Acute and long-term effects elicited by psychoactive drugs on 50-kHz ultrasonic vocalizations in rats: development of a new experimental tool for the study of drug-mediated reward

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Abstract: - Ultrasonic vocalizations (USVs) have recently emerged as an indicator of the emotional state of rats, and the evaluation of the USVs in the 50-kHz range has been proposed as a tool to investigate the affective properties of drugs of abuse. To clarify the relevance of 50-kHz USVs to drug-induced reward, the acute and long-term effects elicited by different psychoactive drugs [amphetamine, 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), methylphenidate, morphine, and nicotine] were characterized in adult male rats. Amphetamine and methylphenidate were the only drugs that stimulated the emission of 50-kHz USVs by rats after their acute administration. Moreover, amphetamine was the only drug that elicited a significant emission of 50-kHz USVs after repeated administration. However, rats in all the treatment groups emitted 50-kHz USVs when later re-exposed to the environment previously paired with repeated drug administration, likely indicative of drug-mediated environmental conditioning. Taken together, these results demonstrate the existence of major differences in the acute and long-term effects of different psychoactive drugs on the emission of 50-kHz USVs by rats. Moreover, these results provide a better understanding of the usefulness of 50-kHz USVs as a new tool for the assessment of drug-mediated reward, with implications for the preclinical study of addictive behaviors.

Key-Words: - Amphetamine, animal model, caffeine, MDMA, methylphenidate, morphine, nicotine, reward

1 Introduction

Several lines of recent evidence have consistently demonstrated that rats emit ultrasonic vocalizations (USVs) when exposed to either appetitive or aversive stimuli. Based on this, USVs are now regarded as a new experimental tool for the study the rats' emotional state [1-4]. Rats generally emit USVs of different frequencies depending on the nature of the stimuli they are exposed to [5,6]. In particular, appetitive stimuli (e.g. social encounters, mating, but also administration of certain drugs) and/or their anticipation selectively elicit the so-called 50-kHz USVs (Fig. 1). Therefore, 50-kHz USVs, besides being regarded as an indicator of the rats' emotional state, have been proposed as a tool for the study of drug-mediated reward [7-10].

Previous studies have shown that both amphetamine and cocaine significantly stimulate the emission of 50-kHz USVs in rats after their acute and repeated administration [9-12]. Conversely, drugs like caffeine and morphine, have been shown not to significantly influence the emission of 50-kHz USVs by rats [13-14]. However, very little is known about the acute and long-lasting effects of drugs other than those mentioned above on the modulation of 50-kHz USVs, and there is the need for a thorough investigation of this issue.

This study evaluated the effects elicited by different drugs with rewarding properties on the emission of 50-kHz USVs, in an attempt to verify the hypothesis that envisions 50-kHz USVs as a straightforward indicator of drug-induced reward. Moreover, the emission of 50-kHz USVs elicited by the re-exposure to an environment previously paired with drug administration was assessed. This procedure was followed to ascertain whether 50-kHz USVs may be useful for the study of context

ISBN: 978-960-474-358-2

conditioning, which is thought to be an important component in drug addiction [13].

Figure 1. Example of 50-kHz ultrasonic vocalizations

2 Problem Formulation

To develop a behavioral model that can be used for studying the effects of drugs bearing rewarding properties and addictive potential. The model should be suited for the evaluation of both the acute and long-lasting effects of drugs, also encompassing the emergence of context conditioning.

3 Problem Solution

Adult male Sprague-Dawley rats were treated either acutely or repeatedly with drugs bearing rewarding properties and addictive potential, and 50-kHz USVs were recorded thereafter. An outline of the experimental plan is reported in Fig. 2. Each animal tested received only a single dose of a given drug, and was no re-utilized for other experiments.

