

Environmental and Pharmacological Modulation of Amphetamine-Induced 50-kHz Ultrasonic Vocalizations in Rats

Henrike Rippberger¹, Marcel M. van Gaalen², Rainer K.W. Schwarting¹ and Markus Wöhr^{1,*}

¹*Behavioral Neuroscience, Experimental and Biological Psychology, Philipps-University of Marburg, Marburg, Germany;* ²*Encepharm GmbH, Göttingen, Germany*

Abstract: Rats emit high-frequency 50-kHz ultrasonic vocalizations (USV) in appetitive situations like social interactions. Drugs of abuse are probably the most potent non-social elicitors of 50-kHz USV, possibly reflecting their euphorogenic properties. Psychostimulants induce the strongest elevation in 50-kHz USV emission, particularly amphetamine (AMPH), either when applied systemically or locally into the nucleus accumbens (Nacc). Emission of AMPH-induced 50-kHz USV depends on test context, such as the presence of conspecifics, and can be manipulated pharmacologically by targeting major neurotransmitter systems, including dopamine (DA), noradrenaline (NA), and serotonin (5-HT), but also protein kinase C (PKC) signaling. Several D₁ and D₂ receptor antagonists, as well as typical and atypical antipsychotics block the AMPH-induced elevation in 50-kHz USV. Inhibiting D₁ and D₂ receptors in the Nacc abolishes AMPH-induced 50-kHz USV, indicating a key role for this brain area. NA neurotransmission also regulates AMPH-induced 50-kHz USV emission given that α_1 receptor antagonists and α_2 receptor agonists exert attenuating effects. Supporting the involvement of the 5-HT system, AMPH-induced 50-kHz USV are attenuated by 5-HT_{2C} receptor activation, whereas 5-HT_{2C} receptor antagonism leads to the opposite effect. Finally, treatment with lithium, tamoxifen, and myricitrin was all found to result in a complete abolishment of the AMPH-induced increase in 50-kHz USV, suggesting the involvement of PKC signaling. Neurotransmitter systems involved in AMPH-induced 50-kHz USV emission only partially overlap with other AMPH-induced behaviors like hyperlocomotion. The validity of AMPH-induced 50-kHz USV as a preclinical model for neuropsychiatric disorders is discussed, particularly with relevance to altered drive and mood seen in bipolar disorder.

Keywords: Amphetamine, antipsychotics, dopamine, lithium, serotonin, ultrasonic vocalizations.

INTRODUCTION

Rats emit different types of vocalizations in the ultrasonic range, commonly referred to as ultrasonic vocalizations (USV; for review see: [1,2]). In juvenile and adult rats, typically two main USV types are differentiated depending on emission context and acoustic features, such as call duration and frequency. Low-frequency USV characterized by long call durations, so called 22-kHz USV, occur in aversive situations and probably reflect a negative emotional state akin anxiety and fear (for review see: [3]). In contrast, high-frequency USV with short call durations, taken together as 50-kHz USV, are emitted in appetitive situations. Under natural conditions, particularly high rates of 50-kHz USV occur during positively reinforcing social interactions, like juveniles rough-and-tumble play (e.g. [4]) and mating in adults (e.g. [5]). Playback studies indicate that 50-kHz USV serve an important communicative function as social contact calls which can maintain or reestablish social proximity by eliciting approach behavior in the recipient (for review see: [6]). Because 50-kHz USV occur almost exclusively in

situations that are positively reinforcing, it was repeatedly suggested that 50-kHz USV might reflect a positive emotional state (e.g. [7]). However, it has to be noted that 50-kHz USV are highly complex and characterized by a huge level of variability. In fact, depending on their shape various subtypes can be discriminated. Typically, the different 50-kHz USV subtypes are clustered into flat (FLAT) and frequency-modulated (FM) calls, with the latter consisting of 50-kHz USV characterized by one or more steps/jumps in frequency, so called frequency step calls, trills, i.e. zigzag-shaped calls, and mixed calls (Fig. 1; e.g. [8-16]), yet more complex classification systems with up to 14 subtypes have also been applied (e.g. [17-21]). While FLAT 50-kHz USV may primarily serve a social coordinating function (e.g. [22,23]), it was suggested that FM 50-kHz USV may in particular or even exclusively reflect a positive affective state (e.g. [24,25]). It is in line with this view that drugs with abuse potential are likely the most potent non-social elicitors of 50-kHz USV, particularly of the FM subtype, possibly reflecting the euphorogenic properties of drugs of abuse.

PHARMACOLOGICAL AGENTS ELICITING 50-kHz USV

Several drugs of abuse elicit 50-kHz USV in rats. In particular, psychostimulants induce a robust elevation of 50-kHz USV emission, most notably d-amphetamine (AMPH).

*Address correspondence to this author at the Behavioral Neuroscience, Experimental and Biological Psychology, Philipps-University of Marburg, Gutenbergstr. 18, 35032 Marburg, Germany; Tel: +6421 28 23612; Fax: +6421 28 23610; E-mail: markus.woehr@staff.uni-marburg.de

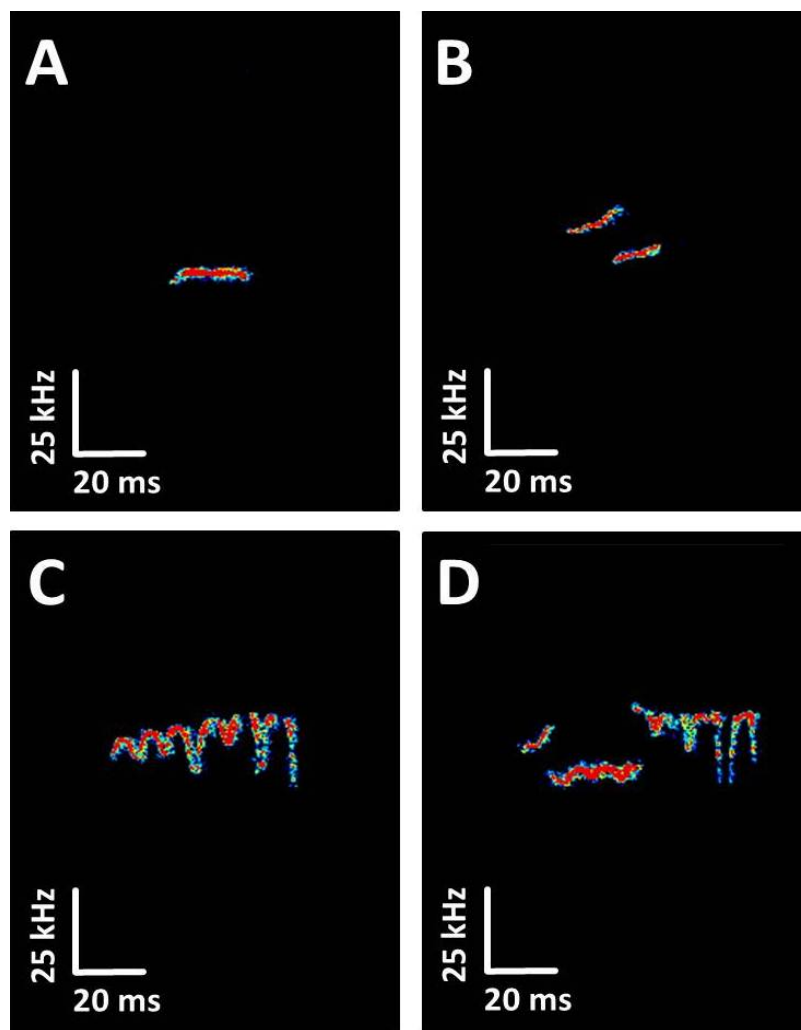


Fig. (1). Exemplary spectrograms of flat (FLAT; A) and frequency-modulated (FM; B-D) 50-kHz ultrasonic vocalizations (USV), with the latter consisting of 50-kHz USV characterized by one or more steps/jumps in frequency, so called frequency step calls (B), trills, i.e. zigzag-shaped calls (C), and mixed calls (D).

Psychostimulants, like AMPH, affect the catecholaminergic system through direct interaction with monoamine transporters, including the dopamine (DA) and the noradrenaline (NA) transporter, which results in increased extracellular DA and NA concentrations (for review see: [26]). High rates of 50-kHz USV can be seen following systemic administration of AMPH [8-31,27-29] or local injection into the nucleus accumbens (Nacc), especially in the shell [30-32]. Relatively low 50-kHz USV emission rates were detected following AMPH injections into the Nacc core [31,32], while AMPH injections into the caudate putamen or the ventral pallidum did not result in 50-kHz USV [30,31]. The level of AMPH-induced 50-kHz USV is clearly dose-dependent, both after systemic [16,21] and local administration in the Nacc [32]. The emission of AMPH-induced 50-kHz USV is typically paralleled by hyperlocomotion [9,11,15], yet rats emitting low versus high rates of AMPH-induced 50-kHz USV do not differ in their locomotor response to AMPH [9,15]. Also, the time course of the 50-kHz USV response differs from the locomotor response profile, with the maximum effect of AMPH on 50-kHz USV occurring earlier than the maximum

locomotor response but being less sustained [27]. In contrast, however, rats selectively bred for low versus high 50-kHz USV emission rates display differences in their locomotor response to AMPH. Rats bred for low 50-kHz USV emission rates show less hyperlocomotion after an AMPH challenge, whereas such challenge induces a stronger locomotor response in rats bred for high levels of 50-kHz USV [33]. Also, rapid and persistent sensitization of locomotor activity and 50-kHz USV emission in response to repeated AMPH treatment was reported in some studies [8,9], but not others [12,16,21]. These inconsistencies could be due to inter-individual differences in response profiles [10,14], changes in the temporal organization of the response [10], or drug treatment protocols [15].

Interestingly, 50-kHz USV emission is not only seen in response to AMPH, but also in anticipation of AMPH administration [9,12,14,15]. Furthermore, in a conditioned place preference paradigm it was shown that highest 50-kHz USV emission rates occur in the drug-paired side during testing [34] or that conditioned place preference is primarily

seen in rats emitting high rates of 50-kHz USV in response to AMPH but not in rats displaying low AMPH-induced call emission levels [9,15]. Together, this suggests that 50-kHz USV may reflect positive affective states associated with drug wanting and/or liking. However, it has to be emphasized that AMPH treatment does not only result in an overall increase in quantity, but also affects the quality of 50-kHz USV. Thus, AMPH particularly increases the proportion of FM 50-kHz USV, most notably trills, whereas the proportion of FLAT 50-kHz USV is typically reduced [12-15,19-21]. Importantly, such AMPH-induced changes in the proportion of 50-kHz USV subtypes are mostly dose-dependent [21] and AMPH-induced sensitization was only seen in FM but not FLAT 50-kHz USV [8,9]. In contrast, acoustic call features were not or only very mildly affected by systemic AMPH treatment, including mean call duration, peak frequency, and frequency modulation [11,13,17,21,29]. AMPH also failed to affect acoustic call features when administered locally into the Nacc [30,32].

Other psychostimulants, which strongly affect catecholamine release, most notably cocaine, also increase 50-kHz USV emission rates, with 50-kHz USV occurring acutely in response to the drug but also in anticipation of it [19,35-45]. Consistently, methamphetamine and cues associated with it also evoke 50-kHz USV [46]. Likewise, methylphenidate, another catecholamine reuptake inhibitor, acutely results in elevated 50-kHz calling [17]. While the above described drugs of abuse that primarily target the catecholamines DA, NA, and less so the indolamine serotonin (5-HT), all elicit 50-kHz USV, other drugs of abuse that are more specifically targeting the 5-HT system, like MDMA, do not affect 50-kHz USV emission, neither in response to the first application [12,17,47] nor during repeated drug exposures [12]. Interestingly, nicotine and caffeine, both having comparably moderate psychostimulatory effects, also do not affect 50-kHz USV emission [12,13,17]. Specifically, neither acute nor repeated nicotine administration induces 50-kHz USV emission [12,17], while for caffeine only acute effects have been tested [13].

