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Research report

Emission of categorized 50-kHz ultrasonic vocalizations in rats repeatedly treated with amphetamine or apomorphine: Possible relevance to drug-induced modifications in the emotional state

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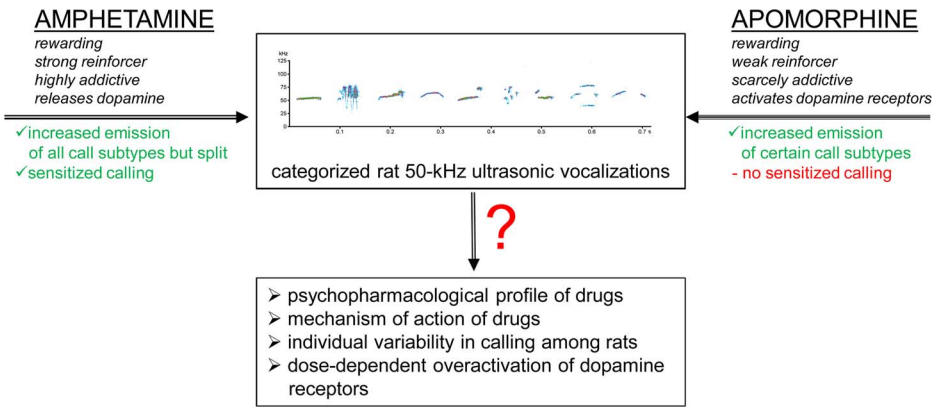
GRAPHICAL ABSTRACT



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ABSTRACT

The emission of 50-kHz ultrasonic vocalizations (USVs) is increasingly emerging as a potential behavioral marker of the subjective effects that psychoactive drugs elicit in rats. However, multiple categories of 50-kHz USVs have been identified, which are thought to possess different behavioral significance. Besides, limited information is available on how psychoactive drugs affect the emission of categorized 50-kHz USVs. To further elucidate this issue, we evaluated the numbers of multiple categories of 50-kHz USVs emitted by rats repeatedly treated with amphetamine (1 or 2 mg/kg, i.p.) or apomorphine (2 or 4 mg/kg, i.p.), two drugs that elicit similar and dissimilar subjective effects. Amphetamine- and apomorphine-treated rats emitted patterns of categorized 50-kHz USVs that varied according to the drug administered, drug dose, and number of drug administrations. Nevertheless, the numbers of several categories of 50-kHz USVs were positively correlated with the number of total calls emitted (i.e., the sum of categorized 50-kHz USVs). Moreover, a marked interindividual variability in the emission of categorized 50-kHz USVs was observed. Taken together, the present results may be relevant to further elucidating the interplay between calling of the 50-kHz USVs group and psychopharmacological profile of drugs.

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1. Introduction

The so-called 50-kHz ultrasonic vocalizations (USVs) are a major means of communication that rats use to maintain the structure of the colony and to communicate positive emotional states to conspecifics [1–4]. USVs of 50-kHz are classically subdivided in the two subfamilies of "flat" and "frequency modulated" (FM) calls, based on the modulation of sound frequency within individual USVs. Flat calls have a sound frequency that remains nearly unchanged [5,6], whereas FM calls have a sound frequency that varies substantially [7]. Besides, FM calls have been further categorized into 13 subtypes that differ in their average sound frequency [8]. It has been hypothesized that flat and FM 50-kHz USVs have dissimilar behavioral significance [3,6,7]. Thus, flat calls are considered to serve mainly a social coordinating function, whereas FM calls are thought to communicate positive emotional states. In this regard, it is noteworthy that rats emit high numbers of the so-called "trill" subtype of FM 50-kHz USVs in situations that are socially rewarding. Accordingly, it has been speculated that trill calls could selectively mark positive emotional states [3,6,7].

The emission of 50-kHz USVs has also been consistently demonstrated in rats treated with psychoactive drugs. This finding has suggested that calling of the 50-kHz USVs group may communicate the positive affect induced by drug administration [9–12]. However, contrasting results have been reported in this respect [13]. Thus, an earlier investigation found that the repeated administration of a moderate dose of amphetamine induced a sensitized emission of trill 50-kHz USVs but not of flat calls [9]. However, sensitization in trill calls was not observed by a subsequent study that used a higher dose of amphetamine [14]. Besides, other investigations have found that rats treated with psychoactive drugs emitted multiple categories of 50-kHz USVs. Thus, the acute administration of amphetamine stimulated the emission of flat calls and of FM call subtypes also other than trills [8,15]. Moreover, rats emitted flat, "non-trill" FM calls, and very few trill FM calls during the self-administration of methamphetamine [16]. Finally, administration of 3,4-methylenedioxymethamphetamine (MDMA), methylphenidate, morphine, and nicotine modified the emission of flat and/or non-trill FM calls [15,17,18]. Taken together, these findings do not allow to unambiguously define the behavioral significance of the categorized 50-kHz USVs that are emitted by rats treated with psychoactive drugs.