A first cohort of rats received the acute administration of either vehicle or one of the following drugs: amphetamine (2 mg/kg, i.p.), 3,4-methylenedioxymethamphetamine (MDMA, ecstasy, 5, 10, 15 mg/kg, i.p.), methylphenidate (2.5, 5, 10 mg/kg, i.p.), morphine (1, 2.5, 5 mg/kg, s.c.), nicotine (0.1, 0.2, 0.4 mg/kg, s.c.). A second cohort of rats received the repeated administration of either vehicle, or one of the following drugs: amphetamine (2 mg/kg, i.p.), MDMA (7.5 mg/kg, i.p.), morphine (7.5, mg/kg s.c.), nicotine (0.4 mg/kg, s.c.). Drug or vehicle administration was performed in an environment (test cage) different from the cage where the rats lived, to promote the onset of drug-induced context conditioning. To avoid the possible emergence of pharmacological tolerance, drugs were given in spaced administration, as reported elsewhere [16]. After 7 days from the end of repeated drug treatment, rats were first re-exposed to the test cage, to evaluate the emission of 50-kHz USVs in response to an environmental cue previously paired with the drug, and then challenged with the drug previously administered, to ascertain the presence of a possible long-lasting sensitization in the emission of 50-kHz USVs stimulated by the drugs evaluated.

For USVs recording, each rat was individually placed in a Plexiglas cylinder (diameter, 25 cm; height, 30 cm) enclosed by four cardboard walls. The cylinder was covered with a lid equipped with an ultrasonic microphone bearing high directional properties (CM16/CMPA, Avisoft, Berlin, Germany), and connected to an ultrasound recording device (UltraSoundGate 116Hb, Avisoft, Berlin, Germany). USVs stimulated by either drug or vehicle were recorded for 1 h, whereas USVs elicited on re-exposure to the drug-paired environment were recorded for 10 min.

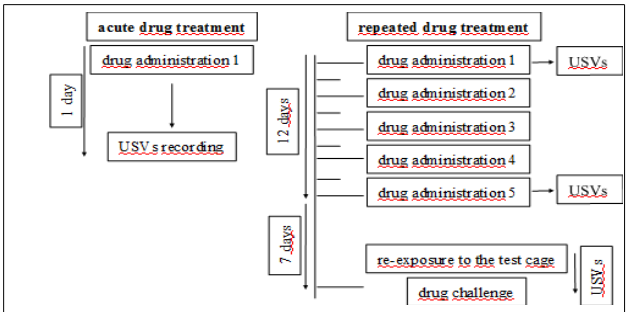

Figure 2 Outline of drug treatment. Rats received either acute or repeated drug treatment as described in the text. Repeated drug treatment was performed on alternate days (every other day)

4 Conclusion

The results obtained in this study showed that amphetamine and methylphenidate were the only drugs that significantly stimulated the emission of 50-kHz USVs by rats following their acute administration. Conversely, MDMA,

ISBN: 978-960-474-358-2

morphine, and nicotine did not elevate the number of 50-kHz USVs emitted, compared with the administration of vehicle (Table 1). As for repeated drug treatment, amphetamine again was the only drug that elicited a significant emission of 50-kHz USVs after its repeated administration and challenge. Nevertheless, all rats that underwent repeated drug treatment, except those that received nicotine, showed a more marked emission of 50-kHz USVs than vehicle-treated rats, when re-exposure to the drug-paired environment (Table 2).

A major mechanism that could explain the different effects of psychoactive drugs on 50-kHz USVs observed in this study may involve dopamine transmission. Thus, it has been consistently demonstrated that stimulation of dopamine transmission in the shell of the nucleus accumbens, an area crucial in drug-mediated reward [13], is associated with the robust emission of 50-kHz USVs [9,12,16,17]. Interestingly, all the drugs evaluated in this study can stimulate dopamine transmission in the shell of the nucleus accumbens, but do this through different mechanisms. Thus, amphetamine and methylphenidate act by directly facilitating dopamine transmission [18]. Conversely, MDMA, morphine, and nicotine modulate dopamine in a more complex fashion, which may involve an amplification by means of indirect mechanism [19]. The different modulation of dopamine transmission could therefore explain the different effects on 50-kHz USVs elicited by the drugs evaluated in this study, even though alternative mechanism cannot be ruled out.

