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ORIGINAL INVESTIGATION

Jeffrey Burgdorf · Brian Knutson · Jaak Panksepp
Toni S. Shippenberg

Evaluation of rat ultrasonic vocalizations as predictors of the conditioned aversive effects of drugs

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Abstract *Rationale*: Since cues that predict aversive outcomes can elicit both avoidance and 20 kHz ultrasonic vocalizations (USVs) in adult rats, 20 kHz USVs may also index the conditioned aversive effects of drugs. *Objective*: We evaluated whether exposure to compartments associated with drugs with aversive effects would selectively increase 20 but not 50 kHz USVs in rats. *Method*: Rats were injected with naloxone (NAL) or lithium chloride (LiCl) and placed in one compartment or with saline (VEH) and placed in another compartment for three 50-min conditioning sessions. 20 kHz USVs, 50 kHz USVs, and time spent in each chamber were recorded during subsequent 15-min testing sessions during which rats had access to both compartments (expt 1) or were confined to the drug- or VEH-paired compartment (expt 2). *Results*: In expt 1, animals conditioned either with NAL (0.3 and 3.0 mg/kg) or LiCl (10 and 30 mg/kg) emitted increased 20 kHz USVs in the drug-paired compartment, relative to VEH-conditioned controls. Conditioning with high doses of both drugs also increased conditioned place aversion and decreased emission of 50 kHz USVs. In expt 2, restriction of animals to the compartment paired with high doses of NAL and LiCl also increased emission of 20 kHz USVs and decreased 50 kHz USVs, relative to VEH-conditioned controls. *Conclusions*: In rats, cues associated with drugs with aversive effects increase 20 kHz USVs and decrease 50 kHz USVs, suggesting that USVs may provide a useful model for predicting the conditioned aversive effects of drugs.

Keywords Naloxone · Lithium chloride · Morphine · Ultrasonic vocalization · Conditioned place aversion · Rat

J. Burgdorf · T.S. Shippenberg
Integrative Neuroscience Unit,
Behavioral Neuroscience Laboratory,
Intramural Research Program, NIDA, NIH,
5500 Nathan Shock Drive, Baltimore, MD 21224, USA

B. Knutson (✉)
Laboratory of Clinical Studies,
Section of Brain Imaging and Electrophysiology,
Intramural Research Program, NIAAA, NIH,
10 Center Drive, Bethesda, MD 20892-1610, USA
e-mail: knutson@odin.niaaa.nih.gov
Tel.: +1-301-4025861, Fax: +1-301-4020445

J. Panksepp
Department of Psychology,
Bowling Green State University, Bowling Green,
OH 43402, USA

Introduction

Animal models of the motivational properties of drugs permit assessment of both appetitive and aversive effects. By extension, these models can provide important information regarding the abuse liability of a drug as well as its capacity to induce side effects that might limit therapeutic use. Several animal models for assessing the aversive effects of drugs exist. Two commonly used paradigms include operant and conditioned avoidance procedures. In operant procedures, administration of a drug that produces aversive effects can suppress operant responding for various types of rewarding stimuli (Rudski et al. 1994). In conditioned avoidance procedures, animals avoid stimuli (e.g., a chamber) associated with drugs that produce aversive effects (Schechter and Calcagnetti 1998; Tzschentke 1998). Unfortunately, test drugs can exert non-specific effects on learning and memory and alter motor behavior, potentially confounding the interpretation of data obtained from both of these procedures. Thus, an index of the aversive effects of drugs that does not require learning or overt locomotor activity might complement existing paradigms. For instance, many mammals exhibit unconditioned emotional responses to biologically relevant stimuli, and these responses may provide a spontaneously occurring behavioral index of aversive drug effects.

In the case of rats, investigators have postulated that certain types of ultrasonic vocalizations (USVs) may unconditionally index different "affective" states. For example, 20 kHz USVs may index negative affective states

in juvenile and adult rats (Miczek et al. 1995), since the expression of these vocalizations correlates with social defeat (Thomas et al. 1983; Tornatzky and Miczek 1995), and episodes of passive avoidance following copulation (Barfield et al. 1979). Rats also make these vocalizations during the delivery of non-social aversive stimuli such as noxious footshock (Cuomo et al. 1988; Tonoue et al. 1986), as well as in response to cues that predict footshock (Antoniadis and McDonald 1999; Burgdorf et al. 2000). Additionally, rats reliably emit these vocalizations during withdrawal from opiates and psychostimulants (Vivian and Miczek 1991; Barros and Miczek 1996; Mutschler and Miczek 1998).