The present study was performed to further elucidate how psychoactive drugs affect the emission of categorized 50-kHz USVs. To this end, we measured the numbers of flat calls and FM call subtypes emitted by rats treated with amphetamine or apomorphine. Either drug was administered according to a protocol that allowed to evaluate calling after acute and repeated drug administration as well as calling emitted on re-exposure to the drug-paired environment, which may reflect drug conditioning [19–21]. Besides, we evaluated the correlation between the numbers of categorized and total 50-kHz USVs (i.e., the sum of all call categories), since psychoactive drugs have been found to modify the total number of 50-kHz USVs emitted by rats [10,19]. Amphetamine and apomorphine were evaluated because those substances induce similar and dissimilar effects on the emotional state of rats, as well as on the emission of total 50-kHz USVs [19], Table 1. In fact, both amphetamine and apomorphine possess rewarding and reinforcing properties. However amphetamine is a strong reinforcer with high abuse potential, whereas apomorphine is a weak reinforcer with scarce abuse potential [22–25]. Moreover, repeated amphetamine administration induces a sensitized increase in the total number of 50-kHz USVs that are emitted, whereas repeated apomorphine administration does not [19]. On this basis, we hypothesized that characterizing the effects of amphetamine and apomorphine on the emission of categorized 50-kHz USVs could be relevant to further elucidating the interplay between calling of the 50-kHz USVs group and psychopharmacological profile of drugs.

2. Materials and methods

2.1. Subjects

The categorized 50-kHz USVs analyzed in the present study were collected from rats used in a previous investigation by our group that evaluated the effects of amphetamine and apomorphine on the total number of 50-kHz USVs emitted [19]. A total of 50 male Sprague–Dawley rats (Harlan, Italy) weighing 275–300 g were included in the present study. Rats were housed in groups of four or five in standard polycarbonate cages with sawdust bedding and maintained on a 12-h light/dark cycle (lights on at 08:00 h). Standard laboratory chow and tap water were freely available except during the experiments, which were performed between 10:00 and 16:00 h. All experiments were conducted in accordance with the guidelines for animal experimentation of the EU directives (2010/63/EU; L.276; 22/09/2010), and with the guidelines approved by the Ethical Committee of the University of Cagliari. Each experimental group included 10 rats.

2.2. Drugs

D-Amphetamine (sulfate) and apomorphine (hydrochloride) were purchased from Sigma–Aldrich (Milan, Italy). Drugs were dissolved in distilled water and administered intraperitoneally (i.p.) in a volume of 3 ml/kg.

2.3. Experimental plan

Rats were handled daily (5 min) for 2 days before experiments. Experiments were structured in five phases: 1) habituation to the test cage (15 min), twice a day × 2 days; 2) acute administration of vehicle (distilled water) in the test cage, to evaluate basal calling; 3) repeated drug administration (×5) in the test cage on alternate days; 4) drug withdrawal (7 days) in the home cage; 5) re-exposure to the test cage (10 min), to evaluate calling that may reflect conditioning to the drug-paired environment [19–21], immediately followed by drug challenge, to evaluate enduring drug effects on calling. Different groups of rats received a single dose of either amphetamine (1 or 2 mg/kg, i.p.) or apomorphine (2 or 4 mg/kg, i.p.). Vehicle-treated rats received distilled water and served as controls. Fig. 1 reports the experimental plan. For further details please refer to [19].

Table 1
Total numbers of 50-kHz ultrasonic vocalizations emitted by rats treated with amphetamine, apomorphine, or vehicle. § indicates $p < 0.05$ vs. vehicle. * indicates $p < 0.05$ vs. first administration within each group. N = 10 rats for each group. Drug doses are expressed in mg/kg (i.p.). ADM = administration; AMPH = amphetamine; APO = apomorphine; CHALL = challenge; RE-EX = re-exposure to the test cage in drug-free conditions; VEH = vehicle.

treatment	ADM 1	ADM 5	CHALL	RE-EX
AMPH 1	230.60 ± 71§	769 ± 158.40§*	1315.4 ± 227.59§*	97.70 ± 29.28§
AMPH 2	464.30 ± 90.86§	699.3 ± 151.42§	878.1 ± 202.41§	84 ± 16.82§
APO 2	278.7 ± 86.51§	133.8 ± 58.82	193.4 ± 68.52§	28 ± 15.34
APO 4	302.9 ± 58.84§	556.6 ± 232.04§	218.9 ± 102.80§	11.8 ± 4.52
VEH	36.4 ± 13.03	49.3 ± 13.39	14.9 ± 4	35 ± 10.32