Another interesting finding of the present study is that rats previously treated with a drug that does not directly stimulate 50-kHz USVs exhibit vocalizations when re-exposed to an environment previously paired with the drug (test cage). Thus, this effect was observed not only in rats treated with amphetamine, that did vocalize in response to the drug, but also in rats that received MDMA or morphine, that did not. A possible explanation of this result could rely in the onset of environmental conditioning [13] following repeated drug administration in a non-familiar environment. In this regard, it is noteworthy that morphine-treated rats showed a most pronounced emission of 50-kHz USVs upon re-exposure to the test cage. This result appears in agreement with the marked ability of opiates to elicit persistent conditioned responses [20].

treatment	number of 50-kHz USVs emitted
VEH	21.1±5.6
AMPH (2 mg/kg, i.p.)	1260.6±245.7*
MPH (2.5 mg/kg, i.p.)	76.8±43.1
MPH (5 mg/kg, i.p.)	203.6±145.8
MPH (10 mg/kg, i.p.)	768.9±234.6*
MDMA (5 mg/kg, i.p.)	12.5±5.6
MDMA (10 mg/kg, i.p.)	26.5±8-9
MDMA (15 mg/kg, i.p.)	18.8±8
MOR (1 mg/kg, s.c.)	16.5±7.8
MOR (2.5 mg/kg, s.c.)	12.5±7.4
MOR (5 mg/kg, s.c.)	5.7±4.6
NIC (0.1 mg/kg, s.c.)	5.5±3.6
NIC (0.2 mg/kg, s.c.)	5.1±4.1
NIC (0.2 mg/kg, s.c.)	12.7±3.6

Table 1 Emission of 50-kHz USVs after acute drug administration. AMPH = amphetamine; MPH = methilphenidate; MOR = morphine; NIC = nicotine. * $p < 0.05$ compared with rats that received vehicle administration. Sample size: N = 5-8.

treatment	USVs 1	USVs 5	USVs re	USVs c
VEH	9.6±3.3	32.5 ± 17.4	13.6±6.9	13.1±8.8
AMPH	914.8 ± 333*	607.5 ± 131.7*	143.8 ± 68.1*	1121 ± 242.9*
MDMA	31±8.4	44±14	72.7±24.7*	78.7±40
MOR	3.1±1.8	42±14.7	135 ± 38.3*	18±6.9
NIC	6.3±1.9	31.7±11.4	48±15.9	25.3±13.2

Table 2 Emission of 50-kHz USVs after repeated drug administration, re-exposure to the drug-paired environment, and drug challenge. AMPH = amphetamine; MOR = morphine; NIC = nicotine; USVs 1 = USVs emitted on the first drug administration; USVs 5 = USVs emitted on the fifth (last) drug administration; USVs re = USVs emitted on re-exposure to the drug-paired environment; USVs c = USVs emitted on drug challenge. * $p < 0.05$ compared with rats that received vehicle administration. Sample size: N = 5-8

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To summarize, the present study provides a thorough evaluation of the acute an long-lasting effects of different drugs bearing rewarding properties and abuse liability on the emission of 50-kHz USVs in rats. The major finding of the present study is that psychoactive drugs differ in their acute and long-lasting effects on 50-kHz USVs. Thus, some drugs (in particular dopaminergic psychostimulants) powerfully stimulate the emission of 50-kHz right after their acute administration, whilst other drugs fail to do so. However, this study also observed that a previous treatment with a drug that does not directly elevate 50-kHz USVs (e.g. morphine and MDMA) does nevertheless result in rats' vocalization upon re-exposure to an environmental stimulus previously paired with the drug. Taken together, the results obtained suggest that 50-kHz USVs can be a new experimental tool that can be useful for the evaluation of the long-lasting effects and conditioning properties of psychoactive drugs, rather than drug-mediated acute effects.

