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Research report

Tolerance to LSD and DOB induced shaking behaviour: Differential adaptations of frontocortical 5-HT_{2A} and glutamate receptor binding sites

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HIGHLIGHTS

- LSD and DOB induce a ketanserin-sensitive increase in shaking behaviour.
- LSD and DOB induced shaking behaviour is undermined by tolerance development.
- Tolerance to DOB correlates with reduced frontocortical 5-HT_{2A} binding sites.
- Tolerance to LSD does not correlate with frontocortical 5-HT_{2A} binding sites.
- Tolerance to LSD correlates with reduced frontocortical glutamate binding sites.

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ABSTRACT

Serotonergic hallucinogens, such as lysergic acid diethylamide (LSD) and dimethoxy-bromoamphetamine (DOB), provoke stereotype-like shaking behaviour in rodents, which is hypothesised to engage frontocortical glutamate receptor activation secondary to serotonin2A (5-HT_{2A}) related glutamate release. Challenging this hypothesis, we here investigate whether tolerance to LSD and DOB correlates with frontocortical adaptations of 5-HT_{2A} and/or overall-glutamate binding sites. LSD and DOB (0.025 and 0.25 mg/kg, i.p.) induce a ketanserin-sensitive (0.5 mg/kg, i.p., 30-min pretreatment) increase in shaking behaviour (including head twitches and wet dog shakes), which with repeated application (7× in 4 ds) is undermined by tolerance. Tolerance to DOB, as indexed by DOB-sensitive [^{3}H]spiroperidol and DOB induced [^{35}S]GTP-gamma-S binding, is accompanied by a frontocortical decrease in 5-HT_{2A} binding sites and 5-HT_2 signalling, respectively; glutamate-sensitive [^{3}H]glutamate binding sites, in contrast, remain unchanged. As to LSD, 5-HT_2 signalling and 5-HT_{2A} binding, respectively, are not or only marginally affected, yet [^{3}H]glutamate binding is significantly decreased. Correlation analysis interrelates tolerance to DOB to the reduced 5-HT_{2A} ($r = .80$) as well as the unchanged [^{3}H]glutamate binding sites ($r = .84$); tolerance to LSD, as opposed, shares variance with the reduction in [^{3}H]glutamate binding sites only ($r = .86$). Given that DOB and LSD both induce tolerance, one correlating with 5-HT_{2A}, the other with glutamate receptor adaptations, it might be inferred that tolerance can arise at either level. That is, if a hallucinogen (like LSD in our study) fails to induce 5-HT_{2A} (down-)regulation, glutamate receptors (activated postsynaptic to 5-HT_{2A} related glutamate release) might instead adapt and thus prevent further overstimulation of the cortex.

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1. Introduction

Serotonergic hallucinogens, such as lysergic acid diethylamide (LSD) or dimethoxy-bromoamphetamine (DOB) share structural elements with serotonin (5-hydroxytryptamine [5-HT]) [1,2], a neurotransmitter involved in mood, (repetitive) gross motor output, vascular tonus, and thermoregulation. Although their structural resemblance to 5-HT renders most hallucinogens prone to bind to diverse 5-HT receptors [3], activation of the 5-HT_{2A} subtype is considered the key mechanism for their human *psychedelic* effect to occur [4,5]. In animals, hallucinogens evoke a variety of stereotype-like motor outputs, including head twitches, wet dog shakes, ear scratches, limb flicking, or backward walking [6]. As *head twitches* in mice and *wet dog shakes* in rats have a very similar pharmacology, with the latter most probably reflecting a more generalised version of the former [7,8], we consider both phenomena analogous, and subsume them under the term *shaking behaviour* [compare 9, 10]. Shaking behaviour is one of the most widely accepted and well-scrutinised model of central hallucinogenic activity [11,12]. It mirrors the human psychedelic effect in its three most important characteristics: It is primarily related to the activation of 5-HT_{2A} receptors [13,14]; it is induced by representatives of the two main groups of serotonergic hallucinogens, the indole- and phenylalkylamines [15–17]; and it rapidly develops tolerance [18,19]. Given its significance for the basic understanding of the human psychedelic effect, the neurophysiological correlates of the hallucinogen induced shaking behaviour are of high interest. In parallel to human research [20,21], and for the following main reasons, the current literature largely focuses on the frontal cortex as a primary correlate: (1) The (frontal) cortex is the region of the brain, where 5-HT_{2A} receptors are most abundantly expressed, notably on cortical output cells (i.e. layer V pyramidal cells) [22,23]. (2) When locally applied into the frontal cortex, hallucinogens evoke shaking behaviour sensitive to systemic 5-HT_{2A} antagonist application [24]. (3) In 5-HT_{2A} knock-out mice, shaking behaviour can be rescued with the expression of 5-HT_{2A} receptors selectively restored to the cortex [16]. Based on the electrophysiological properties of the frontocortical 5-HT_{2A} receptors, shaking behaviour most probably engages a glutamatergic mechanism [25]. In slice preparations of frontocortical layer V pyramidal cells, 5-HT_{2A} receptors increase the frequency of spontaneous excitatory postsynaptic currents/potentials (EPSCs/EPSPs) [26]. As this increase can be counteracted by AMPA receptor blockage or by metabotropic glutamate receptor type$_{2/3}$ ($mGlu_{2/3}$) activation, it is assumed to be accounted for by a 5-HT_{2A} related glutamate release onto AMPA receptors [27,28]; $mGlu_{2/3}$ receptors, in this model, interfere presynaptically with the glutamate release [27] and/or (postsynaptically) with the 5-HT_{2A} signalling [29]. Intriguingly, shaking behaviour has likewise been shown to be sensitive to pharmacological AMPA and $mGlu_{2/3}$ receptor manipulations. Similar to the EPSCs/EPSPs in the pyramidal cells, it can be inhibited by AMPA antagonists [28,30] and $mGlu_{2/3}$ agonists [29,31], but enhanced by $mGlu_{2/3}$ antagonists [32].