Besides amphetamine, another drug of abuse that has been often used in 50-kHz USV experiments is morphine [12,17,18,34,48-50]. Effects of morphine, however, are more complex and less consistent. Thus, it was repeatedly shown that acute treatment with morphine does not lead to enhanced 50-kHz USV emission [12,17,49,50]. In fact, Wright *et al.* [18] demonstrated that acute treatment may even attenuate 50-kHz USV. However, after repeated treatment, 50-kHz USV emission rates increased, either reaching levels of vehicle controls [18] or above [12,49]. Consistently, elevated 50-kHz USV emission rates were also seen during reexposure to the context in which the drug was applied before [48] and in a conditioned place preference paradigm it was shown that highest 50-kHz USV emission rates occur in the drug-paired side during testing [34], yet no such side-dependent differences in 50-kHz USV emission were reported by Wright *et al.* [18].

Together, the present studies show that 50-kHz USV most prominently occur in response to drugs of abuse targeting catecholaminergic systems, with AMPH being the

most potent elicitor. Detailed comparison between AMPH and cocaine indicated that AMPH already at 0.5 mg/kg induced more 50-kHz USV than cocaine at any dose tested [19]. This may explain why the majority of studies on the modulating effects of environmental factors and pharmacological agents on drug-induced 50-kHz USV were performed with AMPH.

ENVIRONMENTAL MODULATION OF AMPH-INDUCED 50-kHz USV

Emission of AMPH-induced 50-kHz USV is context-dependent, with higher rates occurring in presence than in absence of bedding material [27]. When given the choice, rats were found to spend more time and vocalize more in a compartment with bedding material than one without, indicating that 50-kHz USV can be more easily elicited by AMPH in an appetitive environment [27]. Further highlighting the relevance of the test context, rats characterized by low AMPH-induced 50-kHz USV rates in relatively unfamiliar test chambers emit high rates when AMPH is administered in the home cage [9]. In contrast, rats emitting high rates of AMPH-induced 50-kHz USV in the relatively unfamiliar test chambers do so in the home cage as well [9].

Furthermore, the social context plays also an important role, at least in juvenile rats. During rough-and-tumble play behavior in juvenile rats, high rates of 50-kHz USV are typically observed (e.g. [4]). Manduca *et al.* [51] showed that Sprague-Dawley rats emit fewer 50-kHz USV during rough-and-tumble play when pretreated with AMPH, but enhanced levels during non-social activities such as cage exploration or self-grooming. This AMPH-induced inhibition of 50-kHz USV that otherwise parallel rough-and-tumble play is probably due to a strong reduction in play behavior after AMPH treatment. However, it has to be noted that the reduction in 50-kHz USV during rough-and-tumble play was observed in Sprague-Dawley but not Wistar rats. In adult rats, the presence of a conspecific also affected 50-kHz USV emission following AMPH treatment, with rats tested together with a conspecific emitting more 50-kHz USV than when tested individually [19], although this phenomenon was not consistently observed [21]. In the latter study, rats tested under social conditions did not emit more AMPH-induced 50-kHz USV, but displayed a shift in the call emission profile and emitted relatively more FM 50-kHz USV, in particular trills, in response to AMPH than rats tested individually. However, it has to be emphasized that a similar difference was also detected under drug-free conditions, indicating that it is the presence of the conspecific that leads to an increase in FM 50-kHz USV irrespective of drug treatment. Thus, these examples indicate the importance of controlling for environmental and social factors in studies on AMPH-induced USV, and care for these details should be taken when studies are compared.

PHARMACOLOGICAL MODULATION OF AMPH-INDUCED 50-kHz USV

The number of studies investigating the pharmacological modulation of AMPH-induced 50-kHz calling is limited. Most of them use systemic application of test substances [11,16,19,20], and so far only two microinjection studies are

available, in which the substances were injected locally into specific brain areas [29,30,32]. Targeted neurotransmitter systems include DA, NA, and 5-HT, but also glutamate and others (Figs. 2 & 3). Typically, numbers of 50-kHz USV were assessed, with most studies determining changes in the proportions of 50-kHz USV subtypes as well.

Systemic Studies

Dopamine

Wright *et al.* [20] performed an extensive series of experiments investigating the role of DA neurotransmission for AMPH-induced 50-kHz USV emission. Specifically, they studied the effects of blocking DA neurotransmission by various DA receptor antagonists. To this aim, adult male Long-Evans rats were tested in seven different experiments.

For these drug interaction studies, N=10-16 rats were used (within-subject design). AMPH was administered at a dose of 1 mg/kg (IP). AMPH-induced 50-kHz USV were recorded for 20 min immediately following AMPH treatment. Total AMPH-induced 50-kHz USV and the occurrence of the 14 different call subtypes that were described by [21] were determined. Testing took place in the dark phase of the circadian cycle. Importantly, in most of the experiments animals were screened for AMPH-induced 50-kHz calling and low 50-kHz USV emitting animals (approximately 40%) were excluded from the studies. In the other experiments, rats were used that had been exposed to AMPH or morphine before.

The D₁ receptor antagonists SCH 23390 (0, 0.005, 0.01, 0.02 mg/kg; SC) and SCH 39166 (0, 0.03, 0.1, 0.3 mg/kg;

SYSTEMIC STUDIES			Modulation of AMPH-induced 50-kHz USV - Call rate and call profile				REFERENCE				
Substance I	Target	Substance II	Call rate (dose in mg/kg)		Call profile (dose in mg/kg)						
Dopamine (DA)											
SCH 23390 (SC)	D1 antagonist	AMPH (1 mg/kg; IP)	0	0.005	0.01	0.02	0	0.005	0.01	0.02	Wright et al., 2013 [20]
SCH 39166 (SC)	D1 antagonist	AMPH (1 mg/kg; IP)	0	0.03	0.1	0.3	0	0.03	0.1	0.3	Wright et al., 2013 [20]
Haloperidol (IP)	D2 antagonist	AMPH (1 mg/kg; IP)	0	0.1	0.2		0	0.1	0.2		Wright et al., 2013 [20]
Raclopride (SC)	D2 antagonist	AMPH (1 mg/kg; IP)	0	0.1	0.2	0.5	0	0.1	0.2	0.5	Wright et al., 2013 [20]
Eticlopride (IP)	D2 antagonist	AMPH (2 mg/kg; IP)	0	0.01	0.03	0.06	0.09				Wöhr et al., in press [16]
Pimozide (IP)	D2 antagonist	AMPH (1 mg/kg; IP)	0	1			0	1			Wright et al., 2013 [20]
Clozapine (SC)	D2 antagonist	AMPH (1 mg/kg; IP)	0	4			0				Wright et al., 2013 [20]
Risperidone (SC)	D2 antagonist	AMPH (1 mg/kg; IP)	0	0.5			0	0.5			Wright et al., 2013 [20]
Sulpiride (SC)	D2 antagonist	AMPH (1 mg/kg; IP)	0	20	40	80	0	20	40	80	Wright et al., 2013 [20]
GBR 12909 (IP)	DA transporter inhibitor	NO AMPH	0	5	10	20	0	5	10	20	Wright et al., 2013 [20]
Noradrenalin (NA)											
Prazosin (IP)	α1 antagonist	AMPH (1 mg/kg; IP)	0	0.3	1		0	0.3			Wright et al., 2012b [19]
Clonidine (IP)	α2 agonist	AMPH (1 mg/kg; IP)	0	0.01	0.02	0.1	0	0.01			Wright et al., 2012b [19]
Atipamezole (IP)	α2 antagonist	AMPH (1 mg/kg; IP)	0	1			0	1			Wright et al., 2012b [19]
Betaxolol (IP)	β1 antagonist	AMPH (1 mg/kg; IP)	0	1			0	1			Wright et al., 2012b [19]
ICI 118,551 (IP)	β2 antagonist	AMPH (1 mg/kg; IP)	0	0.2			0	0.2			Wright et al., 2012b [19]
Propranolol (IP)	β1/β2 antagonist	AMPH (1 mg/kg; IP)	0	1	3	10	0	1	3	10	Wright et al., 2012b [19]
Betaxolol + ICI 118,551 (IP)	β1+β2 antagonist	AMPH (1 mg/kg; IP)	0	2.5	1	2.5 & 1	0	2.5	1	2.5 & 1	Wright et al., 2012b [19]
Nadolol (IP)	β1/β2 antagonist	AMPH (1 mg/kg; IP)	0	5			0	5			Wright et al., 2012b [19]
Nisoxetine (IP)	NA transporter inhibitor	NO AMPH	0	4	8	16	0	4	8	16	Wright et al., 2013
Serotonin (5-HT)											
NAD-299 (SC)	5-HT1a antagonist	AMPH (1 mg/kg; IP)	0	0.2			0	0.2			Wright et al., 2012b [19]
CP 809,101 (IP)	5-HT2c agonist	AMPH (2 mg/kg; IP)	0	0.3	1	3	10				Wöhr et al., in press [16]
CP 809,101 + SB 242084 (IP)	5-HT2c agonist + antagonist	AMPH (2 mg/kg; IP)	0	3	1	3&1	0	3	1	3&1	Wöhr et al., in press [16]
SB 242084 (IP)	5-HT2c antagonist	NO AMPH	0	0.1	0.3	1.0					Wöhr et al., in press [16]
Antimanic drugs											
Lithium (SC)	Multiple targets	AMPH (2.5 mg/kg; IP)	0	100			0	100			Pereira et al., 2014 [11]
Tamoxifen (SC)	PKC inhibitor	AMPH (2.5 mg/kg; IP)	0	1			0	1			Pereira et al., 2014 [11]
Myricitrin (SC)	PKC inhibitor	AMPH (2.5 mg/kg; IP)	0	10	30		0	10	30		Pereira et al., 2014 [11]

Fig. (2). Mosaic overview on the pharmacological modulation of 50-kHz ultrasonic vocalizations (USV) induced by amphetamine (AMPH): call rate and call profile (systemic studies). Color coding reflects direction and strength of observed effects: yellow = no change; orange = attenuation of AMPH-induced changes; red = complete abolishment of AMPH-induced changes; green = enhancement of AMPH-induced changes (or in absence of AMPH: induction of changes similar to AMPH).

MICROINJECTION STUDIES			Modulation of AMPH-induced 50-kHz USV - Call rate and call profile				REFERENCE			
Substance I	Target	Substance II	Call rate (dose in µg/kg)		Call profile (dose in µg/kg)					
Dopamine (DA)										
SKF-83566 (Nacc)	D1 antagonist	AMPH (7 µg/kg; Nacc)	0	7			0	7		Thompson et al., 2006 [32]
Haloperidol (Nacc)	D2 antagonist	AMPH (7 µg/kg; Nacc)	0	7			0	7		Thompson et al., 2006 [32]
Raclopride (Nacc)	D2 antagonist	AMPH (7 µg/kg; Nacc)	0	7			0	7		Thompson et al., 2006 [32]
Raclopride (Nacc)	D2 antagonist	AMPH (7 µg/kg; Nacc)	0	7						Brudzynski et al., 2011b [30]
Glutamate (AHPOA)		AMPH (1.5, 2, 2.5 mg/kg; IP)	0	17			0	17		Wintink & Brudzynski, 2001 [29]

Fig. (3). Mosaic overview on the pharmacological modulation of 50-kHz ultrasonic vocalizations (USV) induced by amphetamine (AMPH): call rate and call profile (microinjection studies). Color coding reflects direction and strength of observed effects: yellow = no change; orange = attenuation of AMPH-induced changes; red = complete abolishment of AMPH-induced changes; green = enhancement of AMPH-induced changes (or in absence of AMPH: induction of changes similar to AMPH).

