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α- and β-Adrenergic Receptors Differentially Modulate the Emission of Spontaneous and Amphetamine-Induced 50-kHz Ultrasonic Vocalizations in Adult Rats

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Amphetamine (AMPH) increases adult rat 50-kHz ultrasonic vocalizations, preferentially promoting frequency-modulated (FM) calls that have been proposed to reflect positive affect. The main objective of this study was to investigate a possible noradrenergic contribution to AMPH-induced calling. Adult male Long-Evans rats were tested with AMPH (1 mg/kg intraperitoneal) or saline combined with various systemic pretreatments: clonidine (α2 adrenergic agonist), prazosin (α1 antagonist), atipamezole (α2 antagonist), propranolol, betaxolol, and/or ICI 118,551 (β1/β2, β1, and β2 antagonists, respectively), nadolol (β1/β2 antagonist, peripheral only), or NAD-299 ($5HT_{1A}$ antagonist). In addition, effects of cirazoline (α1 adrenergic agonist) and cocaine (0.25–1.5 mg/kg intravenous) were studied alone. AMPH-induced calling was suppressed by low-dose clonidine and prazosin. Cirazoline and atipamezole did not significantly affect calling rate. Propranolol, without affecting the call rate, dose dependently promoted 'flat' calls under AMPH while suppressing 'trills,' thus reversing the effects of AMPH on the 'call subtype profile.' This effect of propranolol seemed to be mediated by simultaneous inhibition of CNS β1 and β2 rather than by $5HT_{1A}$ receptors. Finally, cocaine elicited fewer calls than did AMPH, but produced the same shift in the call subtype profile. Taken together, these results reveal differential drug effects on flat *vs* trill *vs* other FM 50-kHz calls. These findings highlight the value of detailed call subtype analyses, and show that 50-kHz calls are associated with adrenergic α1- and β-receptor mechanisms. These preclinical findings suggest that noradrenergic contributions to psychostimulant subjective effects may warrant further investigation.

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INTRODUCTION

Adult laboratory rats emit two main types of ultrasonic vocalizations (USVs), commonly termed '22-kHz calls' and '50-kHz calls.' Evidence suggests that USVs may have a communicative role (Brudzynski, 2005; Burgdorf *et al*, 2008a; Wohr and Schwarting, 2009). Vocalizations of the 22-kHz type serve as alarm or distress calls (Covington and Miczek, 2003; Litvin *et al*, 2007), whereas 50-kHz calls are frequently elicited by appetitive stimuli (Burgdorf *et al*, 2010; Knutson *et al*, 2002).

The 50-kHz class of adult rat USVs encompasses a wide frequency range (30–90 kHz) (Kaltwasser, 1990; Sales and Pye, 1974; Wright *et al*, 2010) and comprises two main subclasses: flat (ie, constant frequency) and frequency-modulated (FM) calls. These two subclasses seem to differ in their behavioral significance and neurochemical basis (Ahrens *et al*, 2009; Barker *et al*, 2010; Burgdorf *et al*, 2007, 2008a; Burgdorf and Panksepp, 2006; Ciucci *et al*, 2009; Meyer *et al*, 2011; Simola *et al*, 2009; Wohr *et al*, 2008, 2009). FM 50-kHz USVs are diverse, with at least 13 acoustic subtypes, and the prevalent 'trill' call subtype, in particular, consistently occurs in appetitive situations (Burgdorf *et al*, 2008a). On this basis, it has been proposed that FM calls (and especially trill calls) reflect an emotional state homologous to positive affect in humans (Burgdorf and Moskal, 2009; Burgdorf *et al*, 2010).

The prototypical euphoriant D-amphetamine (AMPH) (Foltin and Fischman, 1991) increases the rate of 50-kHz call production in adult rats, both after systemic and central administration (Ahrens *et al*, 2009; Burgdorf *et al*, 2001; Simola *et al*, 2009; Thompson *et al*, 2006; Wintink and Brudzynski, 2001; Wright *et al*, 2010). In addition, AMPH has been shown to modify the 50-kHz call 'profile' (ie, the relative proportion of different call subtypes), preferentially

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increasing trills and decreasing flat calls (Wright *et al*, 2010). Cocaine administration is also reported to promote 50-kHz calling (Barker *et al*, 2010; Browning *et al*, 2011; Ma *et al*, 2010; Maier *et al*, 2010; Mu *et al*, 2009; Williams and Undieh, 2010), and a recent report shows a preferential increase in FM 50-kHz calls in response to intraperitoneal (IP) cocaine (Meyer *et al*, 2011). However, whether intravenous (IV) cocaine mimics the AMPH-induced shift in the call profile has not been reported.

Dopaminergic transmission seems to have a key role in the production of 50-kHz USVs. In particular, dopaminergic manipulations are reported to affect calls elicited by AMPH (Thompson *et al*, 2006), sex-relevant odors (Ciucci *et al*, 2009), tickling (Burgdorf *et al*, 2007), and intracerebral glutamate (Wintink and Brudzynski, 2001). However, the observation that dopamine (DA)-depleting lesions inhibited FM but not flat 50-kHz calls (Burgdorf *et al*, 2007; Ciucci *et al*, 2009) indicates that not all 50-kHz calls are necessarily DA dependent.

AMPH and cocaine promote noradrenergic, as well as dopaminergic neurotransmission (McKittrick and Abercrombie, 2007; Segal and Kuczenski, 1997). However, a possible noradrenergic role in the production of adult rat 50-kHz USVs has not, to our knowledge, been investigated, except in the context of social stress (Tornatzky and Miczek, 1994). This issue is of interest for several reasons. First, recent evidence supports a noradrenergic contribution to conventional reward-related behaviors, notably conditioned place preference (CPP) and reinstatement of IV self-administration (for review, see Weinshenker and Schroeder (2007); also see the 'Discussion' section). Second, noradrenaline (NA) also seems to contribute to the discriminative stimulus effects of AMPH in several species (Snoddy and Tessel, 1983, 1985); these cues potentially model subjective drug effects in humans (Stolerman, 1992). Third, early studies indicated that AMPH euphoria in human subjects is critically dependent on catecholaminergic transmission (Jonsson *et al*, 1969, 1971), and in some studies, AMPH euphoria seemed to be DA independent, suggesting a possible role for NA (Brauer and de Wit, 1997; Rothman *et al*, 2001; Sofuoglu *et al*, 2009).

The main aim of this study was to test the hypothesis that NA (or adrenaline) contributes to the emission of spontaneous and AMPH-induced 50-kHz USVs, potentially in a call subtype-selective manner. To this end, we first examined whether 50-kHz USV emission under AMPH was altered by acute pretreatment with the $\alpha 2$ agonist clonidine, administered at doses that decrease NA release (Schoffelmeer and Mulder, 1984). We then tested the $\alpha 1$ adrenergic antagonist prazosin, the $\alpha 1$ agonist cirazoline, the $\alpha 2$ antagonist atipamezole, and the $\beta 1/\beta 2$ blocker propranolol. Propranolol produced a dose-dependent shift in the call profile under AMPH, and we subsequently investigated the pharmacological mechanism underlying this effect: (1) To test for peripheral mediation, we administered nadolol, a non-selective hydrophilic β-blocker, which does not readily cross the blood–brain barrier (Schiff and Saxey, 1984); (2) We evaluated the contribution of $\beta 1$ *vs* $\beta 2$ receptor blockade using selective antagonists (betaxolol and ICI 118,551); and (3) As propranolol is a weak $5HT_{1A}$ receptor antagonist, we tested a selective antagonist of this receptor (NAD-299) (Ross *et al*, 1999). In a final experiment, we tested whether the call subtype-dependent effects produced by IP administration of AMPH would generalize to the IV route and also to cocaine.

MATERIALS AND METHODS

Subjects

Subjects were 77 male Long-Evans rats (Charles River Laboratories, St Constant, Quebec, Canada), weighing 307–425 g (ie, aged approximately 9–11 weeks) at the start of the experiment. They were housed 2 per cage ($25 \times 48 \times 20\,cm^3$) in a temperature- and humidity-controlled colony room (19–20°C, 50–60%) at the McGill University Animal Research Center. Rats were maintained on a reverse 12 : 12 light/dark cycle, with lights off at 0700 hours. All behavioral testing took place during the dark phase of the cycle. Food and water were available *ad libitum*, except during testing sessions. All procedures were approved by the McGill Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care. In all experiments, rats were initially drug-naive and experimentally naive; Experiments 3 and 6 were each divided into two parts, with part b beginning within a week after the end of part a.

