. Psychostimulants, such as cocaine, have been shown to reliably induce affective distress in self-administering and passively-administered rats during withdrawal. Moreover, withdrawal from opiates and ethanol have been shown to lead to similar states of affective distress as well as changes in neural substrates governing emotion, learning and memory. USVs, specifically those in the 22-kHz frequency range, have been observed in nearly all drug withdrawal states and corroborate human self-reports to characterize affective distress as a cardinal feature of drug withdrawal. To our knowledge, USV recording remains the only method available to non-invasively, and without the requirement of operant behaviors, evaluate changes in affect during psychostimulant withdrawal that are otherwise difficult or impossible to detect. Furthermore, USVs can be used to resolve the magnitude of affective distress from other drugs of abuse, such as opiates and ethanol, and can be observed in concert with somatic withdrawal signs. Future studies can aim to better uncover the gene transcriptional and molecular pathways involved in

acute and protracted withdrawal states from drugs of abuse, and USVs can be used to determine how neural changes mediate affect during drug withdrawal.

ULTRASONIC VOCALIZATIONS, DRUG-RELATED CUES AND REINSTATEMENT

Following periods of abstinence, drug-seeking behavior can be reinstated by drug-associated cues [79, 119-125], by stressful stimuli [120, 125-131], or by re-exposure to the drug itself [119-121, 124, 125, 132] and even other, similar drugs [119, 120]. Thus, studies of cue processing and reinstatement are critical for developing effective treatments for drug dependence. Few studies, however, have examined animals' emotional reactions to drug-associated cues. The emerging evidence suggests that affective processing may play an important role in subjects' interactions with learned cues.

Two of the few studies reporting USVs in response to drug-related cues [65, 133] demonstrated that cues signaling the forthcoming opportunity to self-administer cocaine increase emissions of positive affective USVs. These anticipatory USVs were specifically emitted during the opportunity to self-administer cocaine, as evidenced by fewer emissions in saline-treated and yoked controls. Notably, anticipatory USVs were attenuated when environmental cues had been associated with extinction training instead of cocaine self-administration, demonstrating a modification to the original context-drug (i.e. CS-US) association, whereby context no longer predicted drug reward and thus no longer elicited an anticipatory response. These results are similar to the observation that 50-kHz USVs are elicited following the presentation of cues associated with natural rewards, such as palatable food [134], as well as studies demonstrating the induction of approach behavior from females when pre-copulatory 50-kHz USVs are emitted from males [135]. Together, these studies suggest that learned cue-reward associations for both drug, food, and sexual rewards elicit powerful effects on animals' affective state and behavior but also that the semiotic value of an association can be extinguished following changes in the anticipated outcome.

Studies have also shown that a period of abstinence or extinction training can modulate the emotional states observed during drug anticipation [68, 133]. Specifically, a two-day abstinence period was shown to increase the number of anticipatory, cue-induced positive affective USVs when compared to rates of USVs in sessions that were not preceded by abstinence. Abstinence did not result in any changes in locomotor behavior, adding to results which suggest that affective and behavioral responses to drug-related cues are dissociable (e.g., [7]). Moreover, the presentation of a drug-paired cue was shown to elicit positive affective USVs during a methamphetamine reinstatement test following one week of extinction training, and this effect was enhanced when animals were drug-primed prior to reinstatement testing [68]. Mahler and colleagues [68] further observed that anticipatory positive affective USVs during the reinstatement test with both cue and a methamphetamine priming injection were positively correlated with drug-seeking behavior (i.e. active lever pressing). These results suggest that both drug cues and drug priming are effective at evoking emotional responses and that their ability to produce such a response may 'incubate' or increase in magnitude following the cessation of drug use. Furthermore, drug-seeking effort appears to be associated with the number of 50-kHz USVs observed during reinstatement testing. Anticipatory positive affective USVs may, after all, reflect a combination of positive anticipation [133] and waning negative withdrawal symptoms from prior sessions [26, 86-88]. Thus, a period of abstinence may allow for withdrawal symptoms to subside, resulting in an increase in the net amount of positive anticipation that is observed.