Conversely, a different type of USV of shorter duration and higher frequency (~50 kHz USVs) emitted both by juvenile and adult rats has been postulated to reflect positive affective states (Knutson et al. 1999), since the expression of 50 kHz USVs correlates with approach behavior during play (Knutson et al. 1998), courtship (Barfield et al. 1979) and other appetitive forms of social contact (Panksepp and Burgdorf 2000). Although 50 kHz USVs have also been observed in the context of intermale fighting (Takahashi et al. 1983), their role in this social context may also indicate positive affect, since protection of a defeated males from a former aggressor most potently increases the expression of these USVs in agonistic encounters (Tornatzky and Miczek 1994). Presentation of non-social, yet rewarding stimuli (e.g., cues that predict daily feeding sessions or electrical stimulation of the medial forebrain bundle), also elicits 50 kHz USVs, albeit at lower levels (Burgdorf et al. 2000). Finally, rats emit more 50 kHz USVs in environments where they have previously received prototypical drugs of abuse (i.e., morphine and amphetamine), indicating that these vocalizations may index the conditioned rewarding effects of drugs (Knutson et al. 1999).

If 20 kHz USVs index a negative affective state in rats, they might provide a sensitive tool for evaluating the conditioned aversive effects of certain drugs. In the present studies, we sought to explore this possibility by assessing the effects of lithium chloride (LiCl)- and naloxone (NAL)-induced place conditioning on the expression of USVs. These pharmacological agents were selected because they reliably produce conditioned place aversion in rats (Mucha and Iverson 1984; Shippenberg et al. 1988; Tzschentke 1998). To ensure that changes in vocalizations were not due merely to the place conditioning procedure, the effects of LiCl and NAL conditioning on USVs were compared with vehicle (VEH) conditioning and conditioning with the μ-opioid receptor agonist, morphine (MORPH), which generates place preferences rather than aversion (Kumar 1972; Bardo et al. 1995).

Materials and methods

Subjects

Ninety male Sprague-Dawley rats (Charles River, Wilmington, Mass., USA) weighing 250–300 g were housed two to four per cage (45×24×20 cm) with free access to Purina rodent chow pellets and water in temperature- and humidity-controlled colony room (12/12-h light/dark cycle, 21±1°C, 30–40% humidity). These facilities were accredited by the American Association for the Accreditation of Laboratory Animal Care and all experiments were conducted in accordance with the guidelines of the Institutional Care and Use Committee of the Divisions of Intramural Research/National Institute on Drug Abuse, National Institute of Health.

Apparatus

Ultrasonic vocalization recording

USVs were recorded with a Pettersen D980 ultrasonic detector (Uppsala, Sweden). One channel was tuned so that all USVs could be heard (the broadband channel), whereas the other channel was tuned to the 20-kHz range. USVs that occurred in both channels were determined to be 20 kHz USVs, and the USVs heard only in the broadband channel were determined to be 50 kHz USVs. Both USV types could be discriminated by the experimenter, based on the channel in which they occurred and on distinct temporal characteristics of the USVs (i.e., 50 kHz<300 ms and 20 kHz>300 ms) (Brudzynski et al. 1993). An experimenter who was blind to the condition of each rat coded USVs on-line via headphones. Based on a videotaped sample of animals in these experiments (n=8), in which 50 kHz USVs were transformed to a frequency audible to humans, intra-rater and inter-rater reliability for coding 50 kHz USVs was r^2>0.81, approximating the high reliability values reported in previous studies which have employed these methods (Knutson et al. 1998).

Conditioning chambers

The place conditioning apparatus consisted of two-compartment shuttle boxes made of acrylic (30×60×30 cm), each equipped with a loose-fitting wire mesh lid. One side of the shuttlebox was white and had a textured floor, while the other side was black and had a smooth floor. During conditioning sessions, a partition divided the two sides, creating two different compartments. During the testing sessions of expt 1 (but not expt 2), the partition was replaced with an identical partition with a 20×15 cm door which allowed the animal unrestricted access to either compartment.

Procedure

Place conditioning was conducted using an unbiased procedure as described elsewhere (Shippenberg and Herz 1987). Conditioning sessions were conducted twice a day for 3 days, with VEH injections occurring in the morning and the drug injections occurring in the afternoon. Rats were immediately confined to one compartment following drug injections or the other compartment following VEH injections. A minimum of 6 h separated each 50-min conditioning session. Compartments associated with drug versus VEH were counterbalanced across rats for each drug dose. Each rat was conditioned with only one drug dose. Control groups received VEH in both compartments.

Experiment 1

Rats received three conditioning sessions with NAL (0.3, 3.0 mg/kg; n=8 and 12 per dose), LiCl (10.0, 30.0 mg/kg, n=8 per dose) or VEH (two groups; n=8 per dose). Test sessions were conducted 24 h following the final conditioning session. On the day of the test, uninjected rats were given free access to both compartments for 15 min. Time spent (s) and USVs emitted in each compartment were recorded during these test sessions.