SC) dose-dependently reduced AMPH-induced increases in 50-kHz call emission. Both, SCH 23390 and SCH 39166, also clearly suppressed baseline 50-kHz USV in saline control rats not treated with AMPH, with the highest dose of SCH 39166 reaching statistical significance. Higher doses of the two D₁ receptor antagonists further affected the proportion of the various 50-kHz USV subtypes. Specifically, compared to AMPH alone, the proportion of FM 50-kHz USV, in particular trills, was dose-dependently reduced by SCH 23390 and SCH 39166. For animals that received SCH 39166, this shift was paralleled by a relative increase in FLAT 50-kHz USV.

In addition, the D₂ receptor antagonist raclopride (0, 0.1, 0.2, 0.5 mg/kg; SC) and the typical antipsychotic haloperidol (0, 0.1, 0.2 mg/kg; IP) also led to dose-dependent reductions in AMPH-induced 50-kHz USV emission rates. Baseline 50-kHz USV in saline control rats were also blocked by raclopride, but not by haloperidol. It has to be noted, however, that baseline 50-kHz USV emission in the haloperidol experiment was comparatively low. Both, haloperidol and raclopride, specifically inhibited FM 50-kHz USV, particularly the trill 50-kHz USV subtype. Likewise, a single high dose of the D₂ receptor antagonists pimozide (1 mg/kg; IP), but also the atypical antipsychotics clozapine (4 mg/kg; SC) and risperidone (0.5 mg/kg; SC), blocked the AMPH-induced elevation in 50-kHz USV as well as baseline 50-kHz USV emitted by saline control rats. Pimozide particularly reduced FM 50-kHz USV, most notably the trill 50-kHz USV subtype. In stark contrast, the D₂ receptor antagonist sulpiride (0, 20, 40, 80 mg/kg; SC) did not affect 50-kHz USV emission. Even a single high dose of 80 mg/kg sulpiride, which significantly decreased AMPH-induced hyperlocomotion, did not affect 50-kHz USV emission. In rare cases, sulpiride resulted in a few aversive 22-kHz USV.

Wöhr *et al.* [16] investigated the effect of the D₂ receptor antagonist eticlopride (0, 0.01, 0.03, 0.06, 0.09 mg/kg; IP) on AMPH-induced 50-kHz USV (AMPH: 2 mg/kg; IP) in N=8 unselected young adult male Wistar rats tested during the light phase of the circadian cycle using a within-subject design. AMPH-induced 50-kHz USV were recorded for 30 min immediately following AMPH treatment. Under these conditions, effects of AMPH remained stable after repeated dosing. Eticlopride, dose-dependently attenuated AMPH-induced 50-kHz USV. Detailed analysis of 50-kHz USV subtypes was not performed.

Together, these studies indicate that DA neurotransmission is critically involved in 50-kHz USV emission, both under baseline condition as after AMPH treatment. Although SCH 23390 is not very selective for the D₁ receptor [52,53], comparable effects with the more selective D₁ receptor antagonist SCH 39166 [54,55], strongly suggest that the D₁ receptor is critical for AMPH-induced 50-kHz USV, both for the increase in total 50-kHz USV call rate and the changes in call profile. Furthermore, also D₂ receptor stimulation is essential for AMPH-induced 50-kHz USV, as shown in two independent studies [16,20]. Notably, almost all D₂ receptor antagonists, including a broad range of typical and atypical antipsychotics markedly inhibited or abolished AMPH-induced 50-kHz USV emission, often also affecting the call profile, with the remarkable exception of

sulpiride. Despite a clear inhibition of AMPH-induced hyperlocomotion, sulpiride did not affect 50-kHz USV emission, indicating that 50-kHz USV and behavioral activation are mediated by partly different mechanisms. It is unclear why sulpiride is exceptional in failing to affect AMPH-induced 50-kHz USV. Given its effect on AMPH-induced hyperactivity in the same study, it is unlikely due to under-dosing. Several alternative suggestions have been made, such as a possibly limited selectivity for D₂ receptors, or specific effects of sulpiride on D₂ receptor heteromerization [20,56], but additional experiments are required to come to conclusion. However, although it is unclear why sulpiride does not affect AMPH-induced 50-kHz USV, a broad range of compounds from structurally heterogeneous chemical classes, all having D₂ antagonizing properties, do attenuate 50-kHz USV, suggesting that D₂ receptor stimulation is essential for AMPH-induced 50-kHz USV.

It is of note that the two studies investigating the involvement of DA receptors in AMPH-induced 50-kHz USV used different criteria for subject selection [16,20]. Most of the rats tested by Wright *et al.* [20] were selected for high rates of AMPH-induced 50-kHz USV, which makes it difficult to generalize findings beyond this subpopulation. In fact, with the exception of haloperidol and sulpiride, all substances were exclusively tested in pre-selected rats. In support of a general effect, however, is the finding by Wöhr *et al.* [16] showing that D₂ receptor antagonism can also block AMPH-induced 50-kHz USV in unselected rats. Moreover, D₁ and D₂ receptor antagonism not only blocked AMPH-induced 50-kHz USV emission, but also baseline 50-kHz USV in saline control rats. This indicates that AMPH-induced 50-kHz USV as well as 50-kHz USV emitted in the drug-free state are DA-dependent. However, blocking of baseline 50-kHz USV was only seen in pre-selected rats, i.e. in rats selected for high AMPH-induced 50-kHz USV, and not in the study on the effects of haloperidol using unselected rats. A similar picture was obtained by Scardochio and Clarke [28]. They assessed the effects of the D₁ receptor antagonist SCH 39166 (0.1 mg/kg; SC), the D₂ receptor antagonist L-741,626 (1 mg/kg; SC), and the D₂ receptor antagonist NGB 2904 (2 mg/kg; IP) on baseline 50-kHz USV emission rates in rats pre-selected for high AMPH-induced 50-kHz USV and found clear reductions. This may indicate that the pre-selection procedure of animals, while reducing the generalizability of obtained findings, prevents a floor effect and increases statistical power.

Noradrenaline

The role of NA neurotransmission in modulating AMPH-induced 50-kHz USV emission was studied by Wright *et al.* [19]. Specifically, Wright *et al.* [19] examined the effects of blocking NA neurotransmission by a number of NA receptor antagonists, including α_1 and α_2 adrenergic antagonists, but also β_1/β_2 , β_1 , and β_2 adrenergic antagonists. In addition, a α_2 receptor agonist was used. To this aim, N=8-12 adult male Long-Evans rats were tested per experiment using a within-subject design. Like in the study by Wright *et al.* [20], AMPH was administered at a dose of 1 mg/kg (IP) and AMPH-induced 50-kHz USV were recorded for 20 min immediately following treatment during the dark phase of the circadian cycle. Total AMPH-induced 50-kHz USV and

the occurrence of the 14 different call subtypes described before [21] were determined. Again, rats were screened for AMPH-induced 50-kHz calling and around 40% of the animals characterized by low 50-kHz USV emission rates were excluded.

The α_1 receptor antagonist prazosin (0, 0.3, 1 mg/kg; IP), but also the α_2 receptor agonist clonidine (0, 0.01, 0.02, 0.1 mg/kg; IP) dose-dependently reduced the AMPH-induced increase in 50-kHz call emission. Prazosin and clonidine also clearly suppressed baseline 50-kHz USV in saline control rats not treated with AMPH, with the highest doses of prazosin reaching statistical significance. Furthermore, both substances affected the call profile. Specifically, prazosin blocked the AMPH-induced increase in FM 50-kHz USV, particularly the trill subtype of 50-kHz USV. Clonidine increased the proportion of multi-step calls, while decreasing the proportion of flat-trill combination calls. In contrast, the α_2 receptor antagonist atipamezole (1 mg/kg; IP) did not significantly affect AMPH-induced 50-kHz USV emission, although a trend for more 50-kHz USV was evident in rats not treated with AMPH, indicating that atipamezole alone, i.e. in absence of AMPH, might stimulate 50-kHz USV. Also, the effects on the call profile in rats treated with AMPH were relatively minor, with the percentages of short calls, step-ups, and step-downs being increased.

The β_1/β_2 receptor antagonist propranolol (0, 1, 3, 10 mg/kg; IP) failed to affect AMPH-induced 50-kHz USV, while moderately reducing baseline 50-kHz USV emission in saline control rats. Despite the lack of effect on AMPH-induced 50-kHz USV emission rates, propranolol exerted clear dose-dependent effects on the call profile. Specifically, propranolol led to a dose-dependent increase in FLAT 50-kHz USV and a concomitant decrease in the occurrence of FM 50-kHz USV, particularly the trill subtype, nearly abolishing them, and thus almost completely reversing the AMPH-induced changes in the call profile. However, the selective β_1 receptor antagonist betaxolol (1 mg/kg; IP), the selective β_2 receptor antagonist ICI 118,551 (0.2 mg/kg; IP), and the hydrophilic β_1/β_2 blocker nadolol (5 mg/kg; IP) did not affect AMPH-induced 50-kHz USV emission or baseline 50-kHz calling, with no changes in total 50-kHz USV rates and call profile. While the β_1 receptor antagonist betaxolol and the selective β_2 receptor antagonist ICI 118,551 had no effect when administered alone, their combination (betaxolol: 2.5 mg/kg; ICI 118,551: 1 mg/kg; IP) mimicked the effects of the β_1/β_2 receptor antagonist propranolol and led to a similar change in the AMPH-induced call profile, namely a shift toward FLAT 50-kHz USV at the cost of FM 50-kHz USV, thereby normalizing the AMPH-induced changes. This effect was not due to the higher doses used in the combination experiment, since such higher doses given alone again had no effect.

Together, this indicates that NA neurotransmission also plays a critical role in AMPH-induced 50-kHz calling. Prazosin, a highly selective α_1 receptor antagonist, blocked 50-kHz USV emission. However, it has to be noted that prazosin also binds to the melatonin MT_3 receptor [57-59], of which its functional role is poorly characterized. Clonidine, at the doses used by Wright *et al* [19], acts as an activator of the α_2 autoreceptor [60], thereby inhibiting NA

release and turnover [61,62]. Propranolol selectively blocks β_1 , β_2 , and 5-HT_{1A} receptors [63], with its effects on the AMPH-induced call profile likely being dependent on the combined antagonism of β_1 and β_2 receptors, as suggested by the combination experiment using the selective β_1 receptor antagonist betaxolol and the selective β_2 receptor antagonist ICI 118,551 (see also: serotonin). Propranolol, as well as betaxolol and ICI 118,551, had no effect on 50-kHz USV emission rates. Importantly, the effects of propranolol are mediated by β receptors localized in the central nervous system, as nadolol, a peripheral β_1/β_2 receptor antagonist, did not affect the AMPH-induced call profile. Together, one could conclude that α_1 receptors and α_2 autoreceptors play a role in regulating 50-kHz USV emission rates, while β_1/β_2 receptors in combination modulate their call profile. As for the DA study [20], however, the pre-selection procedure challenges the generalization of the findings obtained by Wright *et al*. [19].