Overview of Experiments

Almost all experiments investigated the effects of various drug pretreatments on the USV response (ie, call rate and acoustic profile) to systemic (IP) AMPH. Exceptionally, Experiment 3a examined the acute USV response to cirazoline and atipamezole alone, and Experiment 7 comprised a dose-response study of IV cocaine given alone. Details of individual experiments are summarized in Table 1.

Protocol for Individual Experiments

AMPH screen. A significant minority of rats emit few USVs in response to systemic AMPH (Wright *et al*, 2010). To identify and exclude such subjects, rats were initially screened for AMPH-induced calling in three 20-min test sessions spaced 2 days apart. Immediately before each session, rats were administered AMPH (1 mg/kg, IP) and then placed in a test chamber. On the intervening days, rats remained in their home cages. Only the third AMPH test session was analyzed because the first two sessions are not necessarily indicative of a rat's subsequent USV response to AMPH (unpublished observation). USVs that were emitted 10–20 min after injection were counted; rats with the lowest rate of calling (ie, 20–43% of rats depending on the experiment) were excluded from subsequent testing. In total, 47 out of 124 rats were excluded on this basis.

Drug testing. Drug testing was initiated 2–5 days after the final AMPH screening session, with the exception of Experiment 7 (ie, 11 days) in which rats needed to recover from surgery before drug testing began. All experiments used a fully parametric within-subject design in which each rat was tested once under each drug condition (see Table 1 for details). Thus, in Experiments 1, 2, 3b, and 4–6, rats received all combinations of pretreatment and treatment

Table 1 Summary of Experiments

Expt	Pretreatment[a]					Treatment[b]				
	Drug		Doses (mg/kg)	Route	Time before treatment (min)	Drug		Doses (mg/kg)	Route	*n*
1	α2 agonist	Clonidine	0, 0.01, 0.02, 0.1	IP	20		AMPH	0, 1	IP	10
2	α1 antagonist	Prazosin	0, 0.3, 1	IP	30		AMPH	0, 1	IP	12
3a	—	—	—	—	—	α1 agonist	Cirazoline	0.5, 1	IP	12
	—	—	—	—	—	α2 antagonist	Atipamezole	0.3, 1	IP	
	—	—	—	—	—		AMPH	0, 1	IP	
3b	α2 antagonist	Atipamezole	0, 1	IP	20		AMPH	0, 1	IP	9[c]
4	β1/β2 antagonist	Propranolol	0, 1, 3, 10	IP	20		AMPH	0, 1	IP	8
5	β1/β2 antagonist	Propranolol	10	IP	20		AMPH	0, 1	IP	11
	β1 antagonist	Betaxolol	1	IP	20		AMPH	0, 1	IP	
	β2 antagonist	ICI 118,551	0.2	IP	20		AMPH	0, 1	IP	
	β1/β2 antagonist (peripheral)	Nadolol	5	IP	20		AMPH	0, 1	IP	
6a	β1/β2 antagonist	Propranolol	10	IP	20		AMPH	1	IP	12
	β1+β2 antagonist	Betaxolol + ICI 118,551	2.5 (BET), 1 (ICI)	IP	20		AMPH	1	IP	
	$5HT_{1A}$ antagonist	NAD-299	0.2	SC	20		AMPH	1	IP	
6b	β1 antagonist	Betaxolol	2.5	IP	20		AMPH	1	IP	
	β2 antagonist	ICI 118,551	1	IP	20		AMPH	1	IP	
7		—	—	—	—		Cocaine	0, 0.25, 0.75, 1.5	IV	12
		—	—	—	—		AMPH	0.5	IV	

[a]For Experiments 5 and 6, each rat was also tested under vehicle pretreatment combined with saline and AMPH treatment.
[b]In all experiments, treatments were administered immediately before placing the rat in the experimental chambers for recording, with the exception of Experiment 3a, in which cirazoline and atipamezole treatments were administered 20 min before. All test session durations were 20 min, except for Experiment 7 (ie, 10 min).
[c]The rats in Experiment 3b were the same as those used in Experiment 3a.

drugs including vehicle controls. Similarly, in Experiments 3a and 7, rats received a test with each of the following: vehicle, AMPH, and each dose of the drug(s) being tested. Within each experiment, the order of testing was counterbalanced as far as possible. Test sessions were of 20 min duration except in Experiment 7; here, subjects were administered IV cocaine or AMPH and were tested only 0–10 min after injection, ie, during the period of drug onset. Test sessions were spaced 2 days apart to minimize possible carry-over effects of the drugs.

For Experiment 7 (IV cocaine and AMPH), rats first underwent IV catheterization surgery (see below). After recovery, the experiment comprised an initial habituation day, whereby rats were placed in the test chambers for 10 min, then removed and immediately injected with 0.1 ml heparin-Baytril-saline solution to maintain catheter patency. On the five test days that followed, each rat received a 10-s infusion of drug directly after they were placed in the test chamber. Immediately after drug infusion, the tubing was disconnected and the session started.

IV Catheterization Surgery

General anesthesia was provided by ketamine HCl (80 mg/kg IP) and xylazine HCl (16 mg/kg IP). A 5-mm incision was made on the right ventral surface of the neck. A chronic indwelling silastic catheter (0.5 mm I.D. and 0.9 mm O.D., Fisher Scientific, Montreal, Quebec, Canada) was inserted in the right jugular vein and secured using silk sutures. The catheter was passed subcutaneously to a 2-cm incision on the head, where it was connected to a modified plastic cannula (Plastics One, Roanoke, VA), which was then anchored to the top of the skull with stainless steel mounting screws (Plastics One) and dental cement (Stoelting, Wooddale, IL). The cannula was blocked using a plastic stopper made from Tygon tubing (Fisher Scientific), and shielded with an aluminum cap when not in use. The analgesic carprofen (5 mg/kg SC) was administered during surgery to alleviate post-surgical pain. In all, 4 rats out of 19 died from anesthetic overdose during surgery. To verify catheter patency, each rat received an infusion of Na methohexital ('Brevital,' 1 mg in 0.1 ml, 2-s infusion, IV) once in their home cage, 3–5 days after surgery; three rats failed to show the expected sedative response and were therefore excluded from the experiment. The remaining rats were allowed 7–9 days of recovery before experimental testing began. Immediately after the habituation session and after each test session, the catheters were flushed with 0.1 ml of a sterile 0.9% saline solution containing 0.2 mg/ml heparin (Sigma-Aldrich, Oakville, Ontario, Canada) and 17 mg/ml Baytril (ICN Biomedicals, Cleveland, OH).

Acquisition and Classification of USVs

Testing took place in clear Plexiglas experimental chambers (ENV-007CT, Med Associates, St Albans, VT), each of

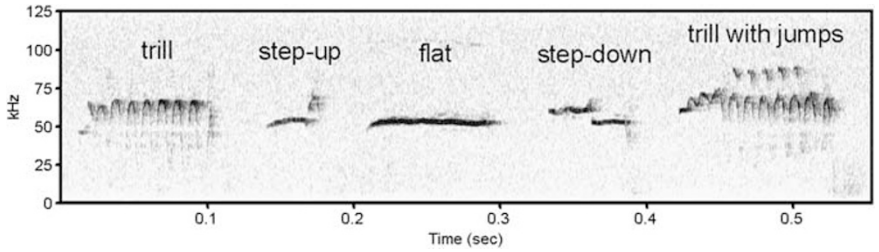

Figure 1 Spectrographic display of individual 50-kHz calls, which are representative of the following subtypes (left to right): trill, step-up, flat, step-down, and trill with jumps. See Wright *et al* (2010) for additional examples of all fourteen 50-kHz call subtypes so far recognized.

which was enclosed in a melamine compartment lined with sound-attenuating acoustic foam (Primacoustic, Port Coquitlam, British Columbia). Condenser ultrasound microphones (CM16/CMPA, Avisoft Bioacoustics, Berlin, Germany) were securely inserted through small (5-cm diameter) holes located centrally in the top panels of the experimental chambers. Consequently, the microphones were 15–30 cm from rats during testing. Microphone signals were fed into an UltraSoundGate 416H data acquisition device (Avisoft Bioacoustics) with a sampling rate of 250-kHz and a 16-bit resolution.