Experiment 2

Rats received three daily conditioning sessions similar to expt 1 with either NAL (3.0 mg/kg, two groups, *n*=8 and *n*=8), LiCl (30.0 mg/kg, *n*=7), MORPH (5.0 mg/kg, *n*=8), or VEH (*n*=7) and three conditioning sessions in the alternate compartment with VEH. Rats were tested 24 h following the final conditioning session. During testing, uninjected rats were confined to the drug-paired compartment for 5 min, except for one of the NAL-paired groups (*n*=8), which was confined to the VEH-paired compartment. USVs were recorded during this 5-min testing period. Additionally, to verify that these drugs had acutely aversive properties, we recorded USVs during the 3 days of conditioning in a subset of the rats conditioned with NAL (*n*=8), both 15 min prior to injection while alone in their home cage, and 15 min after drug injections in the conditioning compartment.

Drug administration

Naloxone hydrochloride (0.3 mg/kg, 3.0 mg/kg; Sigma, St Louis, Mo., USA), LiCl(10 mg/kg, 30 mg/kg; Sigma) and MORPH (5.0 mg/kg; National Institue on Drug Abuse, Baltimore, Md., USA) were dissolved in 0.9% saline solution and were injected subcutaneously (SC: 1.0 ml/kg), using the freebase weight of each compound.

Data analysis

Experiment 1

Place aversion (s), 20 kHz USVs, and 50 kHz USVs (raw counts) emitted in the drug-paired compartment were analyzed using one-way analyses of variance (ANOVA) with dose of NAL or LiCl as an independent variable. In the analyses of 20 kHz USVs, time spent in the drug-paired compartment was entered as a covariate to control for differential amounts of time spent in each compartment, and 20 kHz USVs in the VEH-paired compartment was also entered as a covariate to control for individual differences in overall tendency to express USVs. Similarly, in the analysis of 50 kHz USVs, time spent in the drug-paired compartment and 50 kHz USVs in the VEH-paired compartment were entered as covariates. These ANOVAs were followed by *t*-tests comparing USVs and compartment occupancy of animals that received each dose of drug with animals that received VEH (Bonferroni correction for two comparisons: $P<0.025$, two-tailed).

In addition, to compare the utility of using percentage measures versus raw vocalization counts, similar analyses were performed on percentage of 20 and 50 kHz USVs emitted in the drug-paired compartment, with percentage time spent in the drug-paired compartment entered as a covariate. Percentage scores for 20 kHz USVs were calculated using the formula: %20 kHz USVs=20 kHz USVs drug/[(20 kHz USVs drug+20 kHz USVs VEH)+0.01]. Percentage scores for 50 kHz USVs were calculated using a similar formula: %50 kHz USVs=50 kHz USVs drug/[(50 kHz USVs drug+50 kHz USVs VEH)+0.01] (see Knutson et al. 1999). Again, *t*-tests compared the percentage of USVs and compartment occupancy of animals that received each dose of drug versus animals that received VEH (Bonferroni correction for two comparisons: $P<0.025$, two-tailed).

Experiment 2

The ability of NAL (3.0 mg/kg) and LiCl (30.0 mg/kg) conditioning to increase 20 kHz USVs and decrease 50 kHz USVs, as well as for MORPH (5.0 mg/kg) conditioning to decrease 20 kHz USVs and increase 50 kHz USVs was analyzed with two five-factor (drug type), one-way ANOVAs. Differences between the USVs of animals that received each of these four drug treatments versus animals that received VEH treatment were analyzed with *t*-tests (Bonferroni correction for four comparisons: $P<0.01$, two-tailed). Additionally, the ability of NAL (3.0 mg/kg) to acutely increase 20 kHz USVs and reduce 50 kHz USVs was tested with a 2 (NAL versus VEH; within) by 2 (pre- versus post-injection; within) by 3 (day; within) one-way ANOVA. Differences between the USVs of animals when they received acute NAL versus VEH injections were analyzed with pairwise *t*-tests (Bonferroni correction for six comparisons: $P<0.008$, two-tailed).

Results

Experiment 1

In expt 1, we examined whether cues associated with drugs which have aversive effects would increase 20 kHz USVs and decrease 50 kHz USVs, independent of their ability to induce place aversion.

Naloxone conditioning

Animals that received VEH in each of the compartments exhibited no preference for either of the place cues, while animals that were conditioned with NAL spent less time in the drug-paired compartment than the VEH-paired compartment [$F(2,24)=14.16$, $P<0.001$]. Specifically, animals conditioned with the high [3.0 mg/kg; 134.55±33.21 s; $t(17)=4.77$, $P<0.001$], but not the low dose of NAL (0.3 mg/kg; 319.00±32.62 s), spent significantly less time in the drug-paired compartment than VEH-conditioned controls (mean±SEM=463.12±65.97). Analysis of percentage time spent in the drug-paired compartment yielded similar results [$F(2,24)=17.06$, $P<0.001$]. Due to reductions in variance, animals conditioned with the high dose of NAL spent significantly less time in the drug-paired compartment [15±4%; $t(17)=5.28$, $P<0.001$], while animals conditioned with the low dose showed a trend in this direction [35±3%; $t(14)=2.39$, $P<0.05$], relative to VEH-conditioned animals (53±7%).