Serotonin

The 5-HT_{1A} receptor antagonist NAD-299 (0.2, mg/kg; SC) did not affect AMPH-induced 50-kHz USV in N=12 rats pre-selected for high rates of AMPH-induced 50-kHz USV [19]. The involvement of 5-HT receptors in AMPH-induced 50-kHz USV (AMPH: 2 mg/kg; IP) was also studied in unselected animals by Wöhr *et al*. [16]. Specifically, the effects of the 5-HT_{2C} receptor agonist CP 809,101 and the 5-HT_{2C} receptor antagonist SB 242084 on AMPH-induced 50-kHz USV were tested in two experiments. In the first experiment, the effect of the 5-HT_{2C} receptor agonist CP 809,101 (0, 0.3, 1, 3, 10 mg/kg; IP) was examined in N=8 adult male Wistar rats (approximately 10 weeks old at delivery) tested during the light phase of the circadian cycle using a within-subject design. In the second experiment, N=7 unselected adult male Wistar rats were treated with the 5-HT_{2C} receptor agonist CP 809,101 (3.0 mg/kg; IP), the 5-HT_{2C} receptor antagonist SB 242084 (1.0 mg/kg; IP), or the combination CP 809,101 (3.0 mg/kg; IP) and SB 242084 (1.0 mg/kg; IP), and were compared to vehicle controls. AMPH-induced 50-kHz USV were recorded for 30 min immediately following AMPH treatment. Total AMPH-induced 50-kHz USV were measured in both experiments. In the second experiment, a more detailed analysis was performed and the occurrence of FLAT and FM 50-kHz USV were determined, with the latter consisting of frequency step calls, trills, and mixed calls. Acoustic characteristics of 50-kHz USV were determined in addition.

In the first experiment, the 5-HT_{2C} receptor agonist CP 809,101 dose dependently blocked AMPH-induced 50-kHz USV emission. In the second experiment, CP 809,101 again resulted in a complete inhibition of AMPH-induced calling. In contrast, the 5-HT_{2C} receptor antagonist SB 242084 led to a further enhancement in 50-kHz calling in rats treated with AMPH. When rats were exposed to the combination of CP 809,101 and SB 242084 in addition to AMPH, they displayed 50-kHz USV emission rates similar to the ones observed during exposure to AMPH only. Strong effects on the call profile also were detected. Specifically, the 5-HT_{2C} receptor agonist CP 809,101 led to a clear reduction in proportion of FM 50-kHz USV, most notably of the trill 50-kHz subtype. In contrast, FLAT 50-kHz USV occurred less

often in AMPH-treated rats when exposed to the 5-HT_{2C} receptor antagonist SB 242084 in addition, as compared to AMPH alone, reflecting a shift towards more FM 50-kHz USV, most notably mixed 50-kHz USV. Acoustic characteristics of 50-kHz USV, such as mean call duration, peak frequency, and frequency modulation, were not changed by drug treatment or only mildly affected, as in case of peak amplitude.

Together, the 5-HT_{2C} receptor appears to play an important role in modulating the production of AMPH-induced 50-kHz USV. 5-HT_{2C} receptor activation not only led to an overall reduced AMPH-induced 50-kHz USV emission rate, but also to a clear reduction in the relative proportion of FM 50-kHz USV, most notably of the trill subtype. Importantly, opposite effects were observed following 5-HT_{2C} receptor antagonism. The involvement of 5-HT_{2C} receptors in 50-kHz USV emission is possibly due to the modulation of DA neurotransmission in the mesolimbic system, as electrophysiological and neurochemical studies have revealed that 5-HT_{2C} receptor activation has an inhibitory influence on the activity of midbrain DA systems, with systemic administration of 5-HT_{2C} receptor agonists decreasing the firing rate of VTA DAergic neurons and thus reducing DA release in the Nacc [64,65] (for review see: [66]). The role of 5-HT_{1A} receptors is not yet adequately investigated, and only limited to a single 5-HT_{1A} antagonist [19]. Agonists of this receptor strongly reduce extracellular 5-HT release, thereby reducing 5-HT_{2C} stimulation as well as the activation of many other 5-HT receptors. Additional studies are needed to further elucidate the role of the 5-HT neurotransmitter system in AMPH-induced 50-kHz USV emission.

Antimanic Drugs

As AMPH can produce mania-like symptoms in healthy human subjects and can precipitate manic episodes in patients with bipolar disorder [67,68], AMPH-induced alterations in the behavioral repertoire of rats, most notably AMPH-induced hyperlocomotion, are commonly used in preclinical research [69,70]. However, because mania in humans is not only characterized by elevated drive but also elevated mood, Pereira *et al.* [11] tested whether AMPH-induced 50-kHz USV can be used as a behavioral endpoint with relevance to mania-like changes in mood, in addition to AMPH-induced hyperlocomotion that commonly serves as a measure for mania-like changes in drive. To this aim, Pereira *et al.* [11] studied the effects of the antimanic drugs lithium, tamoxifen, and myricitrin on AMPH-induced 50-kHz USV and hyperlocomotion (AMPH: 2.5 mg/kg; IP) in adult male Wistar rats during the light phase of the circadian cycle in two experiments using a between-subject design with N=7/8 rats per group. Since 50-kHz USV emission rates are characterized by huge levels of inter-individual differences [22,23,71], rats were screened for baseline 50-kHz USV emission rates in the absence of AMPH in the cage test [22,23] in order to assure homogenous experimental groups with similar baseline 50-kHz USV emission rates. No rats were excluded because of low 50-kHz USV emission rates.

In the first experiment, the effects of the mood stabilizer lithium (100 mg/kg; SC) and the protein kinase C (PKC) inhibitor tamoxifen (1 mg/kg; SC) were examined and

compared to vehicle controls. Lithium is the drug of choice for treating bipolar disorder in humans, a disorder characterized by periods of elevated mood and periods of depression [72]. Lithium has an unclear mechanism of action, and efficacy is speculated to be due to targeting inositol monophosphatase and glycogen synthase kinase-3 (GSK-3) among others [72,73], including PKC inhibition [74,75]. In the second experiment, the PKC and nitric oxide inhibitor myricitrin (0, 10, 30 mg/kg; SC) was used. AMPH-induced 50-kHz USV and hyperlocomotion were recorded for 10 min, with the recording starting 10 min following AMPH treatment. Total AMPH-induced 50-kHz USV and the occurrence of FLAT and FM 50-kHz USV were determined, with the latter consisting of frequency step calls, trills, and mixed calls. Acoustic characteristics of 50-kHz USV were measured in addition.

In the first experiment, lithium completely abolished the AMPH-induced increase in 50-kHz USV emission, with rats treated with lithium and AMPH not being different from controls treated with lithium only. Importantly, AMPH-induced changes in the call profile were also reversed by lithium treatment. Specifically, the AMPH-induced increase in the proportion of FM 50-kHz USV was clearly reduced in response to lithium. As expected, lithium significantly reduced AMPH-induced hyperlocomotion. However, it has to be noted that, in contrast to AMPH-induced 50-kHz USV emission, the reduction of AMPH-induced hyperlocomotion was only partial. Locomotor activity was still significantly higher in rats treated with AMPH and lithium than in rats treated with lithium only. Importantly, lithium alone did not affect 50-kHz USV emission or locomotor activity. Lithium is known to induce sickness which may result in non-specific reduction in behavior (e.g. [76]). The effects of lithium reported by Pereira *et al.* [11] are only seen after AMPH treatment, and are, in terms of hyperactivity, only partial, indicating that this is not unspecifically due to sickness. Very similar findings were obtained with tamoxifen. Tamoxifen completely abolished the AMPH-induced increase in 50-kHz USV emission and reduced the AMPH-induced shift toward FM 50-kHz USV, without affecting baseline 50-kHz USV emission. AMPH-induced hyperlocomotion was clearly reduced, but was still higher than in tamoxifen controls not treated with AMPH. Tamoxifen did not affect baseline locomotor activity. In the second experiment, the PKC and nitric oxide inhibitor myricitrin dose-dependently blocked AMPH-induced 50-kHz USV emission, with the highest dose completely blocking the emission of 50-kHz USV in response to AMPH. Myricitrin further reversed the AMPH-induced shift toward FM 50-kHz USV. AMPH-induced hyperlocomotion was only reduced by the highest dose of myricitrin, yet the highest dose exerted a strong effect on locomotor activity in rats treated with AMPH, with locomotor activity levels being similar to myricitrin controls. Similar to lithium and tamoxifen, myricitrin alone did not affect 50-kHz USV emission or locomotor activity. A more detailed analysis of the AMPH-induced changes in acoustic call features revealed that the AMPH-induced increase in frequency modulation is blocked by lithium, tamoxifen, and myricitrin. This inhibition of the AMPH-induced increase in frequency modulation is consistent with the reversal of AMPH-induced changes in the call profile. AMPH, lithium,

tamoxifen, and myricitrin did not affect mean call duration, peak frequency, and call bandwidth.

Together, all of the three antimanic drugs tested blocked the effects of AMPH on 50-kHz calling, both in terms of call number but also call profile. Since all three drugs, lithium, tamoxifen, and myricitrin, have the ability in common to directly or indirectly inhibit PKC [74, 75, 77, 78], it appears likely that the observed effects are due to an inhibition of PKC signaling. Interestingly, the inhibitory effect on 50-kHz calling was even stronger than the one on AMPH-induced hyperlocomotion. Measuring 50-kHz USV appears therefore a promising strategy to assess mania-like changes in mood, in addition to AMPH-induced hyperlocomotion relevant for modelling mania-like changes in drive. Finally, it has to be highlighted that all three substances did not affect baseline 50-kHz USV emission rates and locomotor activity in the absence of AMPH. This indicates that lithium, tamoxifen, and myricitrin specifically block behavioral alterations induced by AMPH.

Microinjection Studies

Local injection of glutamate into the anterior hypothalamic preoptic area (AHPOA) was found to increase 50-kHz USV [79]. More recently, Wintink and Brudzynski [29] investigated if such injections affect AMPH-induced 50-kHz USV emission. To this aim, N=18 adult male Wistar rats were tested using a within-subject design. AMPH was given systemically at the doses of 1.5, 2.0, and 2.5 mg/kg (IP) before glutamate injections into the AHPOA (17 µg; IC). AMPH-induced 50-kHz USV were recorded for 20 min immediately following AMPH treatment. Glutamate was administered after the first 10 min. Total number of 50-kHz USV, the time spent calling, and acoustic characteristics, namely mean call duration and peak frequency, were determined.

The expected additive effect of glutamate injection into the AHPOA on AMPH-induced 50-kHz USV emission rates was not observed in any of the variables analyzed. It is possible that the negative finding is due to the rather low dose of glutamate (17 µg; IC), as a significantly enhanced level of 50-kHz USV was detected in rats treated with a higher dose of glutamate (34 µg; IC). While this higher dose was not combined with AMPH treatment, it was found that the increase in 50-kHz USV in response to the high dose of intra-AHPOA glutamate was blocked by haloperidol (2.0 mg/kg; IP), with the same dose of haloperidol not affecting baseline 50-kHz USV emission rates, indicating again a central role of D₂ receptors of pharmacologically enhanced 50-kHz USV. Acoustic features of 50-kHz USV were not affected by drug treatment.

Thompson *et al.* [32] studied the effects of D₁ and D₂ receptor antagonists directly injected into the Nacc on 50-kHz USV emission elicited by AMPH injections into the same brain site (7 µg/kg; IC). To this aim, N=16 adult male Wistar rats were treated with the D₁ receptor antagonist receptor SKF-83566 (7 µg/kg; IC) and the D₂ receptor antagonists raclopride (7 µg/kg; IC) using a within-subject design. Haloperidol (7 µg/kg; IC) was tested in N=23 adult male Wistar rats. AMPH-induced 50-kHz USV were recorded for 15 min, with recording being started immediately following the administration of the D₁ and D₂ receptor

antagonists. AMPH was administered 5 min later. The total number of 50-kHz USV was determined. In addition, acoustic characteristics, namely mean call duration, peak frequency, frequency bandwidth, and peak amplitude, were determined in a subgroup of 50-kHz USV emitted at the beginning of the experiment. Local injection of AMPH into the Nacc increased 50-kHz USV emission, particularly in the shell but to a lesser degree also in the core region. The D₁ receptor antagonist SKF-83566 and the D₂ receptor antagonist raclopride both blocked the AMPH-induced increase in 50-kHz USV, without affecting baseline 50-kHz USV emission rates. Of note, the inhibitory effects of raclopride on 50-kHz USV elicited by AMPH were recently replicated in a study focusing on the Nacc shell in rats selectively bred for high 50-kHz USV emission rates [30]. Haloperidol, in contrast, failed to antagonize the AMPH effect on 50-kHz USV, with only a minor trend for lower 50-kHz USV levels being evident. Systemic injection of haloperidol does attenuate AMPH induced 50-kHz USV emission [20], which may suggest that the dose of the local injection could have been too low. Surprisingly, however, haloperidol administered alone increased the rate of 50-kHz USV compared to saline [32]. Acoustic call parameters did not differ between drug conditions.