Acoustical analysis was performed using Avisoft SASLab Pro (version 4.2, Avisoft Bioacoustics). Spectrograms were generated with a fast Fourier transform length of 512 points and an overlap of 75% (FlatTop window, 100% frame size). Correspondingly, spectrograms had a frequency resolution of 490 Hz and a time resolution of 0.5 ms. Calls were selected manually from spectrograms by an individual who was masked to the treatment condition. Each identified 50-kHz call was classified into 1 of 14 distinct categories: complex, upward ramp, downward ramp, flat, short, split, step-up, step-down, multi-step, trill, flat–trill combination, trill with jumps, or composite (see Wright *et al* (2010) for criteria for call identification and classification, several examples of each call type, as well as descriptive statistics relating to acoustic parameters). This method of manual call selection has been validated by surgical devocalization, and classification is associated with high inter- and intra-rater reliability (Wright *et al*, 2010). Some representative 50-kHz USVs are shown in Figure 1. 22-KHz calls were rarely observed in this study and were not analyzed further.

Drugs

All test drugs, doses, and routes of administration are shown in Table 1. Drugs were: D-AMPH sulfate (Sigma-Aldrich, Poole, UK); cocaine HCl (Medisca, St-Laurent, Quebec, Canada); clonidine HCl, prazosin HCl, (±)-propranolol HCl, and nadolol (all from Sigma-Aldrich); NAD-299 HCl (ie, Robalzotan), betaxolol HCl, ICI 118,551 HCl, cirazoline HCl, and atipamezole HCl (all from Tocris Bioscience, Ellisville, MO). The doses of prazosin, propranolol, clonidine, and nadolol are expressed as the free base; all other drug doses are expressed as the salt. Drugs were dissolved in sterile 0.9% saline and administered in a volume of 1 ml/kg body weight with the following exceptions: (1) prazosin was dissolved in distilled water, (2) the combination of betaxolol and ICI 118,551 in Experiment 6 was administered in a volume of 4 ml/kg (divided into 2 separate injections), and (3) nadolol (Experiment 5), as well as betaxolol and ICI 118,551 in Experiment 5 were administered in a volume of 2 ml/kg. Control injections were of saline (Experiments 1 and 3–7) or water (for prazosin, Experiment 2) and administered in the same volume as the corresponding drug.

Data Analysis and Statistics

Data were analyzed using commercial software (Systat v11, SPSS, Chicago, IL; GraphPad Prism 4, GraphPad Software, La Jolla, CA). For the IV cocaine dose-response study (Experiment 7), only the USVs emitted during the first 30-s of each minute were analyzed. For Experiment 3a (effects of cirazoline and atipamezole), minutes 3, 8, 13, and 18 were analyzed. For all other experiments, analysis of USVs was restricted to minutes 12, 14, and 16 of the 20-min session to allow time for AMPH to take effect. In the analysis, 'call rate' was defined as the total number of 50-kHz calls (ie, calls of all categories) emitted per minute. ANOVA or Friedman's test was performed, where appropriate, to test the effects of the within-subject factors 'pretreatment' and 'treatment' (see Table 1), for both the call rate and for each call subtype expressed as a proportion of all calls. In addition, for Experiments 4, 6a, and 7, a *post hoc* analysis was performed on non-trill FM calls (ie, all call subtypes except trills, flats, and shorts). All ANOVA p-values were subject to the Huynh–Feldt correction. Multiple comparison tests were performed using Tukey's, Dunnett's, paired t-tests, or Wilcoxon's tests, depending on the type of comparisons to be made and the distribution of the data. For call rate, the latter two tests were subjected to the Holm–Bonferroni (H-B) correction. However, for call subtype analysis, pairwise comparisons were performed using unprotected tests to maintain statistical power. For all tests, a two-tailed p-value $<5\%$ was considered significant.

RESULTS

Experiments 1 and 2: Effects of Clonidine and Prazosin

As expected, AMPH administered alone (ie, with vehicle pretreatment) significantly increased the overall rate of calling (ie, sum of all 50-kHz call categories emitted per minute) (Figure 2a and b). This effect was significantly reduced by the lowest dose of the α2 adrenergic agonist clonidine (ie, 10 μg/kg) and abolished by the two higher doses (20 or 100 μg/kg; Figure 2a). Clonidine also seemed to decrease calls when administered alone (ie, under saline

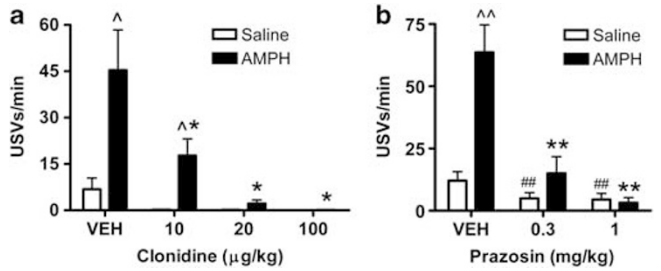

Figure 2 Experiments 1 and 2: Clonidine and prazosin dose dependently decreased the 50-kHz call rate (ie, calls of all categories). The *y* axis represents mean + SEM calls/min. Each rat was tested under all conditions (clonidine group $n = 12$, prazosin group $n = 12$). AMPH administration only significantly increased the call rate when rats were pretreated with vehicle (a, b) or with the lowest dose of clonidine (panel a). Under AMPH treatment, clonidine (panel a) and prazosin pretreatment (panel b) dose dependently reduced the call rate. Prazosin alone (ie, with saline treatment) also decreased the call rate at both doses tested. Clonidine (ie, saline treatment) appeared to decrease calls when administered alone (Friedman test, $p < 0.01$), but the trend did not reach statistical significance for any individual dose ($p > 0.05$). All pairwise comparisons were made by Wilcoxon's tests with Holm–Bonferroni (H-B) correction, $n = 12$ (per experiment). $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$ *vs* the corresponding saline treatment (ie, same pretreatment), $^{\#\#}p < 0.01$ *vs* VEH + saline condition, $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* corresponding VEH pretreatment condition.

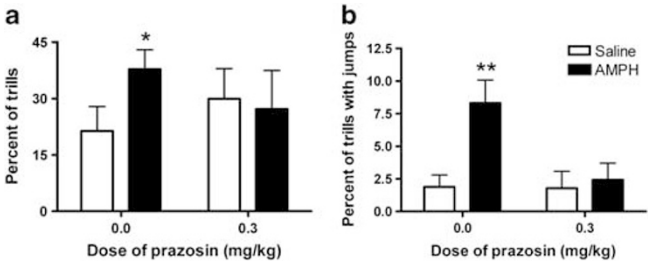

Figure 3 Experiment 2: Prazosin inhibited or blocked the AMPH-induced increase in the percentage of trills (a) and trills with jumps (b). The *y* axes represent mean + SEM percentage. Each rat was tested under all conditions ($n = 12$). Both two-way ANOVA interactions were significant (see main text). $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* corresponding vehicle/saline condition (paired *t*-tests).

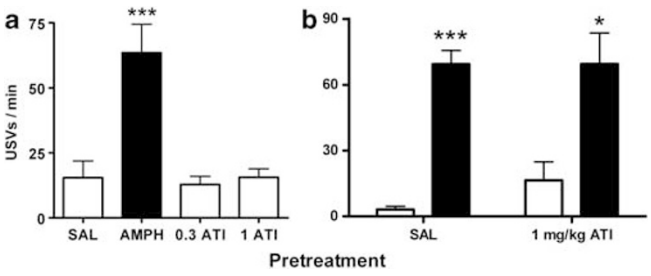

Figure 4 Experiment 3: (a) AMPH increased the call rate, whereas atipamezole (0.3 and 1 mg/kg ATI; $n = 10$) administered alone had no significant effect. The *y* axis represents the mean + SEM call rate per minute. $^{***}p < 0.001$ *vs* saline condition (paired *t*-test) (b) AMPH increased the call rate in rats pretreated with saline or atipamezole. Atipamezole alone did not significantly increase the call rate. The *y* axis represents the mean + SEM call rate per minute. Filled bars correspond to AMPH treatment and open bars correspond to saline treatment. Each rat was tested under all conditions ($n = 9$). $^{*}p < 0.05$, $^{***}p < 0.001$ compared with the same pretreatment with saline challenge (paired *t*-tests).

treatment; Friedman test, $Q_3 = 12.97$, $p < 0.01$; Figure 2a), but no individual dose of clonidine exerted a significant effect (Wilcoxon's tests with H-B correction, $Z \leqslant 2.37$, NS). The $\alpha 1$ antagonist prazosin alone significantly inhibited calling (Friedman test, $Q_2 = 18.48$, $p < 0.001$; Figure 2b), and even the lower dose (0.3 mg/kg) of this drug virtually abolished AMPH-induced calling (Wilcoxon's test with H-B correction, $Z = 2.59$, $p < 0.01$).

