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Review

High-anxiety rats are less sensitive to the rewarding affects of amphetamine on 50 kHz USV

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HIGHLIGHTS

- Amphetamine enhances appetitive 50 kHz vocalisation in rats.
- Amphetamine's effect is stronger in low anxiety (LR) than in high anxiety rats (HR).
- LR had increased basal levels of dopamine in the basolateral amygdala (BLA).
- LR had increased dopamine metabolism in the BLA, in response to aversive context.

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ABSTRACT

This study assessed behaviour, as measured by 50 kHz calls related to positive affect, in rats with different fear conditioned response strengths: low-anxiety rats (LR) and high-anxiety rats (HR), after amphetamine injection in a two-injection protocol (TIPS). The results showed that the first dose of amphetamine evoked similar behavioural effects in frequency-modulated (FM) 50 kHz calls in the LR and HR groups. The second injection of amphetamine resulted in stronger FM 50 kHz calls in LR compared with HR rats. The biochemical data (*'ex vivo'* analysis) showed that the LR rats had increased basal levels of dopamine in the amygdala, and increased homovanilic acid (HVA), dopamine's main metabolite, in the amygdala and prefrontal cortex compared with HR rats. The *'in vivo'* analysis (microdialysis study) showed that the LR rats had increased HVA concentrations in the basolateral amygdala in response to an aversively conditioned context. Research has suggested that differences in dopaminergic system activity in the amygdala and prefrontal cortex may be one of the biological factors that underlie individual differences in response to fear stimuli, which may also affect the rewarding effects of amphetamine.

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1. Introduction

In rats, as in humans, there are a great deal of individual differences in the responses to stress and behavioural responses to the pharmacological activation of the mesolimbic system [1–4]. Our previous studies on high-anxiety rats (HR) and low-anxiety rats (LR), which were selected based on their behaviour in the contextual fear test (i.e., the duration of the freezing response was used as the discriminating variable), showed that HR rats were characterised by an anxiogenic and depressive-like phenotype, using a passive coping strategy, showing decreased activity in the prefrontal cortex and increased activity in the basolateral amygdala (as shown in c-Fos studies) compared with the LR group. In contrast, LR rats were characterised by an active coping strategy, less anxiogenic and depressive-resilience behaviour, and increased activity of the prefrontal cortex and the dentate gyrus (DG) of the hippocampus [5–7].

It is well documented that dopaminergic transmission in the mesolimbic system is important for information processing, emotional responses to environmental changes, and strategies for coping with stress [8–11]. Dopamine plays a critical role in the detection of novel information, which is essential for the consolidation, storage and retrieval of memories, all of which are likely to be impaired in depressed patients [11,12]. Finally, it should be emphasised that dopamine is considered to be the main neurotransmitter involved in the mediation of different natural and drug-induced positive reinforcements [13–16].

The frequency-modulated 50 kHz ultrasonic calls (FM 50 kHz, USV) offer a valuable index of the positive affective states evoked by naturally rewarding stimuli (e.g., food, social contacts) and induced by some psychostimulants (e.g., amphetamine) [17–22].

It is hypothesised that differences in dopaminergic activity may be one of the biological factors underlying individual differences in emotional responses and behavioural sensitisation to psychostimulants. We examined this interesting issue using behavioural responses measured by FM 50 kHz calls in rats with different fear-conditioned response strengths, either low-anxiety rats (LR) or high-anxiety (HR) rats, after amphetamine injection in a two-injection protocol (TIPS). In rats, repeated exposure to psychostimulant drugs results in a long-lasting enhancement of behavioural response, otherwise known as behavioural sensitisation [23,24]. TIPS allows an easy distinction between the induction of sensitisation after the first injection and the drug's expression after a second injection, enabling the assessment of the long-lasting effects of a single exposure to drugs [25]. TIPS has been successfully used for the locomotor sensitisation of mice to morphine and cocaine [25]. It was previously shown that assessment of 50 kHz USV recording in TIPS model provides a good paradigm to observe sensitisation to amphetamine [26,27]. In the literature, there is little information on the role of amygdala in the expression of 50 kHz calls. This fact is particularly interesting given that psychostimulants can produce both positive and aversive effects [28].

Electrophysiological studies have recently found that ultrasonic stimuli applied in two frequency ranges (22 kHz and 50 kHz) produced significant and opposite effects on single-unit responses to these stimuli in the lateral rat amygdala, indicating its contribution to the processing of both ethologically essential social signals [14]. For these reasons, we decided to analyse the role of the prefrontal cortex and basolateral amygdala. We also wanted to determine whether individual predisposition to a stronger fear response influences the development of sensitisation to psychoactive substances. An additional aim of this study was to test a new model of differential susceptibility to psychoactive drugs.

2. Methods

2.1. Animals

Male Wistar rats ($n = 78$, 200–220 g body weight, 8 weeks old at arrival) purchased from the stock of the Centre for Experimental Medicine, Medical University of Białystok, Poland, were housed under standard laboratory conditions with a 12 h light/dark cycle (lights on at 7 a.m.) at a constant temperature ($21 \pm 2\,^{\circ}C$). They had ad libitum access to tap water and standard laboratory rodent chow. 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 at the Medical University of Warsaw, Poland approved all experimental procedures.

2.2. Experimental protocol

After four days of acclimatisation to the *vivarium*, animals were subjected to the conditioned fear test (CFT) to assess their individual responses to aversive conditioned stimuli [29]. Accordingly to their behaviour in the CFT, the animals were divided into high- and low-anxiety rat groups (HR and LR groups). In total, 5 rats did not fulfil the criteria for either group. Subsequently, the rats were randomly divided into the following groups: LR saline group ($n = 10$), HR saline group ($n = 9$), LR amph group ($n = 13$), and HR amph group ($n = 13$). Then, the rats were subjected to a TIPS protocol [26,27]. Additional groups were used for the biochemical analysis: (1) to measure homovanillic acid (HVA) concentration in response to the aversive context ('*in vivo*' study) with microdialysis probe implantation in the basolateral amygdala (BLA), LR ($n = 5$) and HR ($n = 8$); and (2) to measure the basal levels of dopamine (DA) and (HVA) in the prefrontal cortex and the amygdala complex ('*ex vivo*' study), LR ($n = 7$) and HR ($n = 7$) (Fig. 1).

2.3. CFT and the division of the animals into the HR and LR groups

The fear-conditioning experiment was performed in experimental cages (36 cm × 21 cm × 20 cm, w/l/h) under constant white noise conditions (65 dB). The experiment lasted for three consecutive days. On the 1st day, the animals were individually placed in