Together, glutamate directly injected into the AHPOA induced 50-kHz USV, which has also been reported after other pharmacological manipulations in this brain area [80], indicating a key role of the AHPOA in 50-kHz USV emission. However, local glutamate injections did not elevate 50-kHz USV in animals pretreated with AMPH. This may not be due to a ceiling effect after AMPH treatment, as dose-dependent increases in AMPH-induced 50-kHz USV were clearly evident in rats treated with glutamate. Yet, it appears possible that the lack of an additive glutamate effect is due to a rather low dose of glutamate. The main conclusions from these experiments are therefore that both AMPH and glutamate can induce 50-kHz USV but (1) that there is no additive effect and (2) that 50-kHz USV induced by any of these drugs are antagonized by haloperidol, thus 50-kHz USV are mediated by a common DAergic mechanism regardless of the drug initiating the response. Consistently, AMPH applied locally in the Nacc increases 50-kHz USV and D₁ and D₂ receptor antagonism in this brain blocked this effect of AMPH. This indicates that elevation of DA neurotransmission in this brain area critically modulates 50-kHz USV emission. However, haloperidol locally applied in the Nacc failed to block the AMPH effect, which may be due to an insufficient dose. Surprisingly, haloperidol administered into the Nacc alone even increased the rate of 50-kHz USV. While this is in contrast to the study by Wright *et al.* [20] showing that its systemic application reduces AMPH-induced 50-kHz USV, the overall pattern, namely that both D₁ and D₂ receptors need to be available for the AMPH-induced increase in 50-kHz USV, is consistent across systemic and microinjection studies.

DISCUSSION

The studies investigating the pharmacological modulation of AMPH-induced 50-kHz calling are relatively sparse so far, but indicate that major neurotransmitter systems, including DA, NA, and 5-HT, but also PKC signaling, are

involved. Specifically, both D₁ and D₂ receptors play a critical role in the AMPH-induced increase in 50-kHz USV emission. Systemic injection of all D₁ and D₂ receptor antagonists applied so far attenuate the AMPH-induced increase in 50-kHz USV emission, although sulpiride is the exception [16,20]. Effective D₁ receptor antagonists are SCH 23390 and SCH 39166. Effective D₂ receptor antagonists are haloperidol, raclopride, eticlopride, and pimozide. Also, the atypical antipsychotics clozapine and risperidone are effective. The key brain structure for the inhibition of AMPH-induced 50-kHz USV appears to be the Nacc, particularly its shell region, given that local injection of D₁ or D₂ receptors antagonists in the Nacc abolish the AMPH-induced increase in 50-kHz USV [30,32]. Thus, DA neurotransmission in the Nacc correlates positively with 50-kHz USV emission. This is insofar particularly interesting, since it was shown that the perception of 50-kHz USV evokes phasic DA release in the Nacc in the recipient rat [81]. Employing fast-scan cyclic voltammetry, Willuhn *et al.* [81] measured DA in freely-moving rats during playback exposure of four acoustic stimuli, including natural 50-kHz USV and 22-kHz USV, and found that specifically playback of 50-kHz USV provoked phasic DA release in the Nacc, which correlated with social approach behavior. Together with the findings implicating DA release in the production of 50-kHz USV, this suggests therefore that the Nacc may function to close a perception-and-action-loop, linking mechanisms relevant for 50-kHz-USV detection and production. Such a loop might be particularly relevant for appetitive social and reciprocal communicatory signals [50,82,83] (for review see: [6]).

Besides DA, also NA and 5-HT, but also PKC is strongly implicated in the regulation of AMPH-induced 50-kHz USV. The highly selective α_1 receptor antagonist prazosin as well as clonidine, which at the doses used by Wright *et al.* [19] acts as an activator of the α_2 autoreceptor, blocked 50-kHz USV emission. This indicates that α_1 receptors and α_2 autoreceptors play a role in regulating 50-kHz USV emission rates. In contrast, β_1/β_2 receptors modulate the call profile. Propranolol, blocking β_1 and β_2 receptors, shifts the call profile toward FLAT 50-kHz USV at the expense of FM 50-kHz USV. This is mediated by β receptors located in the brain, since nadolol, a peripheral β_1/β_2 receptor antagonist, did not affect the AMPH-induced call profile. Wöhr *et al.* [16] focused on the 5-HT system and found that the 5-HT_{2C} receptor agonist CP 809,101 not only led to an overall reduced AMPH-induced 50-kHz USV emission rate, but also to a clear reduction in the relative proportion of FM 50-kHz USV. Opposite effects were observed after treatment with the 5-HT_{2C} receptor antagonist SB 242084, indicating that modulation of this receptor's activity, can move 50-kHz USV in both directions. Data available do not indicate the involvement of 5-HT_{1A} receptors, although studies are limited to a single antagonist only [19]. Finally, Pereira *et al.* [11] observed a complete abolishment of the AMPH-induced increase in 50-kHz USV following treatment with lithium, tamoxifen, or myricitrin. While these substances have various effects, their common denominator is PKC inhibition [74,75,77,78].

Although the data available on the involvement of DA, NA, 5-HT, and PKC signaling in AMPH-induced 50-kHz

USV indicate a prominent role of all of them, at least two questions arise: (1) How specific are the observed effects? (2) Are increased DA, NA or 5-HT neurotransmission and PKC signaling only required or also sufficient for eliciting 50-kHz USV?

(1) Currently, it is not clear whether the effects on AMPH-induced 50-kHz USV emission are specific for AMPH, or comparable to when elicited by other stimuli, most notably conspecifics. The question of specificity is particularly relevant in the context of preclinical models for neuropsychiatric disorders, including schizophrenia and bipolar disorder, as the optimal outcome would be a complete abolishment of the AMPH-induced elevation in 50-kHz USV emission, without affecting baseline 50-kHz USV and, more importantly, 50-kHz USV uttered in a behaviorally relevant, here social context. It is well established that 50-kHz USV serve an important communicative function as social contact calls [50,82,83] (for review see: [6]) and hence a reduction of 50-kHz USV has to be viewed as a side effect. However, inhibition of AMPH-induced 50-kHz USV might serve as a useful endpoint of a desired drug effect. In all studies focusing on the pharmacological modulation of AMPH-induced 50-kHz USV conducted so far, however, 50-kHz USV emitted in response to AMPH or saline were measured, with no study comparing effects on 50-kHz elicited by AMPH and social stimuli [11,16,19,20,29,32]. When comparing the effects on AMPH-induced USV and baseline 50-kHz USV emission rates, it appears clear that most pharmacological compounds do not selectively affect AMPH-induced 50-kHz USV. Specifically, in the study by Wright *et al.* [20], the only exception with reduced AMPH-induced 50-kHz USV but normal baseline 50-kHz USV rates was the D₂ receptor antagonist haloperidol, which could be due to relatively low baseline 50-kHz USV emission. The low baseline 50-kHz USV emission rate was likely the result of the use of unselected rats. It appears very likely that such a selection for high AMPH-induced 50-kHz emission rates results in high baseline 50-kHz USV levels, since both are characterized by stable and positively correlated inter-individual differences [9,14,15]. In unselected rats tested in a sexual context in response to a female 50-kHz USV, however, D₁ and D₂ receptor antagonism can similarly inhibit 50-kHz USV emission, which may suggest that the pre-selection procedure does explain the discrepancy [84-87]. The same appears to be true for the study by Wright *et al.* [19] focusing on the NA system. Again, pre-selected rats were used and in all cases drugs effective in modulating AMPH-induced 50-kHz USV emission rates also affected baseline 50-kHz USV emission. Specifically, the α_1 receptor antagonist prazosin and the α_2 receptor agonist clonidine clearly suppressed both baseline and AMPH-induced 50-kHz USV. In case of NA, however, it is difficult to judge whether this is due to the pre-selection procedure, since very little is known about the effects of substances modulating NA neurotransmission on 50-kHz USV. In unselected rats exposed to a social defeat paradigm, clonidine did not affect 50-kHz USV prior to social defeat, however, after

defeat the comparatively low level of 50-kHz USV was further decreased by clonidine [88], in line with the suggestion that the pre-selection is not critical. Also, in the study by Wöhr *et al.* [16], drug effects were observed under AMPH and saline conditions. The remarkable exception is the study by Pereira *et al.* [11]. In this study, lithium, tamoxifen, and myricitrin completely abolished the AMPH-induced increase in 50-kHz USV, without altering baseline 50-kHz USV emission rates. It would therefore be highly relevant to test whether this specificity is still detectable when comparing AMPH-induced 50-kHz USV and 50-kHz USV emitted during social interactions with a conspecific. Related to the question whether effects are AMPH specific, is the question whether the concomitant increase in locomotion is modulated in addition to 50-kHz USV. AMPH-induced hyperlocomotion, however, is very rarely examined in studies focusing on 50-kHz USV. Yet, the two studies available indicate that measuring AMPH-induced 50-kHz can provide additional information, as the obtained results do not exactly match the behavioral data profile. For instance, sulpiride reduced AMPH-induced hyperlocomotion, but did not affect 50-kHz USV emission [20], whereas effects of lithium, tamoxifen, and myricitrin on AMPH-induced 50-kHz USV emission appeared to be the more sensitive behavioral endpoint [11].

- (2) The data available on the involvement of D₁ and D₂ receptors in AMPH-induced 50-kHz USV indicate that increased DA neurotransmission is required. Whether increased DA neurotransmission is also sufficient for inducing 50-kHz USV is not clear. The selective DA transporter inhibitor GBR 12909 (5, 10, 20 mg/kg; IP) does not significantly alter 50-kHz USV emission rates in the absence of AMPH [20]. Furthermore, the D₁ receptor agonist A-68930 (0, 0.0625, 0.25, 1, 4 mg/kg; SC), the D₂/D₃ receptor agonist quinpirole (0, 0.033, 0.1, 0.33 mg/kg; IP), the D₃ receptor agonist PD 128907 (0, 0.001, 0.01, 0.1, 1 mg/kg; SC), and the D₄ receptor agonist PD 168077 (0, 0.033, 0.1, 0.33 mg/kg; IP) failed to induce 50-kHz USV when applied individually or in various combinations [28], yet quinpirole (0, 0.06, 0.12, 0.25, 0.50, 1.0, 3.0, 6.0, 10.0, 20.0 µg; IC) was found to promote 50-kHz USV production when administered directly into the shell of the Nacc [89]. Similarly, the NA transporter inhibitor nisoxetine (0, 4, 8, 16 mg/kg; IP) did also not significantly change 50-kHz USV emission rates [20]. Even a combination of the DA transporter inhibitor GBR 12909 (10 mg/kg; IP) and the NA transporter inhibitor nisoxetine (12 mg/kg; IP) did not result in more 50-kHz USV. Finally, the α₁ receptor agonist cirazoline (0, 0.5, 1 mg/kg; IP) and the α₂ receptor antagonist atipamezole (0, 0.3, 1 mg/kg; IP) did not alter 50-kHz USV emission when given alone. Together, these findings strongly indicate that increasing DA and/or NA neurotransmission is not sufficient for eliciting 50-kHz USV. However, microdialysis studies indicate that GBR 12909 increases DA release in a slow and long lasting fashion, whereas compounds like AMPH, methamphetamine, and cocaine have rapid and short-lived effects [90,91]. The maximum effect of GBR

12909 in DA release may last up to 60 min after injection, and therefore, it cannot be ruled out that 50-kHz USV recording was performed before extracellular DA concentrations were sufficiently elevated. In contrast, the 5-HT_{2C} receptor antagonist SB 242084 (0, 0.1, 0.3, 1.0 mg/kg; IP) induced 50-kHz USV in a dose-dependent manner in the absence of AMPH [16]. As described above, it is likely that this is due to the DA modulating effects of 5-HT_{2C} receptors. However, in view of the findings indicating that increased DA neurotransmission is not sufficient for eliciting 50-kHz USV, other mechanisms might be involved as well. Whether activating PKC signaling results in 50-kHz USV is currently not known.