References:

1] Brudzynski SM, Ultrasonic calls of rats as indicator variables of negative or positive states: acetylcholine–dopamine interaction and acoustic coding *Behav. Brain Res*, Vol. 182, No. 2, 2007, pp. 261–273

2] Knutson B, Burgdorf J, Panksepp J, Ultrasonic vocalizations as indices of affective states in rats, *Psychol. Bull*, Vol. 128, No. 6, 2002, pp. 961–977

3] Panksepp J, Psychology. Beyond a joke: from animal laughter to human joy? *Science*, Vol. 308, No. 5718, 2005, pp. 62–63

4] Schwarting RK, Jegan N, Wöhr M, Situational factors, conditions and individual variables which can determine ultrasonic vocalizations in male adult Wistar rats, *Behav Brain Res*, Vol. 182, No. 2, 2007, pp., pp. 208–222

5] Burgdorf J, Knutson B, Panksepp J, Anticipation of rewarding electrical brain stimulation evokes ultrasonic vocalization in rats, *Behav. Neurosci*, Vol. 114, No. 2, 2000, pp. 320–327

6] Knutson B, Burgdorf J, Panksepp J, High-frequency ultrasonic vocalizations index conditioned pharmacological reward in rats, *Physiol. Behav*, Vol. 66, No. 4, 1999, pp. 639–643

7] Ahrens AM, Ma ST, Maier EY, Duvauchelle CL, Schallert T, Repeated intravenous amphetamine exposure: rapid and persistent sensitization of 50-kHz ultrasonic trill calls in rats, *Behav Brain Res*, Vol. 197, No. 1, 2009, pp. 205–209

8] Mu P, Fuchs T, Saal DB, Sorg BA, Dong Y, Panksepp J, Repeated cocaine exposure induces sensitization of ultrasonic vocalization in rats, *Neurosci Lett*, Vol. 453, No. 1, 2009, pp. 31–35

9] Thompson B, Leonard KC, Brudzynski SM, Amphetamine-induced 50 kHz calls from rat nucleus accumbens: a quantitative mapping study and acoustic analysis, *Behav Brain Res*, Vol. 168, No. 1, 2006, pp. 64–73

10] Williams SN, Undieh AS, Brain-derived neurotrophic factor signaling modulates cocaine induction of reward-associated ultrasonic vocalization in rats, *J Pharmacol Exp Ther*, Vol. 332, No. 2, 2010, pp. 463–468

11] Simola N, Ma ST, 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*, Vol. 13, No. 1, 2010, pp. 123–132

12] Wintink AJ, Brudzynski SM, The related roles of dopamine and glutamate in the initiation of 50-kHz ultrasonic calls in adult rats, *Pharmacol Biochem Behav*, Vol. 70, No. 2-3, 2001, pp. 317–323

13] Wöhr M, Schwarting RK, Ultrasonic communication in rats: effects of morphine and naloxone on vocal and behavioral responses to playback of 50-kHz vocalizations, *Pharmacol Biochem Behav*, Vol. 94, No. 2, 2009, pp. 285–295

14] Di Chiara G, Drug addiction as dopamine-dependent associative learning disorder, *Eur J Pharmacol*, Vol. 375, No. 1-3, 1999, pp.13-30

15] Simola N, Cauli O, Morelli M, Sensitization to caffeine and cross-sensitization to amphetamine: influence of individual response to caffeine, *Behav Brain Res*, Vol. 172, No. 1, 2006, pp.72-79

16] Burgdorf J, Knutson B, Panksepp J, Ikemoto S, Nucleus accumbens amphetamine microinjections unconditionally elicit 50-kHz ultrasonic vocalizations in rats, *Behav Neurosci,* Vol. 115, No. 4, 2001, pp. 940-944

17] Burgdorf J, Wood PL, Kroes RA, Moskal JR, Panksepp J, Neurobiology of 50-kHz ultrasonic vocalizations in rats: electrode mapping, lesion, and pharmacology studies, *Behav Brain Res*, Vol. 182, No. 2, 2007, pp.274-283

18] Heal DJ, Cheetham SC, Smith SL, The neuropharmacology of ADHD drugs in vivo: insights on efficacy and safety, *Neuropharmacology*, Vol. 57, No. 7-8, 2009, pp. 608-618

19] Zhu J, Reith ME, Role of the dopamine transporter in the action of psychostimulants, nicotine, and other drugs of abuse, *CNS Neurol Disord Drug Targets*, Vol. 7, No. 5, 2008, pp.393-409

20] Nielsen DA, Kreek MJ, Common and specific liability to addiction: approaches to association studies of opioid addiction, *Drug Alcohol Depend,* 2012, Vol. 123, No. Suppl 1, 2012, pp.S33-S41