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Diverging frequency-modulated 50-kHz vocalization, locomotor activity and conditioned place preference effects in rats given repeated amphetamine treatment

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ABSTRACT

Behavioral sensitization and tolerance to repetitive exposure to addictive drugs are commonly used for the assessment of the early stages of the drug dependence progress in animals. The orchestra of tools for studying the progress of drug dependence in laboratory rodents has been considerably enriched in the 1980s by the introduction of ultrasonic vocalization (USV) detection and characterization. However, the relationship between the results of this technology and those of traditional behavioral tests is not clear. We attempted to elucidate some of the respective ambiguities by comparing the effects of an intermittent amphetamine treatment, which was aimed both at the induction of sensitization and tolerance to this drug and at testing the persistence of these effects, on the locomotor activity and 50-kHz USV responses to both the drug and the context of drug exposure in adult male rats showing diverging susceptibility for sensitization to amphetamine. Categorization of the rats into low and high responders/callers based on sensitization of their frequency-modulated 50-kHz USV responsiveness showed some correspondence with conditioned place preference effects, but not with responses to amphetamine. The study showed distinct changes in the rate and latency of the frequency-modulated 50-kHz USV responses to repetitive amphetamine treatment, which were reminiscent of classical behavioral signs of sensitization and tolerance. These results show the utility of the appetitive USV for monitoring of early phases of complex processes leading to drug dependence. However, USV, locomotor activity and conditioned place preference seem to reflect different aspects of these phenomena.

1. Introduction

The ability to stimulate brain reward system(s) and to produce long-lasting behavioral sensitization and tolerance are typical properties of addictive drugs, which are utilized for modeling, in laboratory animals, early phases of complex processes leading to drug addiction. For many years these characteristics were assessed but indirectly, using, inter alia, locomotor activity- (LA) -related and conditioned place preference (CPP) tests. Since 1980s, a novel approach employing detection and measurement of ultrasonic vocalization (USV) is being developed for animal studies on behavioral effects of drugs of abuse. This technique allows one to assess motivational and emotional states of laboratory rodents by exploiting a variety of USV features (Brudzynski, 2013; Knutson et al., 2002; Wang et al., 2008; Wöhr et al., 2009; Wöhr and Schwarting, 2009). With regard to the assessment of drugs of abuse-induced positive affective states, the most useful is the so-called appetitive 50-kHz USV (Brudzynski, 2009; Burgdorf et al., 2009; Ma et al., 2010; Mahler et al., 2013; Maier et al., 2012; Mu et al., 2009; Simola et al., 2012, 2014; Thompson et al., 2006;

Abbreviations: Amph, amphetamine; ANOVA, analysis of variance; CPP, conditioned place preference; DAMGO, [D-Ala^2, N-MePhe^4, Gly-ol]-enkephalin; FM, frequency-modulated; HC, high callers; LA, locomotor activity; LC, low callers; Sal, physiological saline; TIPS, two-injection protocol of sensitization; USV, ultrasonic vocalization.

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Wright et al., 2010, 2012), which is considered a substitute of drug addicts' self-reporting (Barker et al., in press; Panksepp and Burgdorf, 2000; Mahler et al., 2013).

Fifty-kHz USV reaction to drugs of abuse can picture their rewarding effect and its sensitization as well as the reaction to the context of drug exposure (Barker et al., in press; Hamed et al., 2012; Maier et al., 2012; Taracha et al., 2012). Experimental data evidence a convergence between subjective perception of the psychoactive action of these substances and CPP (Ahrens et al., 2013; Burgdorf et al., 2007), and a positive correlation between the rate of 50-kHz USV and the acquired self-administration of such drugs (Browning et al., 2011; Maier et al., 2010). Our recent study, the results of which have been confirmed in general by the report of Ahrens et al. (2013), has shown that the positive affective state-related FM 50-kHz USV response to amphetamine is greatly diversified among nonselected rats, but shows a substantial intra-individual stability: the rats that experienced a strong sensitization from the first drug dose showed persistence of this effect after consecutive doses, while those resistant to the sensitization did not sensitize during the further drug treatment (Taracha et al., 2012).