Clonidine and prazosin also modified the call profile. As many rats failed to make any calls at higher doses of these drugs, analysis was restricted to the following low-dose conditions: (1) AMPH alone *vs* clonidine (10 μg/kg) + AMPH and (2) all four combinations of vehicle or prazosin (0.3 mg/kg) with saline or AMPH. In the presence of AMPH, clonidine increased the proportion of multi-step calls, while decreasing the proportion of flat–trill combination calls (paired *t*-tests, $t_8 = 2.74$ and $t_8 = 2.83$, respectively, both $p < 0.05$; see Supplementary Figure S1). Prazosin (0.3 mg/kg) blocked the AMPH-induced increase in the proportion of 'trills' and 'trills with jumps' (Figure 3; ANOVA pretreatment × treatment interactions: trills: $F_{1,7} = 6.74$, $p = 0.036$; trills with jumps: $F_{1,7} = 23.31$, $p < 0.01$).

Experiment 3: Effects of Atipamezole and Cirazoline

3a: Effect of atipamezole and cirazoline alone. Neither dose of the $\alpha 2$ antagonist atipamezole (0.3 and 1 mg/kg), administered alone, significantly altered the call rate (Figure 4a) or altered the call profile (data not shown). The $\alpha 1$ agonist cirazoline at both doses tested (0.5 and 1 mg/kg) produced observable changes in the behavior in all rats, such that they disengaged from their cage mate and reared in one corner of the cage. In the first three test days, two rats died shortly after receiving either 0.5 or 1 mg/kg cirazoline, possibly due to pulmonary edema (Micheletti *et al*, 1987). Consequently, saline injection was substituted for cirazoline for the remainder of the experiment. Among rats that received cirazoline ($n = 5$), there was an apparent but non-significant decrease in the call rate (mean ± SEM values for saline and 1 mg/kg cirazoline were 10.7 ± 5.4 and 1.0 ± 1.0 calls/min, respectively).

3b: Effect of atipamezole in combination with AMPH. Atipamezole (1 mg/kg) alone tended to increase the call rate in this experiment, but not significantly (Wilcoxon's test, $Z = 1.89$, $p = 0.0584$; Figure 4b). Atipamezole did not affect the call rate under AMPH (Wilcoxon's test, $Z = 0.41$, NS; Figure 4b), and only had moderate effects on the AMPH call profile. In particular, the percentage of short calls, step-ups, and step-downs was increased by atipamezole (paired *t*-tests, $t_8 = 2.43$–3.08, each $p < 0.05$). Mean ± SEM values in the presence *vs* absence of atipamezole were 17.4 ± 3.1% *vs* 10.4 ± 1.8% (short calls), 11.5 ± 2.3% *vs* 6.5 ± 2.3% (step-ups), and 4.1 ± 0.9% *vs* 2.1 ± 0.7% (step-downs), respectively.

Experiment 4: Effect of Propranolol

Propranolol failed to change the call rate significantly (Figure 5). Although propranolol seemed to depress calling

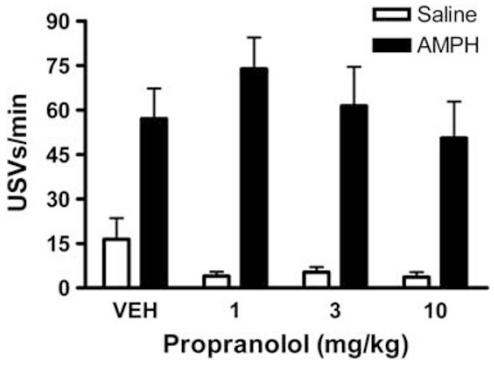

Figure 5 Experiment 4: AMPH-induced 50-kHz call rate was not altered by propranolol. The *y* axis shows mean + SEM calls/min ($n = 8$). Each rat was tested under all conditions. AMPH increased the call rate at all doses of propranolol (Wilcoxon's tests: $p < 0.05$). No dose of propranolol significantly altered the call rate under AMPH (paired *t*-tests, $p > 0.05$) or when administered alone (Wilcoxon's tests, $p > 0.05$). All other pairwise comparisons were subjected to H-B corrections.

Table 2 Effect of Propranolol on Percentage of Flat Calls and Trills under AMPH in Experiment 4

Propranolol dose (mg/kg)	Flat calls	Trills
1	−4.51**	1.86
3	−4.22**	2.7*
10	−9.1***	2.55*

Values in the table are the paired *t*-statistics of the propranolol pretreatment conditions *vs* the saline pretreatment control all under AMPH treatment, df = 7, *$p < 0.05$, **$p < 0.01$,***$p < 0.001$.

when administered alone, no dose differed significantly from saline in this respect, even before correction for multiple comparisons (Wilcoxon's tests, $Z \leqslant 1.96$, NS for each dose). Propranolol also failed to affect 'AMPH-induced' calls (ie, AMPH minus saline difference score; ANOVA $F_{3,21} = 1.86$, NS; uncorrected paired *t*-tests, $t_7 = 0.4$–1.16, NS). In contrast, propranolol had a striking effect on the types of calls emitted (Supplementary Figure S2). In particular, under AMPH, propranolol dose dependently promoted flat calls while nearly abolishing trill calls (ANOVA: flat calls $F_{3,21} = 23.9$, $p < 0.0001$; trills $F_{3,21} = 5.66$, $p < 0.05$; see Table 2 for *t*-statistics comparing each propranolol dose with saline; Figure 6a). In contrast, all other non-trill FM calls collectively remained constant across propranolol doses (ANOVA $F_{3,21} = 0.18$, NS; Figure 6b). The absolute number of trills, flats, and non-trill FM calls are provided in Supplementary Table S1.

Experiment 5: Effects of Betaxolol, ICI 118,551, and Nadolol

In this experiment, the effects of the selective $\beta 1$ adrenergic antagonist betaxolol, the selective $\beta 2$ adrenergic antagonist ICI 118,551, and the hydrophilic $\beta 1/\beta 2$ blocker nadolol were examined. As with propranolol, none of these agents significantly affected the rate of calling after saline or AMPH treatment (Wilcoxon's tests; saline treatment:

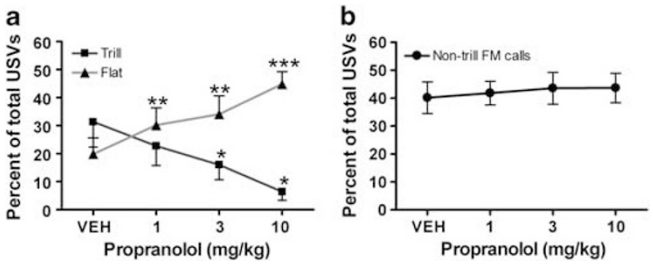

Figure 6 Experiment 4: Propranolol promoted flat calls and inhibited trill calls under AMPH. Line graphs showing (a) the dose-dependent increase in flat calls and concomitant decrease in trills, and (b) no significant difference in non-trill frequency-modulated calls, expressed as mean ± SEM percentage of total calls emitted (ie, calls of all 50-kHz categories). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with vehicle (VEH) pretreatment (paired *t*-tests, $n = 8$).