Covariance analysis that controlled for 20 kHz USVs emitted in the VEH-paired compartment [$t(22)=3.47$, $P<0.005$] as well as for time spent in the drug-paired compartment (NS) indicated that NAL-conditioned animals emitted increased levels of 20 kHz USVs in the drug-paired compartment [$F(2,24)=9.10$, $P<0.005$]. Specifically, animals conditioned with both high [4.64±0.96; $t(17)=3.24$, $P<0.005$] and low doses of NAL [5.87±1.35; $t(14)=3.70$, $P<0.005$] emitted more 20 kHz USVs in the drug-paired chamber than VEH-conditioned animals (0.62±0.42). Analysis of percentage of 20 kHz USVs emitted the drug-paired chamber, covarying for percentage time spent in the drug-paired chamber (NS), yielded similar results [$F(2,24)=29.90$, $P<0.001$]. Animals conditioned with both high [80±8%; $t(17)=6.03$, $P<0.001$] and low doses of NAL [83±4%; $t(14)=9.20$, $P<0.001$] emitted more 20 kHz USVs in the drug-paired compartment than VEH-conditioned animals (10±7%).

Covariance analyses that controlled for 50 kHz USVs emitted in the VEH-paired compartment [$t(22)=4.02$,

$P<0.001$] as well as time spent in the drug-paired compartment (NS), indicated that NAL-conditioned animals showed a trend towards emitting fewer 50 kHz USVs in the drug-paired compartment [$F(2,22)=3.25$, $P=0.06$]. Specifically, animals conditioned with the high [0.36±0.23; $t(17)=2.61$, $P<0.05$] but not the low dose of NAL (3.87±1.23) emitted fewer 50 kHz USVs in the drug-paired compartment, relative to VEH-conditioned animals (3.25±1.26). Analysis of percentage 50 kHz USVs emitted in the drug-paired chamber, covarying for percentage time spent in the drug-paired compartment (NS), yielded more statistically robust findings [$F(2,24)=29.90$, $P<0.001$]. Animals conditioned with the high [5±3%; $t(17)=3.79$, $P<0.005$] but not the low dose of NAL (25±6%) emitted a greater percentage of 50 kHz USVs in the drug-paired compartment relative to VEH-conditioning animals (35±8%).

Lithium conditioning

Again, animals that received VEH in each of the compartments showed no preference for either of the place cues while animals that were conditioned with LiCl spent less time in the drug-paired compartment than the VEH-paired compartment [$F(2,21)=3.91$, $P<0.05$]. Specifically, animals conditioned with the high [30 mg/kg; 326.87±27.76 s; $t(14)=3.24$, $P<0.01$] but not the low dose of LiCl (10 mg/kg; 376.87±43.07 s), spent less time in the drug-paired compartment, relative to VEH-conditioned controls (462.50±31.35 s). Analysis of percentage time spent in the drug-paired compartment revealed a similar pattern [$F(2,21)=3.91$, $P<0.05$]. Animals conditioned with the high [36±3%; $t(14)=3.24$, $P<0.01$] but not the low dose of NAL (42±5%) spent less time in the drug-paired compartment, relative to VEH-conditioned animals (51±3%).

Covariance analysis that controlled for 20 kHz USVs emitted in the VEH-paired compartment [$t(19)=5.40$, $P<0.001$] and time spent in the drug-paired compartment [$t(19)=–2.10$, $P<0.05$] indicated that LiCl-conditioned animals emitted more 20 kHz USVs in the drug-paired compartment [$F(2,19)=3.51$, $P=0.05$]. Specifically, animals conditioned with both high [3.62±0.96; $t(14)=2.61$, $P<0.025$] and low doses of LiCl [6.12±1.31; $t(14)=3.78$, $P<0.005$] emitted more 20 kHz USVs in the drug-paired compartment than VEH-conditioned animals (0.63± 0.62). Analysis of percentage 20 kHz USVs in the drug-paired compartment, covarying for percentage time spent in the drug-paired compartment (NS), revealed a robust effect [$F(2,20)=54.38$, $P<0.001$] such that animals conditioned with both the high [88±5%; $t(14)=11.53$, $P<0.001$] and low doses of LiCl [78±5%; $t(14)=9.60$, $P<0.001$] emitted a greater percentage of 20 kHz USVs in the drug-paired compartment, relative to VEH-conditioned animals (6±6%).