Diverging vulnerability to drug dependence/addiction, which is well documented by both animal and human studies (Cain et al., 2005; Pelloux et al., 2006; Zuckerman, 1984), is known to correlate negatively with the results of anti-addiction therapies. Our recent results (Taracha et al., 2012) opened a perspective for the creation of a rat model that includes this characteristic, and this study represents the next step in the development of such a model. In particular, we intended to verify the validity of our previous categorization of the susceptibility for drug dependence (based on sensitization of FM 50-kHz USV response to amphetamine) by confronting it with the results of classical behavioral tests of proven utility in this regard (CPP and LA), to refine our categorization by incorporating a FM 50-kHz USV and LA response-based assessment of drug tolerance, and possibly to seek an interpretation of the USV-related findings in their comparison with CPP and LA data.

2. Methods and materials

2.1. Subjects

Thirty-six male Sprague–Dawley rats from the stock of the Mossakowski Medical Research Centre, PAS, Warsaw, Poland, were used for the study. The rats were 10–11 weeks old upon arrival and were housed six per opaque plastic cage (55 × 33 cm floor size, $H = 19.5$ cm) in a temperature- and humidity-controlled room (21 ± 2 °C, 60–70% relative humidity) under a 12-h light/12-h dark cycle (lights on at 7 a.m.), with free access to standard laboratory rat chow and tap water. The rats were randomly divided between the vehicle-treated subset (Ctrl, $N = 6$) and the amphetamine-treated subset ($N = 30$); there was no significant difference in body weight between these subsets (mean ± SD: 305 ± 9 g and 288 ± 29 g, respectively, $p = 0.18$).

2.2. Habituation

Before the start of USV/LA tests/recordings, the rats were acclimated for 1 week in the local animal facility. During this period, they were given six 'daily' (excepting weekends) sessions of graded habituation to the procedures related to drug treatment: 2 sessions of simple gentle stroking when on the experimenter's hands (for about 1 min), followed by 2 sessions of being hand-immobilized exactly as for ip amphetamine injections, followed by 2 sessions of being hand-immobilized and pricked with an injection needle of the size intended for ip injections. After each session, the rats from a given cage were placed in a cage identical with the housing cage, with some clean wooden chips on the bottom, and then returned to their home cage. The post-handling cage was replaced with a fresh one for each group of cage-mate rats.

2.3. Experimental design

Amphetamine was given to rats in a way that allows to follow-up the development and to verify the stability of sensitization and tolerance to repeated drug exposures. The procedure involved: 1) the initiation and verification of sensitization to the drug with the so-called two-injection protocol of sensitization (TIPS) that consisted of administration, at 6-day interval, of two drug doses (Amph1 and Amph2); this protocol has been first used for the induction and assessment of locomotor sensitization (with no interfering tolerance) of mice to cocaine and morphine (Valjent et al., 2010), and was next successfully employed (Taracha et al., 2012) for the induction and verification of sensitization of LA and 50-kHz USV responses to amphetamine in rats; 2) the development of tolerance with continuing intermittent drug treatment (Amph3-Amph9); since frequent dosing facilitates the development of tolerance, these doses were given at 1-day intervals, excepting the weekend-related breaks; and 3) the verification of the persistence of these effects with amphetamine challenge (Amph10) given after 2-week withdrawal from the treatment. A complete scheme of the experimental design is shown in Fig. 1. All efforts were taken to minimize animal suffering and the number of rats used. All animal use procedures were in accordance both with the European Communities Council Directive of November 24, 1986, on the protection of laboratory animals (86/609/EEC), and with the current laws of Poland, and were approved by the Bioethical Committee of the Medical University of Warsaw (Certificate of approval No. 47/2012).