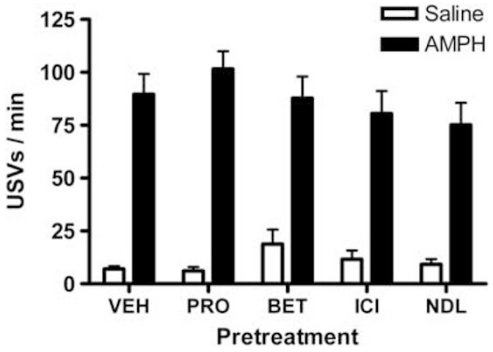

Figure 7 Experiment 5: AMPH-induced 50-kHz calling was not altered by propranolol (PRO; 10 mg/kg, IP), betaxolol (BET; 1 mg/kg, IP), ICI 118,551 (ICI; 0.2 mg/kg, IP), or nadolol (NDL; 5 mg/kg, IP). AMPH robustly increased the call rate under all pretreatment conditions (Wilcoxon's tests: $p < 0.05$–0.003). No pretreatment affected the call rate when administered alone (Wilcoxon's tests: $p > 0.05$) or when combined with AMPH (Wilcoxon's tests: $p > 0.05$). The *y* axis represents mean + SEM calls/min. Each rat was tested under all conditions ($n = 11$). All pairwise comparisons were subjected to H-B corrections.

$Z = 0.62$–0.89, NS; AMPH treatment: $Z = 0.53$–1.95, NS; Figure 7). Analysis of individual call subtypes was restricted to AMPH treatment conditions, as saline test session yielded few calls (Figure 8). Propranolol again caused a highly significant shift in the call profile under AMPH (paired *t*-tests: proportion of (1) trills, $t_{10} = 6.54$, $p < 0.001$; (2) flat calls, $t_{10} = 4.45$, $p < 0.01$) (Figure 8a and b). Here, propranolol also had effects on other call subtypes: propranolol increased the proportion of flat–trill combinations (paired *t*-test, $t_{10} = 2.4$, $p < 0.05$) and split calls (paired *t*-test, $t_{10} = 2.47$, $p < 0.05$) (Figure 8c and d). However, betaxolol, ICI 118,551, and nadolol were all without effect on call profile (Figure 8). The absolute number of trills, flats, flat–trill combinations, and split calls are provided in Supplementary Table S2.

Given the possible sensitizing effects of AMPH on USVs (Ahrens *et al*, 2009), we assessed order effects by examining the call rate under AMPH as a function of the number of times the rat was exposed to AMPH. The call rate did not change significantly over multiple AMPH exposures in this experiment (Supplementary Figure S3).

Experiment 6: Effects of NAD-299 and Higher Doses of Betaxolol and ICI 118,551

The findings of Experiment 5 indicated that the observed effects of propranolol might require simultaneous $\beta1/\beta2$ receptor blockade, or might result from this drug's ability to antagonize $5HT_{1A}$ receptors; alternatively, our doses of betaxolol and ICI 118,551, chosen to ensure $\beta1$ *vs* $\beta2$ selectivity *in vivo* (see 'Notes' in Supplementary Material), might have been insufficient. Therefore, Experiment 6 examined the effects of (1) higher doses of betaxolol and ICI 118,551 alone or in combination and (2) the selective $5HT_{1A}$ antagonist NAD-299.

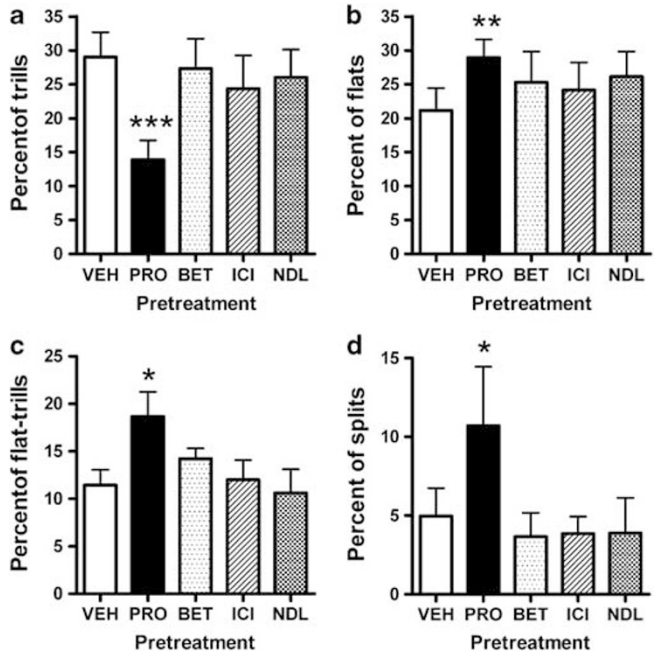

Figure 8 Experiment 5: Propranolol decreased the percentage of trills (a) and increased the percentage of flats, flat–trill combinations, and splits (b–d) under AMPH. The *y* axis represents mean + SEM percentage of total calls (ie, all 50-kHz categories). Each rat was tested under all conditions ($n = 11$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs* vehicle pretreatment condition (paired *t*-tests).

6a: Effects of betaxolol and ICI 118,551 in combination and NAD-299. AMPH treatment again produced a highly significant increase in call rate, and this effect was unaltered by pretreatment with either propranolol, the combination of betaxolol and ICI 118,551, or NAD-299 (Tukey's test: AMPH treatment conditions *vs* saline, $q = 9.07–11.22$, each $p < 0.001$; AMPH treatment alone *vs* drug pretreatment + AMPH, $q = 0.35–1.80$, NS; Supplementary Figure S4). As before, propranolol normalized the trill/flat profile shift induced by AMPH (Figure 9a and b; see Table 3 for statistical details), and in addition, it caused a significant decrease in the proportion of short calls (Figure 9c). The betaxolol/ICI 118,551 combination mimicked these effects of propranolol, whereas NAD-299 was without significant effect (Figure 9a–c). However, propranolol also caused an increase in the proportion of split calls, an effect not observed with the betaxolol/ICI 118,551 combination or with NAD-299 (Figure 9d). There was no significant change in the proportion of non-trill FM calls after any pretreatment in this experiment (Figure 9e).

6b: Effect of betaxolol and ICI 118,551 alone at higher doses. Here, betaxolol or ICI 118,551 was tested individually at the same doses as used in Experiment 6a (2.5 and 1 mg/kg, respectively) in combination with AMPH (1 mg/kg, IP). Neither antagonist affected USV rate or profile (Supplementary Figures S5 and S6).

Experiment 7: Effect of IV Cocaine and AMPH on 50-kHz USVs

The dose of AMPH used in this experiment (0.5 mg/kg IV) was chosen based on a preliminary dose-response study (0.1, 0.5, 1, and 2 mg/kg, IV; Supplementary Figure S7).