Covariance analyses that controlled for 50 kHz USVs emitted in the VEH-paired compartment [$t(19)=9.00$, $P<0.001$] and time spent in the drug-paired compartment (NS), indicated that LiCl-conditioned animals emitted fewer 50 kHz USVs in the drug-paired compartment [$F(2,19)=6.95$, $P<0.05$] (Table 1). However, paired *t*-tests on untransformed data revealed no significant differences between animals conditioned with the high (2.37±1.19) or low dose of LiCl (4.25±1.50) versus VEH-conditioned controls (5.50±1.93). On the other hand, analysis of percentage 50 kHz USVs in the drug-paired chamber, covarying for percentage time spent in the drug-paired chamber, did reveal the expected effect [$F(2,20)=5.09$, $P<0.05$], such that animals conditioned with both high [21±7%; $t(14)=–3.77$, $P<0.005$] and low doses of LiCl [30±7%; $t(14)=2.69$, $P<0.025$] emitted a lower percentage of 50 kHz USVs in the drug-paired compartment, relative to VEH-conditioned animals (49±2%; see Fig. 1).

Table 1 Mean (±SEM) percentage time spent, 50 kHz USVs, and 20 kHz USVs emitted in the drug-paired compartment. *$P<0.05$ relative to VEH, two-tailed, Bonferroni-corrected. *VEH* vehicle, *NAL* naloxone, *LiCl* lithium chloride

	NAL			LiCl		
	0.0	0.3	3.0	0.0	10	30
Time	53±7	35±3	15±4*	51±3	42±5	36±3*
50 kHz USVs	35±8	25±6	5±3*	49±2	30±7*	21±7*
20 kHz USVs	10±7	83±4*	80±4*	6±6	78±5*	88±5*

Experiment 2

In this experiment, after verifying whether NAL could acutely increase 20 kHz USVs and decrease 50 kHz USVs, we investigated whether conditioning with doses of these compounds that induced place aversion in expt 1 (NAL 3.0 mg/kg and LiCl 30 mg/kg) would also increase 20 kHz USVs and decrease 50 kHz USVs if rats were confined to the drug-paired chamber during testing. We also examined whether conditioning with a dose of a drug known to induce conditioned place preference (MORPH 5.0 mg/kg) would exert the opposite effect on USVs (i.e., decrease 20 kHz and increase 50 kHz USVs).

Acute drug effects

As expected, analysis of USVs prior to and after daily NAL (3.0 mg/kg) versus VEH injection revealed a clear influence of acute drug administration on USVs. NAL injection increased 20 kHz USVs relative to VEH injection, and this effect increased over 3 days of conditioning [$F(2,14)=30.05$, $P<0.001$]. Although rats did not emit different levels of 20 kHz USVs prior to injection, rats emitted more 20 kHz USVs after NAL injections than after VEH injections on day 1 [$t(7)=12.83$, $P<0.001$], day 2 [$t(7)=13.12$, $P<0.001$], and day 3 [$t(7)=7.42$, $P<0.001$] of conditioning. Conversely, NAL injections decreased 50 kHz USVs, relative to VEH injec-