CONCLUSION

Emission of AMPH-induced 50-kHz USV depends on test context, such as the presence of conspecifics, and can be manipulated pharmacologically by targeting major neurotransmitter systems, including DA, NA, and 5-HT, but also PKC signaling.

Selective inhibition of the AMPH-induced elevation in 50-kHz USV emission appears to be a useful behavioral endpoint in preclinical models for schizophrenia and bipolar disorder, particularly as it might allow to assess relevant changes in mood in addition to those seen in drive that are typically assessed by measuring AMPH-induced hyperlocomotion.

CONFLICT OF INTEREST

M.M.v.G. is full employee of Encepharm GmbH & Co. KG and shareholder of AbbVie and Abbott. M.W. is scientific advisor of Avisoft Bioacoustics.

ACKNOWLEDGEMENTS

This work was supported by grants from the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG) to M.W. (DFG WO 1732/1-1 and DFG WO 1732/4-1).

REFERENCES

- [1] Brudzynski, S.M. Ethotransmission: communication of emotional states through ultrasonic vocalization in rats. *Curr. Opin. Neurobiol.*, **2013**, *23*(3), 310-317. <http://dx.doi.org/10.1016/j.conb.2013.01.014>
- [2] Wöhr, M.; Schwarting, R.K.W. Affective communication in rodents: ultrasonic vocalizations as a tool for research on emotion and motivation. *Cell Tissue Res.*, **2013**, *354*(1), 81-97. <http://dx.doi.org/10.1007/s00441-013-1607-9>
- [3] Schwarting, R.K.W.; Wöhr, M. On the relationships between ultrasonic calling and anxiety-related behavior in rats. *Braz. J. Med. Biol. Res.*, **2012**, *45*(4), 337-348. <http://dx.doi.org/10.1590/S0100-879X2012007500038>
- [4] Knutson, B.; Burgdorf, J.; Panksepp, J. Anticipation of play elicits high-frequency ultrasonic vocalizations in young rats. *J. Comp. Psychol.*, **1998**, *112*(1), 65-73. <http://dx.doi.org/10.1037/0735-7036.112.1.65>
- [5] Sales, G.D. Ultrasound and mating behaviour in rodents with some observations on other behavioural situations. *J. Zool.*, **1972**, *168*(2), 149-164.
- [6] Seffer, D.; Schwarting, R.K.W.; Wöhr, M. Pro-social ultrasonic communication in rats: Insights from playback studies. *J. Neurosci. Methods*, **2014**, *234*(1), 73-81. <http://dx.doi.org/10.1016/j.jneumeth.2014.01.023>
- [7] Panksepp, J. Beyond a joke: from animal laughter to human joy? *Science*, **2005**, *308*(5718), 62-63. <http://dx.doi.org/10.1126/science.1112066>

- [8] Ahrens, A.M.; Ma, S.T.; Maier, E.Y.; Duvauchelle, C.L.; Schallert, T. Repeated intravenous amphetamine exposure: rapid and persistent sensitization of 50-kHz ultrasonic trill calls in rats. *Behav. Brain Res.*, **2009**, *197*(1), 205-209. <http://dx.doi.org/10.1016/j.bbr.2008.08.037>
- [9] Ahrens, A.M.; Nobile, C.W.; Page, L.E.; Maier, E.Y.; Duvauchelle, C.L.; Schallert, T. Individual differences in the conditioned and unconditioned rat 50-kHz ultrasonic vocalizations elicited by repeated amphetamine exposure. *Psychopharmacology (Berl)*, **2013**, *229*(4), 687-700. <http://dx.doi.org/10.1007/s00213-013-3130-9>
- [10] Lehner, M.H.; Taracha, E.; Kaniuga, E.; Wisłowska-Stanek, A.; Wróbel, J.; Sobolewska, A.; Turzyńska, D.; Skórzewska, A.; Płaźnik, A. High-anxiety rats are less sensitive to the rewarding affects of amphetamine on 50 kHz USV. *Behav. Brain Res.*, **2014**, *275*(1), 234-242. <http://dx.doi.org/10.1016/j.bbr.2014.09.011>
- [11] Pereira, M.; Andreatini, R.; Schwarting, R.K.W.; Brenes, J.C. Amphetamine-induced appetitive 50-kHz calls in rats: a marker of affect in mania? *Psychopharmacology (Berl)*, **2014**, *231*(13), 2567-2577. <http://dx.doi.org/10.1007/s00213-013-3413-1>
- [12] Simola, N.; Frau, L.; Plumitallo, A.; Morelli, M. Direct and long-lasting effects elicited by repeated drug administration on 50-kHz ultrasonic vocalizations are regulated differently: implications for the study of the affective properties of drugs of abuse. *Int. J. Neuropsychopharmacol.*, **2014**, *17*(3), 429-441. <http://dx.doi.org/10.1017/S1461145713001235>
- [13] Simola, N.; Ma, S.T.; Schallert, T. Influence of acute caffeine on 50-kHz ultrasonic vocalizations in male adult rats and relevance to caffeine-mediated psychopharmacological effects. *Int. J. Neuropsychopharmacol.*, **2010**, *13*(1), 123-132. <http://dx.doi.org/10.1017/S1461145709990113>
- [14] Taracha, E.; Hamed, A.; Krząścik, P.; Lehner, M.; Skórzewska, A.; Płaźnik, A.; Chrapusta, S.J. Inter-individual diversity and intra-individual stability of amphetamine-induced sensitization of frequency-modulated 50-kHz vocalization in Sprague-Dawley rats. *Psychopharmacology (Berl)*, **2012**, *222*(4), 619-632. <http://dx.doi.org/10.1007/s00213-012-2658-4>
- [15] Taracha, E.; Kaniuga, E.; Chrapusta, S.J.; Maciejak, P.; Sliwa, L.; Hamed, A.; Krząścik, P. Diverging frequency-modulated 50-kHz vocalization, locomotor activity and conditioned place preference effects in rats given repeated amphetamine treatment. *Neuropharmacology*, **2014**, *83*(1), 128-36. <http://dx.doi.org/10.1016/j.neuropharm.2014.04.008>
- [16] Wöhr, M.; Rippberger, H.; Schwarting, R.K.W.; van Gaalen, M.M. Critical involvement of 5-HT2c receptor function in amphetamine-induced 50-kHz ultrasonic vocalizations in rats. *Psychopharmacology (Berl)*, in press. <http://dx.doi.org/10.1007/s00213-014-3814-9>
- [17] Simola, N.; Fenu, S.; Costa, G.; Pinna, A.; Plumitallo, A.; Morelli, M. Pharmacological characterization of 50-kHz ultrasonic vocalizations in rats: comparison of the effects of different psychoactive drugs and relevance in drug-induced reward. *Neuropharmacology*, **2012**, *63*(2), 224-234. <http://dx.doi.org/10.1016/j.neuropharm.2012.03.013>
- [18] Wright, J.M.; Deng, L.; Clarke, P.B. Failure of rewarding and locomotor stimulant doses of morphine to promote adult rat 50-kHz ultrasonic vocalizations. *Psychopharmacology (Berl)*, **2012a**, *224*(4), 477-487. <http://dx.doi.org/10.1007/s00213-012-2776-z>
- [19] Wright, J.M.; Dobosiewicz, M.R.; Clarke, P.B. α - and β -Adrenergic receptors differentially modulate the emission of spontaneous and amphetamine-induced 50-kHz ultrasonic vocalizations in adult rats. *Neuropsychopharmacology*, **2012b**, *37*(3), 808-821. <http://dx.doi.org/10.1038/npp.2011.258>
- [20] Wright, J.M.; Dobosiewicz, M.R.; Clarke, P.B. The role of dopaminergic transmission through D1-like and D2-like receptors in amphetamine-induced rat ultrasonic vocalizations. *Psychopharmacology (Berl)*, **2013**, *225*(4), 853-868. <http://dx.doi.org/10.1007/s00213-012-2871-1>
- [21] Wright, J.M.; Gourdon, J.C.; Clarke, P.B. Identification of multiple call categories within the rich repertoire of adult rat 50-kHz ultrasonic vocalizations: effects of amphetamine and social context. *Psychopharmacology (Berl)*, **2010**, *211*(1), 1-13. <http://dx.doi.org/10.1007/s00213-010-1859-y>
- [22] Schwarting, R.K.W.; Jegan, N.; Wöhr, M. Situational factors, conditions and individual variables which can determine ultrasonic vocalizations in male adult Wistar rats. *Behav. Brain Res.*, **2007**, *182*(2), 208-222. <http://dx.doi.org/10.1016/j.bbr.2007.01.029>
- [23] Wöhr, M.; Houx, B.; Schwarting, R.K.W.; Spruijt, B. Effects of experience and context on 50-kHz vocalizations in rats. *Physiol. Behav.*, **2008**, *93*(4-5), 766-776. <http://dx.doi.org/10.1016/j.physbeh.2007.11.031>
- [24] Burgdorf, J.; Panksepp, J. The neurobiology of positive emotions. *Neurosci. Biobehav. Rev.*, **2006**, *30*(2), 173-187. <http://dx.doi.org/10.1016/j.neubiorev.2005.06.001>
- [25] Burgdorf, J.; Panksepp, J.; Moskal, J.R. Frequency-modulated 50 kHz ultrasonic vocalizations: a tool for uncovering the molecular substrates of positive affect. *Neurosci. Biobehav. Rev.*, **2011**, *35*(9), 1831-1836. <http://dx.doi.org/10.1016/j.neubiorev.2010.11.011>
- [26] Hutson, P.H.; Tarazi, F.I.; Madhoo, M.; Slawewski, C.; Patkar, A.A. Preclinical pharmacology of amphetamine: Implications for the treatment of neuropsychiatric disorders. *Pharmacol. Ther.*, **2014**, *143*(3), 253-264. <http://dx.doi.org/10.1016/j.pharmthera.2014.03.005>
- [27] Natusch, C.; Schwarting, R.K.W. Using bedding in a test environment critically affects 50-kHz ultrasonic vocalizations in laboratory rats. *Pharmacol. Biochem. Behav.*, **2010**, *96*(3), 251-259. <http://dx.doi.org/10.1016/j.pbb.2010.05.013>
- [28] Scardochio, T.; Clarke, P.B. Inhibition of 50-kHz ultrasonic vocalizations by dopamine receptor subtype-selective agonists and antagonists in adult rats. *Psychopharmacology (Berl)*, **2013**, *226*(3), 589-600. <http://dx.doi.org/10.1007/s00213-012-2931-6>
- [29] Wintink, A.J.; Brudzynski, S.M. The related roles of dopamine and glutamate in the initiation of 50-kHz ultrasonic calls in adult rats. *Pharmacol. Biochem. Behav.*, **2001**, *70*(2-3), 317-323. [http://dx.doi.org/10.1016/S0091-3057\(01\)00615-3](http://dx.doi.org/10.1016/S0091-3057(01)00615-3)
- [30] Brudzynski, S.M.; Silkstone, M.; Komadoski, M.; Scullion, K.; Duffus, S.; Burgdorf, J.; Kroes, R.A.; Moskal, J.R.; Panksepp, J. Effects of intraaccumbens amphetamine on production of 50 kHz vocalizations in three lines of selectively bred Long-Evans rats. *Behav. Brain Res.*, **2011b**, *217*(1), 32-40. <http://dx.doi.org/10.1016/j.bbr.2010.10.006>
- [31] Burgdorf, J.; Knutson, B.; Panksepp, J.; Ikemoto, S. Nucleus accumbens amphetamine microinjections unconditionally elicit 50-kHz ultrasonic vocalizations in rats. *Behav. Neurosci.*, **2001a**, *115*(4), 940-944. <http://dx.doi.org/10.1037/0735-7044.115.4.940>
- [32] Thompson, B.; Leonard, K.C.; Brudzynski, S.M. Amphetamine-induced 50 kHz calls from rat nucleus accumbens: a quantitative mapping study and acoustic analysis. *Behav. Brain Res.*, **2006**, *168*(1), 64-73. <http://dx.doi.org/10.1016/j.bbr.2005.10.012>
- [33] Brudzynski, S.M.; Gibson, B.; Silkstone, M.; Burgdorf, J.; Kroes, R.A.; Moskal, J.R.; Panksepp, J. Motor and locomotor responses to systemic amphetamine in three lines of selectively bred Long-Evans rats. *Pharmacol. Biochem. Behav.*, **2011a**, *100*(1), 119-124. <http://dx.doi.org/10.1016/j.pbb.2011.08.006>
- [34] Knutson, B.; Burgdorf, J.; Panksepp, J. High-frequency ultrasonic vocalizations index conditioned pharmacological reward in rats. *Physiol. Behav.*, **1999**, *66*(4), 639-643. [http://dx.doi.org/10.1016/S0031-9384\(98\)00337-0](http://dx.doi.org/10.1016/S0031-9384(98)00337-0)
- [35] Barker, D.J.; Bercovicz, D.; Servilio, L.C.; Simmons, S.J.; Ma, S.; Root, D.H.; Pawlak, A.P.; West, M.O. Rat ultrasonic vocalizations demonstrate that the motivation to contextually reinstate cocaine-seeking behavior does not necessarily involve a hedonic response. *Addict. Biol.*, **2014a**, *19*(5), 781-790. <http://dx.doi.org/10.1111/adb.12044>
- [36] Barker, D.J.; Root, D.H.; Ma, S.; Jha, S.; Megehee, L.; Pawlak, A.P.; West, M.O. Dose-dependent differences in short ultrasonic vocalizations emitted by rats during cocaine self-administration. *Psychopharmacology (Berl)*, **2010**, *211*(4), 435-442. <http://dx.doi.org/10.1007/s00213-010-1913-9>
- [37] Barker, D.J.; Simmons, S.J.; Servilio, L.C.; Bercovicz, D.; Ma, S.; Root, D.H.; Pawlak, A.P.; West, M.O. Ultrasonic vocalizations: evidence for an affective opponent process during cocaine self-administration. *Psychopharmacology (Berl)*, **2014b**, *231*(5), 909-918. <http://dx.doi.org/10.1007/s00213-013-3309-0>
- [38] Browning, J.R.; Browning, D.A.; Maxwell, A.O.; Dong, Y.; Jansen, H.A.T.; Panksepp, J.; Sorg, B.A. Positive affective vocalizations during cocaine and sucrose self-administration: a model for spontaneous drug desire in rats. *Neuropharmacology*, **2011**, *61*(1-2), 268-275. <http://dx.doi.org/10.1016/j.neuropharm.2011.04.012>

