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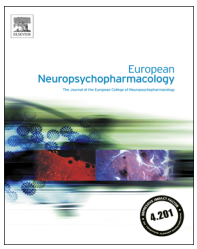

Repeated amphetamine administration and long-term effects on 50-kHz ultrasonic vocalizations: Possible relevance to the motivational and dopamine-stimulating properties of the drug

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Abstract
Ultrasonic vocalizations (USVs) of 50 kHz are thought to indicate positive affective states in rats, and are increasingly being used to investigate the motivational properties of drugs of abuse. However, previous studies have observed that only dopaminergic psychostimulants of abuse, but not other addictive drugs, stimulate 50-kHz USVs immediately after their administration. This would suggest that 50-kHz USVs induced by addictive dopaminergic psychostimulants might reflect rewarding dopaminergic effect, rather than motivational effect. To elucidate this issue, our study compared the effects of the psychostimulant of abuse amphetamine and the dopamine receptor agonist apomorphine on 50-kHz USVs. Rats that received five drug administrations on alternate days in a novel test-cage were first re-exposed to the test-cage 7 days after treatment discontinuation to assess drug-conditioning, and then received a drug challenge. USVs were recorded throughout the experiments together with locomotor activity. To further clarify how amphetamine and apomorphine influenced 50-kHz USVs, rats were subdivided into "low" and "high" vocalizers, and time-dependence of drug effects was assessed. Amphetamine and apomorphine stimulated both 50-kHz USVs and locomotor activity, though they elicited dissimilar changes in these behaviors, depending on drug dose, rats' individual predisposition to vocalize, and time. Moreover, only amphetamine-treated rats displayed both sensitized 50-kHz USVs emission and conditioned vocalizations on test-cage re-exposure. These results indicate that the effects of amphetamine on 50-kHz USVs

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are not mimicked by a dopaminergic agonist with a low abuse potential, and may further support the usefulness of 50-kHz USVs in the study of the motivational properties of psychoactive drugs.

1. Introduction

Several studies suggest that the emission of ultrasonic vocalizations (USVs) may reflect changes in the emotional state of rats (Brudzynski, 2007, 2013; Panksepp, 2005; Schwarting et al., 2007). Therefore, USVs have recently emerged as a tool for investigating both euphoric and dysphoric states, and the stimuli that produce these states. In particular, 50-kHz USVs, contained within the 35–80 kHz frequency range (Brudzynski, 2013), are thought to indicate positive affective states, since rats emit them in response to, or anticipation of, pleasurable stimuli, including mating, heterospecific play ("tickling" by a human hand), and non-aggressive social encounters (Brudzynski, 2005; Burgdorf et al., 2008, 2011). Moreover, 50-kHz USVs are induced by certain drugs of abuse (Mu et al., 2009; Simola et al., 2012; Wintink and Brudzynski, 2001); hence they are increasingly being used to study the motivational properties of drugs (Ahrens et al., 2009; Barker et al., 2014; Hamed et al., 2012; Ma et al., 2010; Mahler et al., 2013).

While amphetamine and dopaminergic psychostimulants of abuse elicit 50-kHz USVs immediately after their administration (Mu et al., 2009; Simola et al., 2012; Wintink and Brudzynski, 2001), other drugs, such as 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"), caffeine, morphine, and nicotine do not (Hamed et al., 2012; Sadananda et al., 2012; Simola et al., 2010, 2014). On one hand, this could stem from differences in drugs' mechanisms of action but, on the other it might partially question the relevance of 50-kHz USVs in the study of all the motivational properties of drugs. Notably, while in some instances the 50-kHz USVs emission parallels other behavioral measures of drug-induced reward and reinforcement, in others it does not. A simultaneous sensitization in 50-kHz USVs and locomotor activity, the latter being regarded as an index of progressive increase in the motivational properties of drugs of abuse with repeated experience (Robinson and Berridge, 2001), has been observed in cocaine-treated rats (Mu et al., 2009). Conversely, others found that doses of morphine that increase locomotor activity and elicit conditioned place preference do not stimulate 50-kHz USVs (Wright et al., 2012a). Furthermore, no 50-kHz USVs sensitization was observed in rats that self-administered escalating doses of methamphetamine (Mahler et al., 2013). Finally, and remarkably, direct non-selective dopaminergic agonists with a low abuse potential, such as apomorphine and quinpirole, also elicit 50-kHz USVs (Brudzynski et al., 2012; Williams and Undieh, 2010), although others failed to observe this effect with subtype selective dopaminergic agonists (Scardochio and Clarke, 2013). Collectively, these findings could suggest that 50-kHz USVs observed immediately after the administration of dopaminergic psychostimulants of abuse may reflect rewarding dopaminergic effect, and not be necessarily related to the motivational properties of these drugs.

To clarify this issue, the present study investigated the modifications in 50-kHz USVs elicited by the repeated administration of either the dopaminergic psychostimulant drug of abuse amphetamine or the dopaminergic agonist apomorphine, which has a low abuse potential. The procedure used in this investigation aimed to study both the direct (observed immediately after drug administration) and long-lasting conditioned effects of these drugs on 50-kHz USVs (see Simola et al. (2014)). Rats received repeated treatment in a novel environment (5 administrations on alternate days) followed (7 days after discontinuation) by re-exposure to the drug-paired environment, and thereafter drug challenge. Long-term modifications in drug-induced locomotor activity were also measured throughout the treatment, to obtain an additional behavioral measure that is considered to correlate with changes in the motivational properties of drugs with repeated administration (Robinson and Berridge, 2001), and to verify whether modifications in this behavior paralleled those involving 50-kHz USVs. Moreover, to further elucidate the effects of amphetamine and apomorphine on 50-kHz USVs, rats were subdivided in "low" and "high" vocalizers, and the time-dependence of drug effects was evaluated.

2. Experimental procedures

2.1. Subjects

Male Sprague–Dawley rats (Harlan, Italy) weighing 275–300 g were used. Four or five rats per cage were housed 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 from 10:00 to 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. Efforts were made to minimize animal discomfort and reduce the number of animals used.

2.2. Drugs

D-Amphetamine (sulfate) and apomorphine (hydrochloride) were purchased from Sigma–Aldrich (Milan, Italy). Drugs were dissolved in distilled water (vehicle) and administered intraperitoneally (i.p.), in a 3 ml/kg volume. Apomorphine solutions contained 0.05% ascorbic acid to prevent autoxidation. The doses were based on previous studies by us and others that investigated the effects of amphetamine and apomorphine on 50-kHz USVs emission (Simola et al., 2012, 2014; Williams and Undieh, 2010), and on preliminary experiments performed in our laboratories. Apomorphine was selected as a term of comparison to study the effects of amphetamine on 50-kHz USVs, since both these drugs act by activating