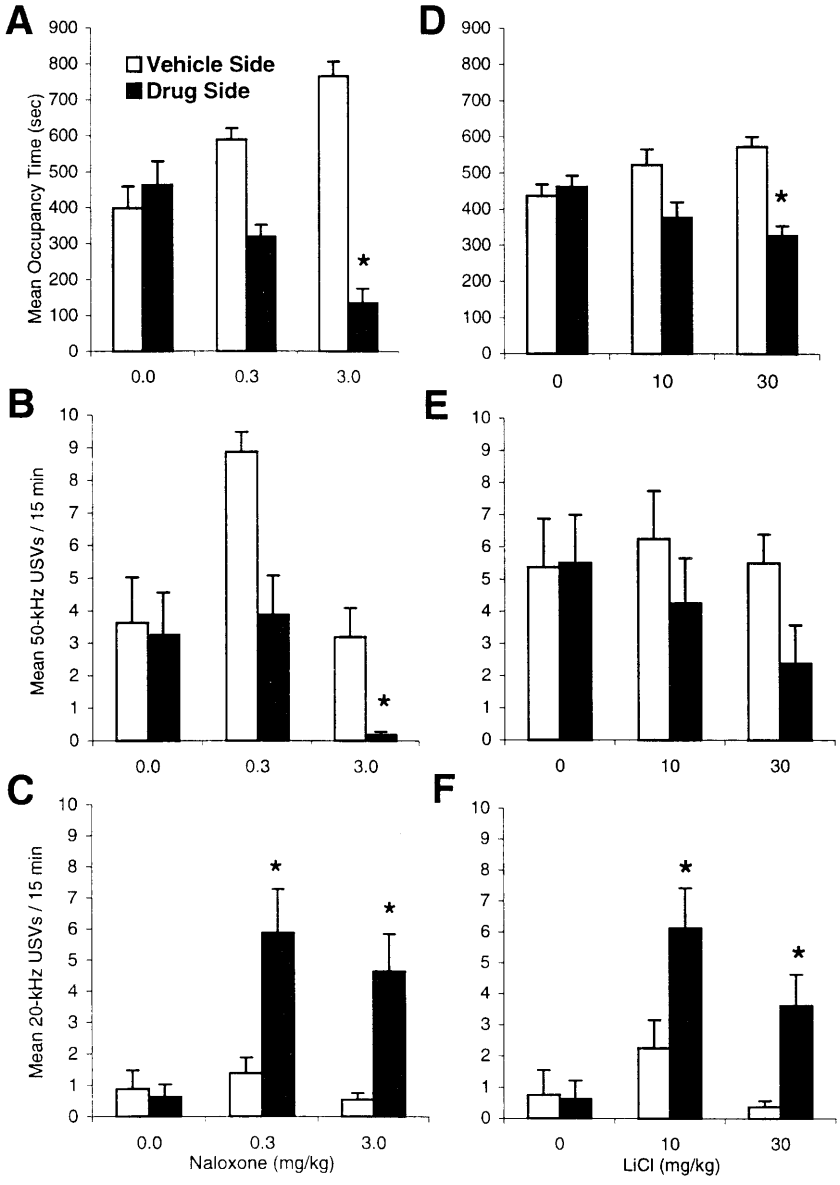

Fig. 1 Mean (±SEM) time spent (**A**, **D**), 50 kHz USVs (**B**, **E**), and 20 kHz USVs (**C**, **F**) in the drug- and vehicle-paired chamber during a 15-min conditioned place aversion testing procedure with naloxone (**A**, **B**, **C**) or lithium chloride (**D**, **E**, **F**). **P*<0.05 relative to VEH, two-tailed, Bonferroni-corrected

tions. Although rats did not differ in their production of 50 kHz USVs prior to injection, NAL-injected animals emitted fewer 50 kHz USVs than VEH-injected controls after injections on day 1 [$t(7)=7.39$, $P<0.001$], day 2 [$t(7)=3.91$, $P<0.006$], and day 3 [$t(7)=16.17$, $P<0.001$] of conditioning (see Fig. 2).

Conditioned drug effects

Even while confined to the conditioning chamber during testing, animals that were conditioned with drugs showed changes in USVs relative to those that were conditioned with VEH [$F(4,33)=10.51$, $P<0.001$]. Specifically, both NAL- [6.00±1.05; $t(13)=4.45$, $P<0.001$] and LiCl-conditioned animals [18.43±5.39; $t(12)=3.26$, $P<0.01$] emitted more 20 kHz USVs than VEH-conditioned controls (0.86±0.26) when tested in the conditioning chamber. These USVs appeared to be specifically evoked by the chamber associated with prior drug conditioning, since a separate NAL-conditioned group which was instead tested in a VEH-paired chamber instead of the NAL-paired chamber (1.00±0.33, NS) did not differ from VEH-conditioned controls. MORPH-conditioned animals (0.87±0.35, NS) also did not differ from VEH-conditioned controls in their production of 20 kHz USVs, since neither group emitted appreciable levels of these vocalizations.

Drug conditioning also altered the production of 50 kHz USVs relative to VEH conditioning [$F(4,33)=14.83$, $P<0.001$]. Both NAL- [1.75±0.49; $t(13)=-3.22$, $P<0.01$] and LiCl-conditioned animals [2.43±.87; $t(12)=-2.43$, $P=0.01$] emitted fewer 50 kHz USVs than VEH-conditioned controls (16.57±4.91) when tested in the conditioning chamber. Again, the NAL-conditioned group that was tested in a VEH-paired chamber (13.37±