2.4. Drugs

D-Amphetamine sulfate (Sigma, St. Louis, MO, USA) was dissolved (1.5 mg/ml) in sterile aqueous 0.9% NaCl solution (Sal; Polpharma, Starogard Gdański, Poland) and injected ip at the dose of 1.5 mg/kg body weight. As a rule, the preferred compartment of CPP apparatus (see below) was used for Sal injections and post-Sal USV/LA recording sessions, and the non-preferred section was used for amphetamine injections and post-drug USV/LA recording sessions. However, the sessions preceding Amph2, Amph9 and Amph10 injections were carried out with the entire CPP apparatus open for the subject rats.

2.5. CPP apparatus and test procedure

The apparatus consisted of a plywood box (with 34 cm high walls) divided into two main compartments (35.5 × 20 cm floor size) separated by a smaller section (10 × 20 cm floor size) with vertically sliding matt black doors and floor and walls coated with clear lacquer. One main compartment had a rough plywood floor and both its floor and the walls were painted matt black. The other main compartment had a smooth black plastic floor and its walls were also painted matt black, but the lowermost part of its long walls carried four 4 cm high white paint rectangles of 10, 5.5, 5.5 and 8 cm length (listed from the most proximal to the most distant with regard to the door), spaced 2 cm one from another. The CPP test was performed in a 4.4 × 2.8 × 2.9 m ($L \times W \times H$) room with ceiling and walls painted white and lit with four reflectors (each facing different wall) fixed centrally 48 cm below the ceiling, each equipped with an incandescent 40 W matt white light bulb. Two CPP apparatuses were used simultaneously, which were thoroughly cleaned after each rat. The apparatuses were separated with a 1.1 × 0.7 $m \times (L \times H)$ sound-attenuating wall made of a 2 cm thick particle board with black veneer on both sides; the wall has been verified to prevent the microphones used for USV recording from collecting calls emitted by the rat residing in the CPP apparatus behind the wall. Rats' LA was registered with a ceiling-fixed model EV-650CG video camera (Sony, Japan) connected to a PC equipped with the EthoVision® XT Video Tracking System v.7 (Noldus Information Technology B.V., Wageningen, The Netherlands).

Initial place preference was determined in a 15-min pre-test, with no USV and LA recording. On the next day, the rats were given an ip Sal injection and were instantly confined to the preferred section of the apparatus for a 40-min session of LA and USV recording. One day later, the Ctrl rats and the rats scheduled for drug treatment were given their next Sal dose and the first amphetamine dose, respectively, and were immediately confined to the non-preferred section of the apparatus for another 40-min session of LA/USV recording. Seven days after the initial Sal injection, all rats were again given access to open CPP apparatus for 20 min (of which the first 15 min were taken for CPP analysis) for the assessment of their place preference and LA/USV activity. CPP was calculated as the difference between the times spent in the drug-paired section during the test and pre-test.

2.6. USV recording

USVs were recorded with a single CM16 condenser microphone (Avisoft Bioacoustics, Germany) placed 35 cm above the cage/testing box floor, centrally with regard to the rat-accessible area. The microphone was sensitive to frequencies of 15–180 kHz, had a flat response characteristic (±6 dB) within the 25–140 kHz frequency range, and was connected to a custom-made amplifier of 600 Ω input impedance, 16 V/V (12 dB) voltage gain, and ±0.1 dB (30 Hz–100 kHz) frequency response. The amplified signals were passed to an adjacent room, processed with a custom-made antialiasing filter, and then sent to a PC equipped with PCI-703-16A acquisition board (14-bit, 400 kHz; Eagle Technology, Eagle River, WI, USA) and a custom-written software (Rat-Rec Pro 5.0), processed using a fast Fourier transform (1024 or 512, Hamming or Hann window) and displayed as a color spectrogram. Frequency-modulated (FM) and non-FM ("flats") 50 kHz calls were identified as specified elsewhere (Brudzynski, 2013). Since the number of flats is not affected by amphetamine treatment (Ahrens et al., 2009; also confirmed in our lab), only FM 50 kHz calls were analyzed.