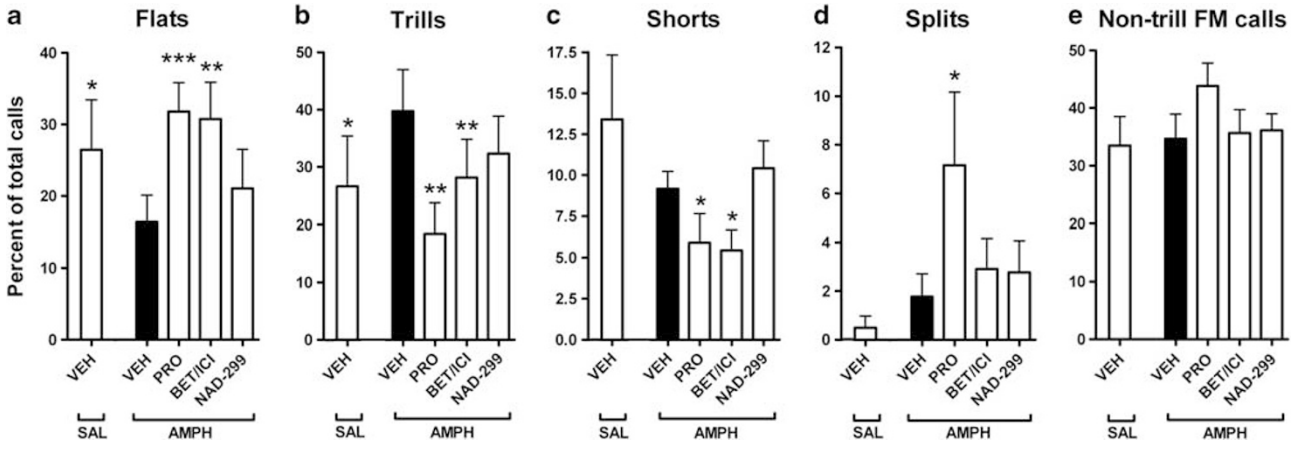

Figure 9 Experiment 6a: Propranolol (PRO; 10 mg/kg, IP) and the combination of betaxolol and ICI 118,551 (BET/ICI; 2.5 and 1 mg/kg IP, respectively) increased the percentage of flat calls under AMPH (a) while decreasing the percentage of trills (b) and shorts (c). In this experiment, propranolol also significantly increased the percentage of split calls (d), an effect not observed with the betaxolol/ICI 118,551 combination. There was no significant effect of any pretreatment on non-trill frequency-modulated calls (e). All pairwise comparisons between the PRO *vs* BET/ICI conditions were non-significant (paired *t*-tests, $p = 0.07–0.81$). NAD-299 failed to affect the percentage of any calls emitted. The *y* axis shows mean + SEM percent of total calls (ie, all subtypes) ($n = 12$). Pretreatments are listed immediately below the *x* axes, and saline or AMPH treatment conditions are indicated underneath each graph. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the VEH/AMPH condition.

Table 3 Call Profile Shifts in Experiment 6a

Pretreatment	Treatment	AMPH treatment alone				
		Flats	**Trills**	**Shorts**	**Splits**	**Non-trill FM calls**
Vehicle	Saline	2.48*	2.25*	0.95	1.17	0.2
Propranolol	AMPH	4.85***	4.06**	2.33*	2.41*	2.12
Betaxolol/ICI 118,551	AMPH	4.31**	3.23**	3.05*	1.36	0.29
NAD-299	AMPH	1.31	1.78	0.64	1.11	0.44

Values in the table refer to the paired *t*-statistics comparing the percentage of each call subtype under AMPH treatment alone with percentage under the pretreatment/treatments listed in the first two columns. df = 11, *$p<0.05$, **$p<0.01$,***$p<0.001$.

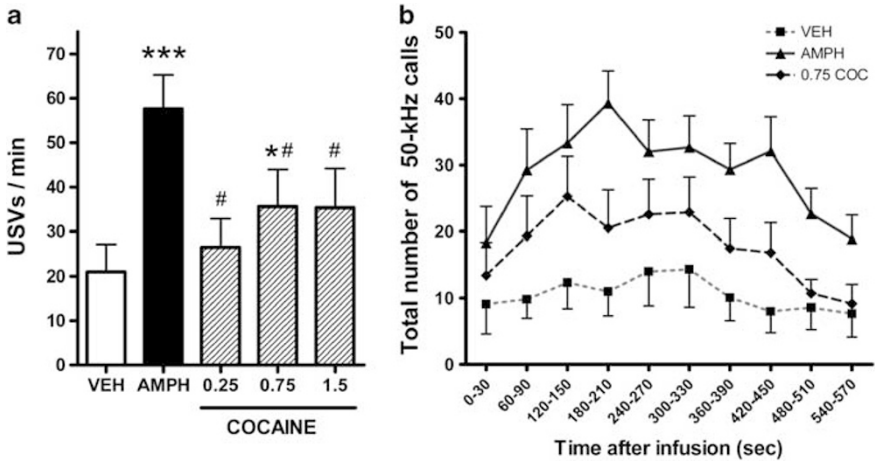

Figure 10 Experiment 7: Cocaine (0.25, 0.75, 1.5 mg/kg, IV) dose dependently increased the number of USVs emitted by rats, but significantly less so than amphetamine (0.5 mg/kg IV; AMPH). (a) The rate of 50-kHz calling was averaged 0–10 min after injection and is expressed as calls/min (mean + SEM). Each rat was tested under all conditions ($n=12$). Only AMPH and 0.75 mg/kg cocaine significantly increased the call rate. *$p<0.05$, ***$p<0.001$ *vs* corresponding saline (VEH) condition, $^{\#}p<0.001$ *vs* the corresponding AMPH condition (Tukey's test). (b) Time course of the call rate after AMPH (0.5 mg/kg, IV) or cocaine (0.25, 0.75, and 1.5 mg/kg, IV) administration. The *x* axis refers to the time after the end of the 10-s infusion. For visual clarity, only the VEH, AMPH, and the 0.75 mg/kg doses of cocaine (ie, the most effective dose of cocaine on the call rate) are illustrated.

Only AMPH and the 0.75 mg/kg dose of cocaine significantly increased the call rate compared with saline treatment, and cocaine was less effective than AMPH in this regard (Tukey's test: AMPH *vs* saline, $q=10.19$, $p<0.001$; 0.75 mg/kg cocaine *vs* saline, $q=4.08$, $p<0.05$; AMPH *vs* each cocaine dose, $q=6.11$–8.66, all $p<0.001$; Figure 10a). Analysis restricted to FM calls showed the same pattern of effects (Supplementary Figure S8). Under AMPH, the call rate increased detectably within the first 30 s after infusion (paired *t*-test *vs* saline, $t_{11}=3.12$, $p<0.01$), and this drug effect peaked between 180 and 210 s (Figure 10b). Cocaine (0.75 mg/kg) produced a significant increase in the call rate 60–90 s after the infusion (paired *t*-test *vs* saline, $t_{11}=2.27$, $p<0.05$), and this effect peaked between 120 and 150 s (Figure 10b).

Although cocaine only modestly affected call rate, it produced a highly significant shift in the call profile at all doses tested (Figure 11). In this respect, it closely mimicked the effect of AMPH, such that trill calls proportionally increased while flat calls decreased (Dunnett's tests *vs* saline: trills, $q=2.67$–4.35, $p<0.01$–0.05; flat calls, $q=3.25$–4.53, $p<0.01$ for each comparison). There was no change in the proportion of non-trill FM calls under AMPH or cocaine (Dunnett's test *vs* saline: $q=0.35$–2.05, NS).

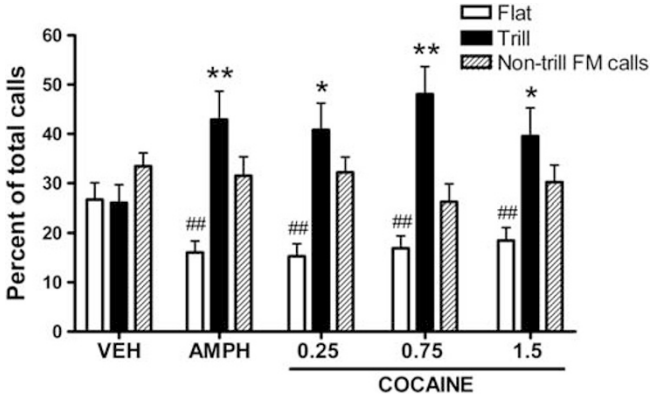

Figure 11 Experiment 7: AMPH (0.5 mg/kg, IV) and all doses of cocaine (0.25, 0.75, and 1.5 mg/kg, IV) promoted trill calls while suppressing flat calls. The *y* axis represents the percentage of the total calls that were trills, flat calls, and non-trill frequency-modulated calls for each drug/dose condition (mean + SEM, $n=12$). *$p<0.05$, **$p<0.01$, $^{\#\#}p<0.01$ *vs* corresponding VEH (ie, saline) condition (Dunnett's tests).

DISCUSSION

Previous investigations relating noradrenergic mechanisms to rat USV emission have almost exclusively focused on

adult 22-kHz USVs (McIntosh and Barfield, 1984) or pup calls (Blumberg *et al*, 2005); both types of call appear to be functionally distinct from the 50-kHz calls emitted by adult rats (Portfors, 2007). To our knowledge, the only previous report of a potential noradrenergic contribution to adult rat 50-kHz USVs was in the context of social stress (Tornatzky and Miczek, 1994). Hence, this study is the first to examine the association between NA and 50-kHz USV production in unstressed adult rats.