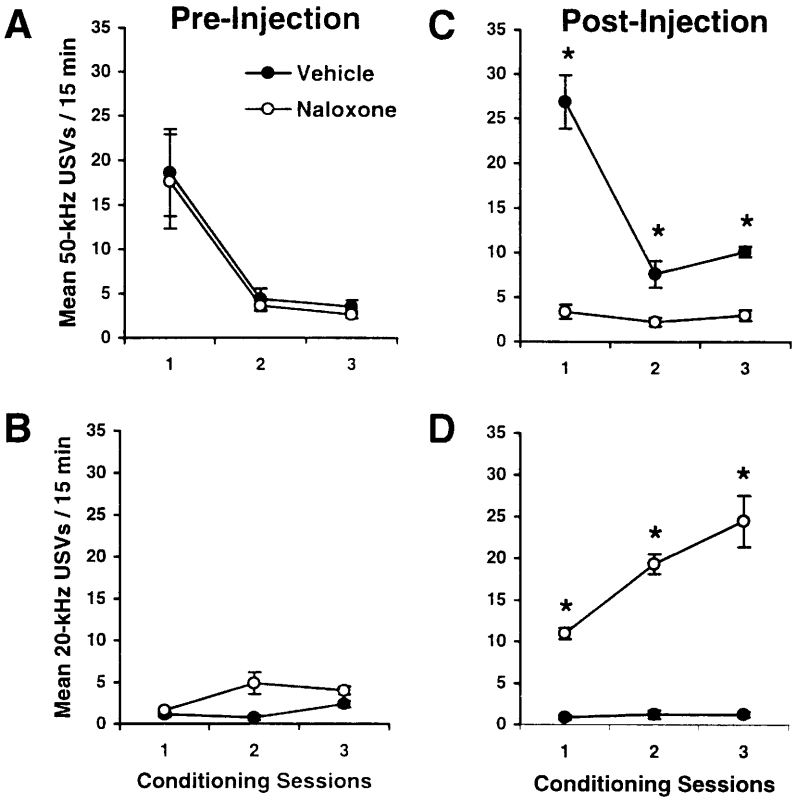

Fig. 2 Mean (±SEM) 50 kHz (**A**) and 20 kHz (**B**) USVs 15 min prior to drug or vehicle injection in the home cage and 50 kHz (**C**) and 20 kHz (**D**) USVs during the first 15 min after drug or vehicle injection in the conditioning chamber. **P*<0.05 relative to VEH, two-tailed, Bonferroni-corrected

1.47, NS) did not differ from VEH-conditioned controls. However, MORPH-conditioned animals [59.00±12.32; $t(13)=3.03$, $P<0.01$] did emit significantly more 50 kHz USVs than VEH-conditioned controls during testing (see Fig. 3).

Discussion

These data replicate prior demonstrations that the administration of NAL or LiCl can induce conditioned place aversion in rats (Mucha and Iverson 1984; Shippenberg et al. 1988). Additionally, administration of these compounds can elicit conditioned USVs. In expt 1, both NAL- and LiCl-conditioned animals emitted more 20 kHz USVs and fewer 50 kHz in drug-paired chambers than did VEH-conditioned controls. The effects of drug conditioning on vocalization could not be statistically accounted for by different amounts of time spent in the drug versus VEH-paired compartments. Notably, animals that received intermediate conditioning doses (i.e., 0.3 mg/kg NAL; 10 mg/kg LiCl) emitted significantly more 20 kHz USVs in the drug-paired compartment, even though they did not show significant place aversion. Because of variance due to individual differences in the tendency to produce USVs, percentage measures of USVs provided a clearer distinction between VEH and drug conditioning, validating prior uses of this measure (Knutson et al. 1999).

In expt 2, NAL- and LiCl-conditioned animals emitted more 20 kHz USVs and fewer 50 kHz USVs than VEH-conditioned controls even when they were confined to the drug-paired compartment during testing. However, NAL-conditioned animals that were tested in a VEH-paired compartment did not show alterations in USV emission, suggesting that drug-paired environmental cues triggered the USVs. Finally, animals that were conditioned with a drug that elicits place preference rather than aversion (MORPH) showed an opposite pattern of USVs, emitting increased 50 kHz USVs relative to VEH-conditioned animals. These findings suggest that the changes in USVs due to NAL and LiCl conditioning cannot be attributed to nonspecific effects of drug conditioning per se. Instead, the ability of an environment to elicit a specific pattern of USVs depended on the appetitive or aversive effects of the drug with which it was previously associated.

Although we have argued that USVs can index affective states, and have implicated associated central nervous system substrates (Knutson et al. 1999; Burgdorf et al. 2000), changes in USVs might also occur secondary to changes in thermoregulation that result from drug conditioning. This hypothesis would accord with research which demonstrates that infant rats, who cannot regulate their own body temperature, show increased 40 kHz "distress calls" upon lowering of the ambient temperature (Allin and Banks 1971; Carden and Hofer 1992). Additionally, researchers have found that acute administration of MORPH can induce hyperthermia (Drawbaugh and Lal 1974), while acute administration of NAL and LiCl can induce hypothermia in adult rats (Tulunay 1976; Eikelboom 1987).