- [39] Ma, S.T.; Maier, E.Y.; Ahrens, A.M.; Schallert, T.; Duvauchelle, C.L. Repeated intravenous cocaine experience: development and escalation of pre-drug anticipatory 50-kHz ultrasonic vocalizations in rats. *Behav. Brain Res.*, **2010**, *212*(1), 109-114. <http://dx.doi.org/10.1016/j.bbr.2010.04.001>
- [40] Maier, E.Y.; Abdalla, M.; Ahrens, A.M.; Schallert, T.; Duvauchelle, C.L. The missing variable: ultrasonic vocalizations reveal hidden sensitization and tolerance-like effects during long-term cocaine administration. *Psychopharmacology (Berl)*, **2012**, *219*(4), 1141-1152. <http://dx.doi.org/10.1007/s00213-011-2445-7>
- [41] Maier, E.Y.; Ahrens, A.M.; Ma, S.T.; Schallert, T.; Duvauchelle, C.L. Cocaine deprivation effect: cue abstinence over weekends boosts anticipatory 50-kHz ultrasonic vocalizations in rats. *Behav. Brain Res.*, **2010**, *214*(1), 75-79. <http://dx.doi.org/10.1016/j.bbr.2010.04.057>
- [42] Meyer, P.J.; Ma, S.T.; Robinson, T.E. A cocaine cue is more preferred and evokes more frequency-modulated 50-kHz ultrasonic vocalizations in rats prone to attribute incentive salience to a food cue. *Psychopharmacology (Berl)*, **2012**, *219*(4), 999-1009. <http://dx.doi.org/10.1007/s00213-011-2429-7>
- [43] Mu, P.; Fuchs, T.; Saal, D.B.; Sorg, B.A.; Dong, Y.; Panksepp, J. Repeated cocaine exposure induces sensitization of ultrasonic vocalization in rats. *Neurosci. Lett.*, **2009**, *453*(1), 31-35. <http://dx.doi.org/10.1016/j.neulet.2009.02.007>
- [44] Reno, J.M.; Marker, B.; Cormack, L.K.; Schallert, T.; Duvauchelle, C.L. Automating ultrasonic vocalization analyses: the WAAVES program. *J. Neurosci. Methods*, **2013**, *219*(1), 155-161. <http://dx.doi.org/10.1016/j.jneumeth.2013.06.006>
- [45] Williams, S.N.; Undieh, A.S. Brain-derived neurotrophic factor signaling modulates cocaine induction of reward-associated ultrasonic vocalization in rats. *J. Pharmacol. Exp. Ther.*, **2010**, *332*(2), 463-468. <http://dx.doi.org/10.1124/jpet.109.158535>
- [46] Mahler, S.V.; Moorman, D.E.; Feltenstein, M.W.; Cox, B.M.; Ogburn, K.B.; Bachar, M.; McGonigal, J.T.; Ghee, S.M.; See, R.E. A rodent "self-report" measure of methamphetamine craving? Rat ultrasonic vocalizations during methamphetamine self-administration, extinction, and reinstatement. *Behav. Brain Res.*, **2013**, *236*(1), 78-89. <http://dx.doi.org/10.1016/j.bbr.2012.08.023>
- [47] Sadananda, M.; Natusch, C.; Karrenbauer, B.; Schwarting, R.K.W. 50-kHz calls in rats: effects of MDMA and the 5-HT_{1A} receptor agonist 8-OH-DPAT. *Pharmacol. Biochem. Behav.*, **2012**, *101*(2), 258-264. <http://dx.doi.org/10.1016/j.pbb.2012.01.012>
- [48] Burgdorf, J.; Knutson, B.; Panksepp, J.; Shippenberg, T.S. Evaluation of rat ultrasonic vocalizations as predictors of the conditioned aversive effects of drugs. *Psychopharmacology (Berl)*, **2001b**, *155*(1), 35-42. <http://dx.doi.org/10.1007/s002130100685>
- [49] Hamed, A.; Taracha, E.; Szyndler, J.; Krząścik, P.; Lehner, M.; Maciejak, P.; Skórzewska, A.; Plaźnik, A. The effects of morphine and morphine conditioned context on 50 kHz ultrasonic vocalisation in rats. *Behav. Brain Res.*, **2012**, *229*(2), 447-450. <http://dx.doi.org/10.1016/j.bbr.2012.01.053>
- [50] Wöhr, M.; Schwarting, R.K.W. Ultrasonic communication in rats: effects of morphine and naloxone on vocal and behavioral responses to playback of 50-kHz vocalizations. *Biochem. Behav., Pharmacol.* **2009**, *94*(2), 285-295. <http://dx.doi.org/10.1016/j.pbb.2009.09.008>
- [51] Manduca, A.; Campolongo, P.; Palmery, M.; Vanderschuren, L.J.; Cuomo, V.; Trezza, V. Social play behavior, ultrasonic vocalizations and their modulation by morphine and amphetamine in Wistar and Sprague-Dawley rats. *Psychopharmacology (Berl)*, **2014**, *231*(8), 1661-1673. <http://dx.doi.org/10.1007/s00213-013-3337-9>
- [52] Bischoff, S.; Heinrich, M.; Sonntag, J.M.; Krauss, J. The D-1 dopamine receptor antagonist SCH 23390 also interacts potently with brain serotonin (5-HT₂) receptors. *Eur. J. Pharmacol.*, **1986**, *129*(3), 367-37. [http://dx.doi.org/10.1016/0014-2999\(86\)90449-8](http://dx.doi.org/10.1016/0014-2999(86)90449-8)
- [53] Nicklaus, K.J.; McGonigle, P.; Molinoff, P.B. [³H]SCH 23390 labels both dopamine-1 and 5-hydroxytryptamine_{1c} receptors in the choroid plexus. *J. Pharmacol. Exp. Ther.*, **1998**, *247*(1), 343-348
- [54] Alburges, M.E.; Hunt, M.E.; McQuade, R.D.; Wamsley, J.K. D1-receptor antagonists: comparison of [³H]SCH39166 to [³H]SCH23390. *J. Chem. Neuroanat.*, **1992**, *5*(5), 357-366. [http://dx.doi.org/10.1016/0891-0618\(92\)90051-Q](http://dx.doi.org/10.1016/0891-0618(92)90051-Q)
- [55] McQuade, R.D.; Duffy, R.A.; Coffin, V.L.; Chipkin, R.E.; Barnett, A. *In vivo* binding of SCH 39166: a D-1 selective antagonist. *J. Pharmacol. Exp. Ther.*, **1991**, *257*(1), 42-49.
- [56] Maggio, R.; Aloisi, G.; Silvano, E.; Rossi, M.; Millan, M.J. Heterodimerization of dopamine receptors: new insights into functional and therapeutic significance. *Parkinsonism Relat. Disord.*, **2009**, *15*(S4), 2-7. [http://dx.doi.org/10.1016/S1353-8020\(09\)70826-0](http://dx.doi.org/10.1016/S1353-8020(09)70826-0)
- [57] Doxey, J.C.; Lane, A.C.; Roach, A.G.; Virdee, N.K. Comparison of the alpha-adrenoceptor antagonist profiles of idazoxan (RX 781094), yohimbine, rauwolscine and corynanthine. *Naunyn Schmiedebergs Arch. Pharmacol.*, **1984**, *325*(2), 136-144. <http://dx.doi.org/10.1007/BF00506193>
- [58] Molinari, E.J.; North, P.C.; Dubocovich, M.L. 2-[125I]iodo-5-methoxycarbonylamino-N-acetyltyptamine: a selective radioligand for the characterization of melatonin ML₂ binding sites. *Eur. J. Pharmacol.*, **1996**, *301*(1-3), 159-168. [http://dx.doi.org/10.1016/0014-2999\(95\)00870-5](http://dx.doi.org/10.1016/0014-2999(95)00870-5)
- [59] Pickering, D.S.; Niles, L.P. Pharmacological characterization of melatonin binding sites in Syrian hamster hypothalamus. *Eur. J. Pharmacol.*, **1990**, *175*(1), 71-77. [http://dx.doi.org/10.1016/0014-2999\(95\)00870-5](http://dx.doi.org/10.1016/0014-2999(95)00870-5)
- [60] Drew, G.M.; Gower, A.J.; Marriott AS. Alpha 2-adrenoceptors mediate clonidine-induced sedation in the rat. *Br. J. Pharmacol.*, **1979**, *67*(1), 133-141.
- [61] Anden, N.E.; Corrodi, H.; Fuxe, K.; Hokfelt, B.; Hokfelt, T.; Rydin, C.; Svensson, T. Evidence for a central noradrenaline receptor stimulation by clonidine. *Life Sci.*, **1970**, *9*(9), 513-523. [http://dx.doi.org/10.1016/0024-3205\(70\)90207-9](http://dx.doi.org/10.1016/0024-3205(70)90207-9)
- [62] Sacchetti, G.; Bernini, M.; Gobbi, M.; Parini, S.; Pirona, L.; Mennini, T.; Samanin, R. Chronic treatment with desipramine facilitates its effect on extracellular noradrenaline in the rat hippocampus: studies on the role of presynaptic alpha₂-adrenoceptors. *Naunyn Schmiedebergs Arch. Pharmacol.*, **2001**, *363*(1), 66-72. <http://dx.doi.org/10.1007/s002100000334>
- [63] Middlemiss, D.N.; Tricklebank, M.D. Centrally active 5-HT receptor agonists and antagonists. *Neurosci. Biobehav. Rev.*, **1992**, *16*(1), 75-82. [http://dx.doi.org/10.1016/S0149-7634\(05\)80054-5](http://dx.doi.org/10.1016/S0149-7634(05)80054-5)
- [64] Di Giovanni, G.; Di Matteo, V.; Di Mascio, M.; Esposito, E. Preferential modulation of mesolimbic vs. nigrostriatal dopaminergic function by serotonin(2C/2B) receptor agonists: a combined *in vivo* electrophysiological and microdialysis study. *Synapse*, **2000**, *35*(1), 53-61. [http://dx.doi.org/10.1002/\(SICI\)1098-2396\(200001\)35:1<53::AID-SYN7>3.0.CO;2-2](http://dx.doi.org/10.1002/(SICI)1098-2396(200001)35:1<53::AID-SYN7>3.0.CO;2-2)
- [65] Di Matteo, V.; Di Giovanni, G.; Di Mascio, M.; Esposito, E. Biochemical and electrophysiological evidence that RO 60-0175 inhibits mesolimbic dopaminergic function through serotonin(2C) receptors. *Brain Res.*, **2000**, *865*(1), 85-90. [http://dx.doi.org/10.1016/S0006-8993\(00\)02246-0](http://dx.doi.org/10.1016/S0006-8993(00)02246-0)
- [66] Di Matteo, V.; Cacchio, M.; Di Giulio, C.; Esposito, E. Role of serotonin(2C) receptors in the control of brain dopaminergic function. *Pharmacol. Biochem. Behav.*, **2002**, *71*(4), 727-734. [http://dx.doi.org/10.1016/S0091-3057\(01\)00705-5](http://dx.doi.org/10.1016/S0091-3057(01)00705-5)
- [67] Smith, R.; Daves, J.M. Comparative effect of d-amphetamine, l-amphetamine and methylphenidate on mood in man. *Psychopharmacology (Berl)*, **1977**, *53*(1), 1-12. <http://dx.doi.org/10.1007/BF00426687>
- [68] Willson, M.C.; Bell, E.C.; Dave, S.; Asghar, S.J.; McGrath, B.M.; Silverstone, P.H. Valproate attenuates dextroamphetamine-induced subjective changes more than lithium. *Eur. Neuropsychopharmacol.*, **2005**, *15*(6), 633-639. <http://dx.doi.org/10.1016/j.euroneuro.2005.04.015>
- [69] Harrison-Read, P.E. Models of mania and antimanic drug actions: progressing the endophenotype approach. *J. Psychopharmacol. (Oxf)*, **2009**, *23*(3), 334-337. <http://dx.doi.org/10.1177/0269881108089840>
- [70] Young, J.W.; Henry, B.L.; Geyer, M.A. Predictive animal models of mania: hits, misses and future directions. *Br. J. Pharmacol.*, **2011**, *164*(4), 1263-1284. <http://dx.doi.org/10.1111/j.1476-5381.2011.01318.x>
- [71] Wöhr, M.; Kehl, M.; Borta, A.; Schänzer, A.; Schwarting, R.K.W.; Höglinger, G.U. New insights into the relationship of neurogenesis and affect: Tickling induces hippocampal cell proliferation in rats emitting appetitive 50-kHz ultrasonic vocalizations. *Neuroscience*, **2009**, *163*(4), 1024-1030. <http://dx.doi.org/10.1016/j.neuroscience.2009.07.043>