Studies using USVs have also illustrated important individual differences in the incentive motivational properties accrued by drug-related cues. Studies by Robinson and colleagues have revealed that certain animals utilize cues as effective conditioned reinforcers ('sign-trackers') while other animals instead track the outcome or goal ('goal-trackers') or, indeed, use a mixed strategy [67, 136-140]. USVs in a cocaine-paired environment were shown to be greater for sign-tracking animals than for goal-tracking animals or for sign-tracking animals for whom stimuli were administered without cocaine (i.e., explicitly unpaired; [67]). Specifically, USVs and behavioral activity were measured for sign- and goal-tracking animals using a conditioned place preference paradigm. Sign-tracking animals developed a place preference for a cocaine paired environment, whereas goal-tracking animals did not. Moreover, while all animals showed an increase in positive affective USVs during drug administration sessions, positive affective USVs during a drug-free test in the presence of drug-paired environmental cues were significantly increased in sign-tracking animals [67], suggesting that conditioned cues serve to generate a greater number of 50-kHz USVs, more so in sign-trackers than goal-trackers.

Drug-paired contexts can induce drug-seeking behavior and affective reactions following abstinence. Our laboratory has previously observed that cocaine-experienced animals vocalize in both 22- and 50-kHz frequency ranges when re-exposed to the self-administration chamber context at 30- and 60-days after cocaine self-administration [7]. It was further observed that cocaine-seeking behavior is not necessarily associated with either 50- or 22-kHz USVs. These findings show that drug anticipation can elicit positive affective reactions [28, 65, 141] or negative affective responses. Notably, negative affective responses may be the result of learned associations with drug-paired cues or may be the result of reward omission [10, 142]. Despite the absence of a clear, unidirectional emotional response, drug-seeking behavior was nonetheless observed at 30- and 60-day reinstatement tests, suggesting a long-lasting context-drug association. A different study showed that a morphine-paired context elicited significantly more anticipatory positive affective USVs after a two-week abstinence period relative to the positive anticipatory affective reactions prior to the two-week abstinence period [143]. Taken together, these studies show that drug-paired contexts reliably elicit affective reactions in rodents following a period of abstinence, but that the strength of CS-US associations and that the duration of abstinence prior to reinstatement testing may modulate the

magnitude and relative presence of positive and negative affective states.

In combination, the aforementioned results demonstrate that rats develop conditioned emotional responses to drug-related cues. Conditioned responses to cues predicting impending drug availability consistently demonstrate that animals exhibit a positive anticipation of forthcoming drug. Notably, the magnitude of this anticipation may be modulated by the experimental schedule, given evidence showing an increase in the magnitude of emotional responses following a brief period of abstinence. Data also suggest that the nature of the association between cues and drug rewards reflects animals' propensities to administer drugs and perhaps ultimately their propensities to relapse.

CONSIDERATION AND CONCLUSIONS

Considerations and Limitations

Early studies using USVs often selected for animals with sufficiently high rates of USV emission in order to reliably detect changes in calling behavior. While an important first step, studies demonstrating that high- and low-calling animals represent different genotypes and phenotypes, particularly their differing degrees of anticipating stimulant drugs [81], suggest that future studies must sample the full range of vocalization rates in order to avoid targeting one particular phenotype/genotype. Also, studies of drug abuse often focus on the 'rewarding' rather than 'reinforcing' properties of drugs. However, data from studies using USVs suggests that both positive and negative affect play a role in drug-seeking behaviors. Specifically, available data suggest that, following positive reinforcement during initial load-up, long-access drug seeking behaviors during a binge are maintained *via* a negative, rather than positive, reinforcement mechanism [8]. Along these lines, it is important that studies analyze the full spectrum of rat vocalizations, including both 22- and 50-kHz frequency ranges. Also, for some time the role of short 22-kHz vocalizations remained unknown. However, our current understanding of these calls has suggested that: 1) short 22-kHz USVs occur only during aversive situations and have yet to be recorded during a situation that is explicitly positive; 2) global pharmacological manipulations can change the duration of USVs [9], suggesting that short 22-kHz USVs observed under the influence of psychomotor stimulants are categorically the same as the longer aversive vocalizations observed under naturalistic conditions; 3) short 22-kHz USVs elicit a greater defensive response (i.e., retreat/hiding) from conspecifics [19]. Ultimately, all types of vocalizations should be considered in order to avoid the loss of crucial information.