Pharmacological Considerations

As discussed below, the effects of prazosin, clonidine, and propranolol observed in this study are likely mediated through $\alpha1$, $\alpha2$, and $\beta1/\beta2$ adrenergic receptors, respectively. Doses of prazosin were based on the drug's potency in the following *in vivo* assays: $\alpha1$ radiotracer binding (Couch *et al*, 1988), antagonism of an $\alpha1$ agonist cue (Schechter, 1991), and inhibition the psychomotor stimulant effects of AMPH (Selken and Nichols, 2007; Vanderschuren *et al*, 2003). Prazosin, at the doses used in this study, is highly $\alpha1$-selective, with negligible affinity for $\alpha2$ or β adrenergic, DA, and serotonin receptors (Balle *et al*, 2003; Clineschmidt *et al*, 1979; Miach *et al*, 1980; Sanger, 1989) or for imidazoline sites (Angel *et al*, 1995). However, prazosin also binds to melatonin MT_3 receptors, although with significantly lower affinity than to $\alpha1$ receptors (Doxey *et al*, 1984; Molinari *et al*, 1996; Pickering and Niles, 1990). The function of the MT_3 receptor remains poorly characterized, except in the regulation of intraocular pressure (Pintor *et al*, 2001). Therefore, on present evidence it is not clear whether MT_3 antagonism would produce detectable behavioral effects.

Clonidine acts as a potent agonist at both $\alpha2$ adrenergic and I_1-imidazoline receptors (Edwards *et al*, 2001). In the dose range administered (0.01–0.1 mg/kg), clonidine would be expected to dose dependently stimulate $\alpha2$ autoreceptors (Drew *et al*, 1979), thereby inhibiting release and turnover of NA (Anden *et al*, 1970; Sacchetti *et al*, 2001). Moreover, within this dose range, clonidine (0.04 mg/kg) produced an $\alpha2$ receptor-mediated drug cue without detectable $\alpha1$- or β-receptor activity (Bennett and Lal, 1982). However, clonidine probably also activated I_1-imidazoline receptors. These receptors have been proposed to contribute to the CNS control of blood pressure (Holt, 2003) and to modulate aversive effects of opiate withdrawal (Georges *et al*, 2005). As the neuropharmacological and behavioral consequences of I_1-imidazoline receptor stimulation are largely unknown, we cannot exclude their possible role in USV inhibition by clonidine.

Propranolol selectively antagonizes $\beta1$, $\beta2$, and $5HT_{1A}$ receptors (Middlemiss and Tricklebank, 1992), while possessing much lower affinity for $\beta3$ receptors (Baker, 2005). Several observations suggest that $5HT_{1A}$ receptors did not contribute to the call profile-changing effect of propranolol under AMPH. First, the highly selective $5HT_{1A}$ antagonist NAD-299 (Ross *et al*, 1999) failed to affect USVs in this study, even when administered in a dose (0.2 mg/kg) beyond that required to inhibit *in vivo* responses to the $5HT_{1A}$ agonist 8-OH-DPAT (Arborelius *et al*, 1999; Johansson *et al*, 1997). Second, the highest dose of propranolol used here (ie, 10 mg/kg) did not inhibit 8-OH-DPAT effects on 5HT release (Sharp *et al*, 1989). Third, the effects of propranolol observed in this study were mimicked by co-administration of selective $\beta1$ and $\beta2$ antagonists (ie, betaxolol and ICI 118,551), neither of which interact significantly with the $5HT_{1A}$ receptor (Middlemiss *et al*, 1985). Finally, our negative finding with nadolol, a non-CNS penetrant β-adrenergic antagonist (Schiff and Saxey, 1984), suggests that propranolol's effects on ultrasonic calling depend on central $\beta1$ and/or $\beta2$ receptors.

Behavioral Considerations

Clonidine and prazosin. Both clonidine and prazosin, when administered alone, inhibited USV emission. An inhibitory effect of high-dose clonidine (ie, 0.1 mg/kg) is consistent with its known sedative effects (Drew *et al*, 1979). The inhibitory effects of lower doses of clonidine (ie, 0.01 and 0.02 mg/kg IP) are perhaps attributable to mild sedation, which has been seen in some (Carey *et al*, 2008; Drew *et al*, 1979; Sara *et al*, 1995) but not in all (De Luca *et al*, 1999; Skolnick *et al*, 1978) studies. Prazosin, in contrast, inhibited 50-kHz calling at doses that are clearly non-sedative (Drouin *et al*, 2002; Vanderschuren *et al*, 2003).

Both clonidine and prazosin dose dependently inhibited AMPH-induced calling, with partial-to-complete block even at low doses. It is unlikely that these drugs produced aversive effects, which might have inhibited 50-kHz calling. Clonidine, for example, is self-administered IV (Davis and Smith, 1977) and induces CPP (Asin and Wirtshafter, 1985; Cervo *et al*, 1993) in rats, whereas prazosin seems motivationally neutral (Forget *et al*, 2009; Zarrindast *et al*, 2002). The inhibitory effect of prazosin is potentially interesting in view of its reported failure to block either the discriminative stimulus effects of AMPH in rats (Arnt, 1996; West *et al*, 1995) or the acquisition of AMPH CPP (Hoffman and Donovan, 1995).

Although clonidine and prazosin, at doses used here, also suppress AMPH-induced locomotion (Drouin *et al*, 2002; Vanderschuren *et al*, 2003), the act of locomotion *per se* does not seem to cause rats to emit ultrasonic calls (Knutson *et al*, 2002).

Cirazoline and atipamezole. The $\alpha1$ agonist cirazoline failed to increase the call rate significantly or modify the call profile, when administered alone. However, cirazoline (0.5. and 1 mg/kg) produced major adverse side effects after injection, most likely due to its action on peripheral $\alpha1$ receptors (Micheletti *et al*, 1987). Thus, it remains unclear whether activation of central $\alpha1$ receptors without the peripheral side effects would elicit 50-kHz USVs. Surprisingly, comparable or even higher doses of cirazoline have been used in several other studies of conscious rats (Alsene *et al*, 2006; Sebban *et al*, 1999; Swerdlow *et al*, 2006).

In contrast, the highly selective $\alpha2$ antagonist atipamezole (Virtanen *et al*, 1989) did not produce any observable changes in behavior. Doses of atipamezole were chosen based on previous studies showing increased extracellular NA levels in the brain (Bondi *et al*, 2010; Wortley *et al*, 1999). The lack of effect of atipamezole on call rate suggests that increased NA release resulting from $\alpha2$ receptor antagonism is not sufficient to elicit USVs. Moreover, the effect of atipamezole on USVs under AMPH suggests that $\alpha2$

receptor inhibition does not affect AMPH-induced call rate, but may modestly contribute to AMPH's ability to modify the call profile.

Propranolol. This study reveals potentially novel psychostimulant effects that are mediated by CNS β-receptors. Propranolol profoundly altered the call profile in rats that were acutely challenged with AMPH. Thus, propranolol suppressed trill calls and promoted flat calls, effectively countering the profile-altering effects exerted by AMPH alone. Additional tests with betaxolol, ICI 118,551, nadolol, and NAD-299 implicated centrally located β-receptors. In contrast, propranolol did not inhibit the AMPH-induced enhancement of call rate, a result that may possibly be related to propranolol's inability to inhibit behavioral stimulant effects of AMPH (Simon *et al*, 1972; Vanderschuren *et al*, 2003). As both USVs and discriminative stimulus (cue) properties have been proposed to model subjective effects of drugs, it is of interest that propranolol antagonized AMPH's effects on call profile (this study) at doses that failed to inhibit the AMPH cue (West *et al*, 1995).

Remarkably, the effects of β-blockers on conventional measures of psychostimulant reward or aversion have received little attention in animals. For example, there seem to be no reports of CPP/aversion testing using propranolol. In an initial study, acute propranolol administration inhibited IV self-administration of AMPH in rats (Yokel and Wise, 1976). In addition, propranolol substantially reduced cocaine IVSA (Harris *et al*, 1996). Thus, in light of these findings, CNS β-adrenergic receptors warrant further attention in the context of psychostimulant reward and aversion.

