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Research report

# Non-parametric analysis of neurochemical effects and Arc expression in amphetamine-induced 50-kHz ultrasonic vocalization

Adam Hamed[a,b,*], Patrycja Daszczuk[c], Miron Bartosz Kursa[d], Danuta Turzyńska[b], Alicja Sobolewska[b], Małgorzata Lehner[b], Paweł M. Boguszewski[e], Janusz Szyndler[f]

[a] Laboratory of Spatial Memory, Department of Cellular and Molecular Biology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
[b] Department of Neurochemistry, Institute of Psychiatry and Neurology, Warsaw 02-957, 9 Sobieskiego Street, Poland
[c] Laboratory of Synaptogenesis, Department of Cell Biology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
[d] Interdisciplinary Centre for Mathematical and Computational Modelling, University of Warsaw, Pawinskiego 5A, 02-106 Warsaw, Poland
[e] Laboratory of Animal Models, Neurobiology Center, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
[f] Department of Experimental and Clinical Pharmacology, Centre for Preclinical Research and Technology CePT, Medical University of Warsaw, Banacha 1B, 02-097 Warsaw, Poland

## HIGHLIGHTS

- Re-exposure to amphetamine increased 50-kHz USVs comparing to control.
- 50-kHz USVs do not correlate with distance covered by the investigated animals.
- Noradrenaline in the NAcc strongly correlated with the number of 50-kHz USVs.
- NAcc noradrenaline negatively correlated with of dopamine and dopamine metabolites.
- NAcc NA correlated with the concentration of GABA and 5-HIAA in this structure.
- Amphetamine indicates cross-talk between NA, DA, 5-HT and GABA in the NAcc.

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## ABSTRACT

A number of studies have identified the importance of dopaminergic, opioid, serotonergic, noradrenergic and glutamatergic neurotransmission in amphetamine-induced "50-kHz" ultrasonic vocalizations (USVs). Amphetamine became a topic of interest for many researchers interested in USVs due to its ability to induce 50-kHz USVs. To date, it has been difficult to identify the neurotransmitters responsible for this phenomenon. The aim of this study was to determine the following: (i) concentrations of neurotransmitters in selected structures of the rat brain after re-exposure of the rats to amphetamine administration; (ii) changes in Arc in the medial prefrontal cortex, striatum, nucleus accumbens core and shell, hippocampus, amygdala and ventral tegmental area; and (iii) a biological basis for differences in 50-kHz USV emissions in response to amphetamine administration. Re-exposure to amphetamine increased 50-kHz USVs. This parameter do not correlate with distance covered by the investigated animals. An increased concentration of noradrenaline in the nucleus accumbens (NAcc) strongly correlated with the number of 50-kHz USVs. We found that NAcc noradrenaline concentrations negatively correlated with the concentration of dopamine and dopamine metabolites and positively correlated with the concentration of GABA and 5-HIAA (serotonin metabolite) in this structure. We have also identified a positive correlation between striatal 3-MT (dopamine metabolite) concentrations and Arc expression in the hippocampal DG as well as a negative correlation between the concentration of GABA in the amygdala and Arc expression in the central amygdala. Thus, the relationship between the emission of 50-kHz USVs and the neurochemical changes that occur after re-exposure to amphetamine indicates cross-talk between NA, DA, 5-HT and GABA neurotransmission in the NAcc.



* Corresponding author at: Laboratory of Spatial Memory, Department of Cellular and Molecular Biology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur Street, 02-093 Warsaw, Poland.
*E-mail addresses:* a.hamed@nencki.gov.pl, adamhamed2005@yahoo.com (A. Hamed).

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## 1. Introduction

The phenomenon of ultrasonic vocalization (USV) in animals has been studied since the 1970s [1]. Due to modern techniques, researchers have managed to extract two main patterns of USV calls, including 22-kHz alarm calls, which express negative emotions, and 50-kHz calls, which indicate positive emotions [2–5]. A number of studies have demonstrated the importance of the dopaminergic system in the 50-kHz USV [6–10], and some studies have directly linked an increase in dopamine (DA) levels in the nucleus accumbens (NAcc) with an increase in USVs in the 50-kHz band [11–14]; however, studies of amphetamine administration have suggested the involvement of other neurotransmitter systems, i.e., noradrenaline (NA) and GABA, in the effects of psychostimulants [4,15,16]. Simultaneously, detailed studies have reported the influence of the opioid, dopaminergic, serotonergic and glutamatergic systems on 50-kHz USV emissions [17–19].

Amphetamine, which has been in use since the early 1920s, is a sympathomimetic drug that influences many neurotransmitter systems, i.e., dopaminergic, noradrenergic, and serotonergic [15]. This substance became a topic of interest for many researchers interested in the USVs of rats due to its ability to induce 50-kHz USVs [3,20–22]. Playback of 50-kHz USV emissions was also demonstrated to increase DA levels in the NAcc [12].