Current data implicate dopaminergic and noradrenergic systems in the production of positive affective vocalizations [32, 33, 70], as well as the ascending cholinergic system in the production of aversive vocalizations [11; Brudzynski, this issue]. However, it is also known that manipulations of these systems can affect motor systems and thus the acoustic quality of USVs (e.g., [43]). With this in mind, future pharmacological studies might focus on targeting specific circuits in order to elucidate key differences in the limbic and motor contributions to psychostimulant-induced changes in USVs. Second, studies of drug self-administration and studies incorporating models of relapse/reinstatement are still relatively sparse. Thus, further study may be needed in order to elucidate the role of animals' emotional responses during these processes.

Conclusions

Given the relationships between psychostimulant use and both positive and negative affective states, models of affect in preclinical studies have recently gained attention [5-9, 25-30, 32-34, 60, 61, 64-69, 133]. Based on available data, USVs provide an objective measure from which an animal's emotional response can be inferred. Indeed, USVs suggest that animals show an initial positive affective response to psychostimulant administration and are capable of identifying individual differences in the animals response to the drug upon initial exposure. Moreover, USVs have been used to demonstrate that animals' emotional and behavioral responses become sensitized to psychostimulants over acute exposure before eventually exhibiting signs of drug tolerance. Perhaps most importantly, the development of tolerance represents a point of divergence between affective and behavioral responses to psychostimulants. That is, drug-seeking behaviors (e.g., lever responses) continue to escalate, while affective responses decline. Such divergence is a strong endorsement of the potential utility in adding USV assessment to available behavioral measures. In the drug-dependent animal, affective responses indicate a sequence of positive and negative affective responses to psychomotor stimulants, predominantly characterized by an initial bout of positive affect followed by an opponent negative emotional state, mirroring affective responses observed in human addicts (e.g., [50]). Periods of abstinence are known to produce a period of acute withdrawal symptoms (i.e., ~72 h), during which negative affective states are transiently observed. Finally, it has been shown that drug-paired cues produce a learned, positive anticipatory response during the course of drug administration, and that the presentation of these cues following abstinence produces both positive affect and reinstatement behavior.

The available data also provide a number of important insights for future studies of drug abuse. First, it is clear that animals' affective responses are different depending on the class of abused drug and may therefore provide subtle insights into the differences between drug types. Indeed, data have suggested that opiates and psychostimulants produce some similar behavioral responses (i.e., CPP and hyperlocomotion) but induce differential USV responses. Along these lines, evidence also suggests that motivated behaviors (e.g., operant responding) are dissociable from affective responses (i.e., USVs). Thus, the integration of USVs into well-designed behavioral paradigms allows for insights that cannot be ascertained from behavioral measures alone. With this in mind, the incorporation of USVs may provide insights into the differences between circuits involved in motivation and those involved in processing emotion. Finally, USVs provide a mechanism for identifying individual phenotypes and genotypes which show traits that are relevant to addiction and might identify subjects that are

susceptible to substance dependence or are otherwise relapse-prone. Consequently, an understanding of these differences could allow for the identification of the analogous phenotype (and possibly genotype) in humans in order to develop targeted therapies.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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