- [72] Oruch, R.; Elderbi, M.A.; Khattab, H.A.; Pryme, I.F.; Lund, A. Lithium: A review of pharmacology, clinical uses, and toxicity. *Eur. J. Pharmacol.*, **2014**, *740*(1), 464-473. <http://dx.doi.org/10.1016/j.ejphar.2014.06.042>
- [73] Can, A.; Schulze, T.G.; Gould, T.D. Molecular actions and clinical pharmacogenetics of lithium therapy. *Pharmacol. Biochem. Behav.*, **2014**, *123*(1), 3-16. <http://dx.doi.org/10.1016/j.pbb.2014.02.004>
- [74] Hahn, C.G.; Umapathy, H.Y.; Wang, R.; Koneru, D.F.; Friedman, L.E. Lithium and valproic acid treatments reduce PKC activation and receptor-G protein coupling in platelets of bipolar manic patients. *J. Psychiatr. Res.*, **2005**, *39*(4), 355-363. <http://dx.doi.org/10.1016/j.jpsychires.2004.10.007>
- [75] Manji, H.K.; Lenox, R.H. Protein kinase C signaling in the brain: molecular transduction of mood stabilization in the treatment of manic-depressive illness. *Biol. Psychiatry*, **1999**, *46*(10), 1328-1351. [http://dx.doi.org/10.1016/S0006-3223\(99\)00235-8](http://dx.doi.org/10.1016/S0006-3223(99)00235-8)
- [76] Boland, F.J.; Stern, M.H. Suppression by lithium of voluntary alcohol intake in the rat: mechanism of action. *Pharmacol. Biochem. Behav.*, **1980**, *12*(2), 239-248. [http://dx.doi.org/10.1016/0091-3057\(80\)90363-9](http://dx.doi.org/10.1016/0091-3057(80)90363-9)
- [77] Meotti, F.C.; Luiz, A.P.; Pizzollati, M.G.; Kassuya, C.A.L.; Calixto, J.B.; Santos, A.R.S. Analysis of the antinociceptive effect of the flavonoid myricitrin: evidence for a role of the l-arginine-nitric oxide and protein kinase C pathways. *J. Pharmacol. Exp. Ther.*, **2006**, *316*(2), 789-796. <http://dx.doi.org/10.1124/jpet.105.092825>
- [78] Pereira, M.; Siba, I.P.; Chioca, L.R.; Correia, D.; Vital, M.A.; Pizzollati, M.G.; Santos, A.R.; Andreatini, R. Myricitrin, a nitric oxide and protein kinase C inhibitor, exerts antipsychotic-like effects in animal models. *Prog. Neuropsychopharmacol. Biol. Psych.*, **2011**, *35*(7), 1636-1644. <http://dx.doi.org/10.1016/j.pnpbp.2011.06.002>
- [79] Fu, X.W.; Brudzynski, S.M. High-frequency ultrasonic vocalization induced by intracerebral glutamate in rats. *Pharmacol. Biochem. Behav.*, **1994**, *49*(4), 835-841. [http://dx.doi.org/10.1016/0091-3057\(94\)90231-3](http://dx.doi.org/10.1016/0091-3057(94)90231-3)
- [80] Brudzynski, S.M. Ultrasonic vocalization induced by intracerebral carbachol in rats: localization and a dose-response study. *Behav. Brain Res.*, **1994**, *63*(2), 133-143. [http://dx.doi.org/10.1016/0166-4328\(94\)90084-1](http://dx.doi.org/10.1016/0166-4328(94)90084-1)
- [81] Willuhn, I.; Tose, A.; Wanat, M.J.; Hart, A.S.; Hollon, N.G.; Phillips, P.E.M.; Swarting, R.K.W.; Wöhr, M. Phasic dopamine release in the nucleus accumbens in response to pro-social 50-kHz ultrasonic vocalizations in rats. *J. Neurosci.*, **2014**, *34*(32), 10616-10623. <http://dx.doi.org/10.1523/JNEUROSCI.1060-14.2014>
- [82] Wöhr, M.; Swarting, R.K.W. Ultrasonic communication in rats: can playback of 50-kHz calls induce approach behavior? *PLoS One*, **2007**, *2*(12), e1365. <http://dx.doi.org/10.1371/journal.pone.0001365>
- [83] Wöhr, M.; Swarting, R.K.W. Testing social acoustic memory in rats: effects of stimulus configuration and long-term memory on the induction of social approach behavior by appetitive 50-kHz ultrasonic vocalizations. *Neurobiol. Learn. Mem.*, **2012**, *98*(2), 154-164. <http://dx.doi.org/10.1016/j.nlm.2012.05.004>
- [84] Ciucci, M.R.; Ma, S.T.; Fox, C.; Kane, J.R.; Ramig, L.O.; Schallert, T. Qualitative changes in ultrasonic vocalization in rats after unilateral dopamine depletion or haloperidol: a preliminary study. *Behav. Brain Res.*, **2007**, *182*(2), 284-289. <http://dx.doi.org/10.1016/j.nlm.2012.05.004>
- [85] Ciucci, M.R.; Ahrens, A.M.; Ma, S.T.; Kane, J.R.; Windham, E.B.; Woodlee, M.T.; Schallert, T. Reduction of dopamine synaptic activity: degradation of 50-kHz ultrasonic vocalization in rats. *Behav. Neurosci.*, **2009**, *123*(2), 328-336. <http://dx.doi.org/10.1037/a0014593>
- [86] Ringel, L.E.; Basken, J.N.; Grant, L.M.; Ciucci, M.R. Dopamine D1 and D2 receptor antagonism effects on rat ultrasonic vocalizations. *Behav. Brain Res.*, **2013**, *252*(1), 252-259. <http://dx.doi.org/10.1016/j.bbr.2013.06.006>
- [87] Hori, M.; Shimoju, R.; Tokunaga, R.; Ohkubo, M.; Miyabe, S.; Ohnishi, J.; Murakami, K.; Kurosawa, M. Tickling increases dopamine release in the nucleus accumbens and 50 kHz ultrasonic vocalizations in adolescent rats. *Neuroreport*, **2013**, *24*(5), 241-245. <http://dx.doi.org/10.1097/WNR.0b013e32835edbf8>
- [88] Tornatzky, W.; Miczek, K.A. Behavioral and autonomic responses to intermittent social stress: differential protection by clonidine and metoprolol. *Psychopharmacology (Berl)*, **1994**, *116*(3), 346-356. <http://dx.doi.org/10.1007/BF02245339>
- [89] Brudzynski, S.M.; Komadoski, M.; St Pierre, J. Quinpirole-induced 50 kHz ultrasonic vocalization in the rat: role of D2 and D3 dopamine receptors. *Behav. Brain Res.*, **2012**, *226*(2), 511-518. <http://dx.doi.org/10.1016/j.bbr.2011.10.004>
- [90] Desai, R.I.; Paronis, C.A.; Martin, J.; Desai, R.; Bergman, J. Monoaminergic psychomotor stimulants: discriminative stimulus effects and dopamine efflux. *J. Pharmacol. Exp. Ther.*, **2010**, *333*(3), 834-843. <http://dx.doi.org/10.1124/jpet.110.165746>
- [91] Matthews, M.; Bondi, C.; Torres, G.; Moghaddam, B. Reduced presynaptic dopamine activity in adolescent dorsal striatum. *Neuropsychopharmacology*, **2013**, *38*(7), 1344-1351. <http://dx.doi.org/10.1038/npp.2013.32>