The majority of the structures that have been suggested to be involved in the emission of 50-kHz USVs are associated with the reward system (i.e., the medial prefrontal cortex, or mPFC; NAcc; and ventral tegmental area, or VTA). In this study, we sought to identify plastic changes in the rat brain that occurred in response to the administration of a second dose of amphetamine by measuring the expression of Arc, which is associated with brain synaptic activity changes related to learning and memory [23,24].

By taking advantage of the high inter-individual variation in the response to amphetamine re-exposure, as measured by 50-kHz USV emissions [21], we aimed to correlate biochemical and immunohistochemical observations with the level of 50-kHz USV emission, as measured by the number of emission episodes. We also used machine learning methods to investigate indirect, multivariate associations.

The aim of this study was to determine the following: (i) concentrations of neurotransmitters in selected structures of the rat brain after re-exposure of the rats to amphetamine administration; (ii) changes in Arc in the medial prefrontal cortex, striatum, nucleus accumbens core and shell, hippocampus, amygdala and ventral tegmental area; and (iii) a biological basis for differences in 50-kHz USV emissions in response to amphetamine administration.

## 2. Materials and methods

### 2.1. Animals

Adult male *Sprague-Dawley* rats (n = 16; 180 ± 20 g) were used in all experiments. The animals were purchased from a licensed breeder (the Polish Academy of Science Medical Research Center, Warsaw, Poland). The animals were housed under standard laboratory conditions that included a 12 h:12 h light:dark cycle (lights on at 7 a.m.), a constant temperature (21 ± 2 °C) and 70% humidity. The rats had free access to food and water. The experiments were performed in accordance with the European Communities Council Directive of November 24, 1986 (86/609 EEC). The Local Committee for Animal Care and Use of Warsaw Medical University approved all experimental procedures involving animal subjects. Before the study, the rats received seven sessions of handling and habituation. One rat (from the amphetamine group) was eliminated from the experiment due to emission of 22-kHz calls after first injection of amphetamine.

### 2.2. Experimental protocol

#### 2.2.1. Procedure

The *Sprague-Dawley* rats were housed in groups of eight (amphetamine or saline group). On the first day (D1) of drug treatment, the rats were brought to the testing room, placed individually in clean plastic cages (56 × 34 × 20 cm) with no bedding, and tested for USV emissions both before (20 min) and after (20 min) the injection of amphetamine or saline (vehicle). Then, the rats were returned to their home cages in the housing room for five days. One rat (from the amphetamine group) was eliminated from the experiment due to emission of 22-kHz calls after first injection of amphetamine. During the second day of drug treatment (D7), the rats were brought to the testing room, placed individually in clean plastic cages (56 × 34 × 20 cm) with no bedding, and tested for USV emissions before (20 min) and after (20 min) the injection of amphetamine or saline. Ninety minutes after the injection (D7), the rats were decapitated. Brain tissues were frozen in dry, ice-cold isopentane, and stored at −70 °C for neurochemical and immunocytochemical analysis.

### 2.3. Drugs

D-amphetamine sulfate (Sigma-Aldrich) was dissolved (2 mg/ml) in saline solution (0.9%) and injected into the rats at a dose of 1.5 mg/kg (i.p.).

### 2.4. Apparatus and USV recordings

The all subtypes of 50 kHz rat calls were recorded using an UltraSoundGate Condenser Microphone CM16 (Avisoft Bioacoustics, Berlin, Germany) that was positioned 25–30 cm above the floor of the cage. This microphone was sensitive to frequencies of 15–180 kHz with a flat frequency response (±6 dB) of between 25 and 140 kHz. It was connected to an amplifier (custom-made, Warsaw) that had the following parameters: a voltage gain of 16 V/V (12 dB), a frequency response of ±0.1 dB, a range of 30 Hz to 120 kHz, and an input impedance of 600 Ω. The signal was then transferred through a 120 kHz anti-aliasing filter (custom-made, Warsaw). The filtered sounds were sent to a PCI-703-16A data acquisition board (Eagle Technology, USA). This board was a 14-bit 400-kHz analogue input and analogue output board for PCI-based systems. The recorded data were processed using the RAT-REC PRO 5.0 software (custom-made, Warsaw). The signals were processed through a fast Fourier-transformation (1024 or 512, Hamming or Hann window) and displayed as color spectrograms. Each signal was manually marked (Fig. 1) with the section label included in the automated parameter measurement. Various parameters were determined automatically, including the number of USV calls, the total calling time (s), the mean call length (s), the frequency bandwidth (kHz), the number of gaps, the mean gap length (s), and the mean peak frequency (kHz). Only the number of calls is included as a main parameter in the results presented here. All measured types of USVs calls had their frequency bandwidth over 5 kHz (most of them were trills, splits and inverted-U shaped calls). The multiplicity of 50 kHz calls did not allow for a clear association them with a given type of behaviour, moreover we found intra-individual differences between rats in emitting different types of 50-kHz USVs calls. For that reasons all 50 kHz USVs calls were counted together (without calls with a frequency bandwidth below 5 kHz). The signal from the microphone was sent to another room where the computer and observer were situated [5,25].