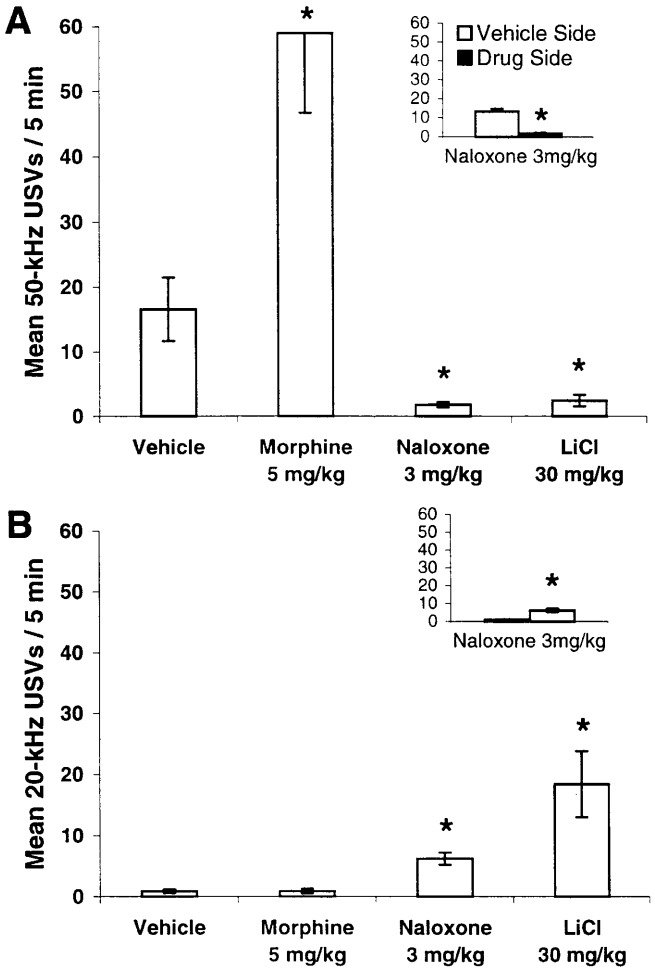

Fig. 3 Mean (±SEM) 50 kHz (**A**) and 20 kHz USVs (**B**) in a drug-free state when rats were placed back in the conditioning compartment where they had been conditioned with vehicle, morphine, naloxone, or lithium chloride (*LiCl*). *Inserts*: mean (±SEM) 50 kHz (**A**) and 20 kHz USVs (**B**) of rats who had been conditioned with naloxone in one compartment and vehicle in another compartment, with one group being tested in the naloxone-paired compartment and the other in the vehicle-paired compartment. **P*<0.05 relative to vehicle, two-tailed, Bonferroni corrected

However, drug-paired cues do not necessarily elicit the same thermoregulatory responses as acute administration of the same drugs. As Schwarz and Cunningham (1990) have noted, cues associated with drugs as diverse as morphine, amphetamine, ethanol, pentobarbitol, and chlordiazepoxide all induce hyperthermia, yet these agents have diverse acute effects on thermoregulation. Further, cues associated with food delivery and electric shock delivery both elicit conditioned hyperthermia (Cunningham and Schwarz 1989), yet have divergent effects on USVs. While cues associated with shock (an aversive incentive) increase 20 kHz USVs and decrease 50 kHz USVs, cues associated with food (an appetitive incentive) have the opposite effect of decreasing 20 kHz USVs and increasing 50 kHz USVs (Burgdorf et al. 2000). Although we did not measure body temperature in these experiments, these data suggest while body temperature may provide a useful marker of general arousal, it is unlikely to distinguish between appetitive and aversive forms of arousal, as do different types of USVs.

Even if USVs can distinguish between aversive and appetitive conditioned effects, several pragmatic constraints may limit their utility as an experimental measure of drug conditioning. Consistent with our hypothesis that USVs index appetitive or aversive states, rats may not continuously emit USVs, but rather sporadically emit them in the context of situations that also elicit avoidant or appetitive behaviors (Knutson et al. 1998). Thus, assuming a low base rate of USVs, we would predict that 20 kHz USVs provide the best index of the aversive effects of a drug, while 50 kHz USVs provide a better index of the appetitive effects of a drug (Knutson et al. 1999). Additionally, adult rats show large and stable individual differences in their tendency to produce USVs. Use of percentage measures or appropriate within-subject controls can help reduce this variation.

In spite of these caveats, USVs may offer some advantages over existing methods of measuring conditioned drug effects. Since they are an unconditioned response, USVs require no prior training. USVs can be rapidly and relatively easily assessed without complicated experimental procedures. Their expression does not appear to depend on gross motor activity or occupancy of certain environments. Although we focused primarily on conditioned USVs in this paper, USVs could be used to assess both conditioned and acute drug effects, whereas place preference can only index conditioned drug effects, and self-administration paradigms can only index acute drug effects. Thus, measurement of USVs may complement existing measures of aversive and appetitive drug effects.

In summary, the present experiments demonstrate that environmental cues associated with drugs which produce aversive effects can increase the expression of 20-kHz USVs and decrease the expression of 50-kHz USVs in adult rats. These findings support our hypothesis that 20 kHz USVs might index conditioned aversive effects of drugs, whereas 50 kHz USVs instead index conditioned appetitive effects of drugs (Knutson et al. 1999). We also introduce a simplified procedure for measuring conditioned USV effects, compared to existing conditioned place preference paradigms. In the future, USVs may provide a rapid and highly sensitive index for detecting the conditioned aversive and appetitive effects of drugs in rats.

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