50-KHz USVs in Relation to Subjective Drug Effects in Humans

In human subjects, there is considerable debate as to the relative importance of dopaminergic and noradrenergic mechanisms in the positive subjective effects of AMPH (Abi-Dargham *et al*, 2003; Brauer and de Wit, 1997; Dlugos *et al*, 2007; Jonsson, 1972; Leyton *et al*, 2007; Lott *et al*, 2005; Nurnberger *et al*, 1984; Rothman *et al*, 2001; Sofuoglu *et al*, 2009). For example, dopaminergic antagonists have failed to reduce psychostimulant euphoria in most studies (Brauer and de Wit, 1995, 1996, 1997; Gawin, 1986; but see Gunne *et al* (1972) and Jonsson (1972)). Moreover, human and animal studies suggest that DA transmission does not contribute to the hedonic impact of psychostimulants, but rather to the incentive salience of reward-related cues (Berridge and Robinson, 1998; Leyton *et al*, 2005, 2007). In contrast, several observations point to possible noradrenergic mediation of AMPH euphoria (Dlugos *et al*, 2007; Rothman *et al*, 2001; Sofuoglu *et al*, 2009); although preliminary studies using α- or β-receptor antagonists have been largely negative, only low antagonist doses were used (Brauer and de Wit, 1995; Jonsson, 1972; Nurnberger *et al*, 1984).

FM 50-kHz calls have been proposed as an index of positive affect in rats (Burgdorf *et al*, 2010). Accordingly, this study confirmed that AMPH selectively promotes trill calls (this study; Wright *et al*, 2010) at doses that are comparable to euphorigenic doses in human studies (Grilly and Loveland, 2001). Propranolol countered this call profile shift. In humans, the impact of β-receptor blockade on the euphoric effect of AMPH has been investigated in only two studies (Jonsson, 1972; Nurnberger *et al*, 1984), to our knowledge. Both studies used propranolol and were ostensibly negative. However, in the first of these, an unusually high dose of AMPH (200 mg, ie, $\sim$3 mg/kg IV) was combined with only moderate doses of propranolol (20 and 40 mg PO). In the second, the dose of AMPH was lower (0.3 mg/kg IV), but subjective effects were inferred only from the subjects' behavior; here, too, it is not clear whether propranolol (0.1 mg/kg IV) was administered in a sufficiently high dose. Therefore, our preclinical findings suggest that CNS β-receptor mechanisms would merit further study in humans under AMPH challenge.

Possible (Nor)Adrenaline–DA Interactions

USV emission by adult rats is not only influenced by (nor)adrenergic mechanisms (this study) but is also strongly DA dependent (see the 'Introduction' section). These neurotransmitter systems are extensively coupled (for review, see Weinshenker and Schroeder (2007)); for example, a number of studies have shown a critical role of noradrenergic transmission in AMPH-induced mesoaccumbens DA release (Darracq *et al*, 1998; Pan *et al*, 1996). However, it seems unlikely that clonidine, prazosin, or propranolol interfered with dopaminergic agonist actions of AMPH in this study. For example, prazosin (0.5 mg/kg IP) did not affect extracellular DA in the nucleus accumbens after systemic AMPH administration (Darracq *et al*, 1998). Similarly, clonidine failed to alter AMPH-induced extracellular DA levels (Florin *et al*, 1994; Tanda *et al*, 1996). Finally, propranolol administration did not inhibit several DA-dependent behavioral effects of AMPH, ie, locomotor stimulation (Simon *et al*, 1972; Vanderschuren *et al*, 2003), stereotypy (Simon *et al*, 1972), and cue properties (West *et al*, 1995).

Generalization to IV Cocaine and AMPH

Acute IP cocaine administration reportedly increases 50-kHz call rate (Williams and Undieh, 2010). Previous studies using IV cocaine have been performed in the context of self-administration (and its anticipation) and sensitization (Barker *et al*, 2010; Browning *et al*, 2011; Ma *et al*, 2010; Maier *et al*, 2010). Here, we provide the first report of the effects of non-contingent IV administration of cocaine on USVs. Cocaine increased the call rate at the 0.75 mg/kg dose, with a rapid onset (peak effect 120–150 s after infusion). Although this USV rate-enhancing effect of cocaine was less pronounced than that of AMPH, cocaine nevertheless produced a profound AMPH-like shift in the call profile at all doses tested (ie, 0.25–1.5 mg/kg). In this dose range, cocaine maintains self-administration (Roberts *et al*, 2007) and induces CPP (Nomikos and Spyraki, 1988; Sellings *et al*, 2006), but is also anxiogenic (Ettenberg, 2004); how these effects may relate to USV emission merits further investigation.

Information Gained from 50-kHz Call Subtype Analysis *vs* Call Rate

Several findings of this study highlight the importance of detailed call subtype analysis. First, both cocaine and

propranolol changed the propensity to emit different call subtypes at doses that did not significantly change the call rate. These results add to evidence that call rate and profile can be manipulated independently by drugs or lesions (Ciucci *et al*, 2009, 2007). Moreover, although several groups currently distinguish between FM and flat 50-kHz calls (Ahrens *et al*, 2009; Burgdorf *et al*, 2007, 2008a; Burgdorf and Panksepp, 2006; Simola *et al*, 2009; Wohr *et al*, 2008), only a few investigators have extended their analysis beyond those two classes (Ciucci *et al*, 2009; Kaltwasser, 1990; Takahashi *et al*, 2010; Vivian and Miczek, 1993; White *et al*, 1990; Wright *et al*, 2010). Importantly, our detailed analysis reveals that the prevalent trill call subtype (Wright *et al*, 2010) is not representative of all FM calls.

Human psychostimulant abusers cannot readily discriminate between cocaine and AMPH (Fischman *et al*, 1976). In this study, cocaine affected the FM call rate less than AMPH, yet produced an equivalent shift in the call profile (ie, preferentially promoting trills over flat calls). Therefore, insofar as FM 50-kHz calls convey information about positive affect in rats (as proposed by Burgdorf *et al* (2010)), the call profile might be more pertinent than the absolute FM call rate.

Limitations and Methodological Considerations

Adult rats vary considerably in their USV response to various stimuli including systemic AMPH (Burgdorf and Panksepp, 2006; Schwarting *et al*, 2007; Wohr *et al*, 2008; Wright *et al*, 2010). To study the effects of drugs on AMPH-induced calling, it was necessary to exclude low responders based on an initial test screen. However, it is important to bear in mind that low- and high-calling rats may differ in other behavioral or neurochemical respects (Burgdorf *et al*, 2008b). The test screen likely explains why we did not subsequently observe sensitization with repeated exposure to AMPH during the experiment, as USV sensitization seems to occur mainly within the first three exposures to cocaine or AMPH (Ahrens *et al*, 2009; Meyer *et al*, 2011; Mu *et al*, 2009).

These findings strongly indicate a (nor)adrenergic role in AMPH-induced 50-kHz USVs. However, the evidence for α1 receptor mediation rests on the use of a single drug—prazosin. Although prazosin is a well-characterized and selective α1 receptor antagonist (see above), it would have been desirable to test other drugs of the same class. However, other currently available α1 antagonists are either less α1-selective (eg, phentolamine), α1 subtype selective (eg, tamsulosin), brain impenetrant (eg, doxazosin), or little characterized in the rat (eg, HEAT).

In this study, only prazosin significantly inhibited 50-kHz calling when administered alone. However, rates of spontaneous calling were generally low, making it hard to detect potential suppressive effects of other drugs. To determine whether noradrenergic transmission has a wider role in USV production, it would be informative to test these drugs in combination with non-pharmacological stimuli that evoke high rates of 50-kHz calling (Ciucci *et al*, 2007; Panksepp and Burgdorf, 2000).

Conclusions

These findings provide the first evidence of (nor)adrenergic involvement in the elicitation of adult rat 50-kHz USVs by AMPH. Furthermore, USV emission seems to be differentially associated with α1- *vs* β-receptor mechanisms, whereby (nor)adrenergic transmission through α1 receptors principally modulates the call rate, whereas NA (or adrenaline) acting on β-receptors affects the acoustic subtypes of 50-kHz calls emitted.

In the context of drug addiction, psychostimulants reinforce self-administration behavior and acutely promote positive affect. At present, it is not clear how these two effects are related. Dopaminergic transmission in the brain seems critical to motivation, but has not been convincingly linked to psychostimulant euphoria. Preliminary evidence points to a noradrenergic contribution to euphorigenic effects of AMPH, but receptor mechanisms have not been identified. These findings suggest that CNS β-adrenergic receptors merit further attention in this regard.

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DISCLOSURE

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)