In the current work, we address the tolerance phenomenon characteristic for repeated hallucinogen application [for a review see 5, 33, 34]. Tolerance to hallucinogen induced shaking behaviour has often been associated with a downregulation of frontocortical 5-$HT_{2(A)}$ receptors [35–39]. However, mathematical correlations for this receptor-behaviour association, apart from one study on antagonist related regulation of both parameters [40], have not been presented. Also, concomittant adaptations of the (downstream) glutamatergic system are largely obscure. Thus, assuming – as indicated by the above listed evidence – that shaking behaviour primarily relates to $mGlu_{2/3}$-sensitive glutamate release downstream of frontocortical 5-HT_{2A} activity, we here investigate whether behavioural tolerance to LSD and DOB co-occurs with adaptations of 5-HT_2 and $mGlu_{2/3}$ signalling, or of 5-HT_{2A} and/or overall-glutamate binding sites of the frontal cortex. To characterise the relationship between neurochemistry and behaviour more closely, we in addition probe the results by correlation analysis.

2. Methods and materials

2.1. Animals and housing

For all experiments, male *Sprague Dawley* rats (MolTac: SD, Taconic Denmark) (av. 10 weeks, av. 380 g) were used. They were housed in groups of five animals per cage, and held under controlled laboratory conditions (temperature 20 ± 2 °C, air humidity 55–60%, light/dark cycle 12:12 [light on at 6 a.m.]) with standard food pellets (ssniff SM/R/NH, 10 mm; ssniff Spezialdiäten GmbH, Soest, Germany) and tap water ad libitum. All experiments performed comply with the regulations of the *National Act on the Use of Experimental Animals* (Germany), as approved by the *Tierschutzkommission* Sachsen-Anhalt.

2.2. Behavioural experiments

2.2.1. Treatment

LSD tartrate, DOB hydrochloride (both from THC Pharm, Frankfurt am Main, Germany), and ketanserin tartrate (Biozol, Eching, Germany) were dissolved in isotonic saline, and applied into the peritoneum (i.p.) (10 ml/kg). Adequate dosing was determined by dose–response curves (LSD and DOB), or extrapolated from literature (ketanserin: 0.5 or 1.0 mg/kg, 30 min before agonist) [e.g. 17]. For *tolerance* experiments, both hallucinogens were applied seven times over four consecutive days. Every morning before observation (at ~10 a.m.), a low dose was given (0.025 mg/kg LSD vs. 0.25 mg/kg DOB); in the evening of days 1–3 (at ~10 p.m.), an additional high dose (0.25 mg/kg LSD vs. 0.75 mg/kg DOB) followed. Control animals were treated alike but received pure saline.

To estimate whether psychological habituation to the experimental setting might contribute to tolerance development, a fourth group of rats experienced a four-days habituation phase before the above mentioned LSD treatment began. In this phase, the rats received daily saline injections, were put into the experimental cages, and observed as if they were in the actual LSD experiment.

2.2.2. Shaking behaviour

Shaking behaviour was defined as brisk rotational movement of the head (with or without propagation to shoulders and trunk [wet dog shakes vs. head twitches]) around the long axis of the rat's body. For 30 min, starting right after agonist application, the occurrence of shaking behaviour was continuously registered by a trained observer, and validated via digital camera recordings. For dose–response curve experiments, rats were observed individually, i.e. one animal per cage (acryl cylinder: 19 cm Ø, 23 cm H). For antagonist and tolerance experiments, rats were observed in larger Plexiglas cages (36 cm L × 38 cm H × 20 cm W), with groups of 2–3 animals per cage. To avoid grooming related shaking behaviour, no sawdust bedding was provided. For general habituation, all animals were repeatedly exposed to the experimenter, and put into the room of experimentation a few days beforehand.

2.3. Neurochemical experiments

2.3.1. 5-HT_{2A} and glutamate receptor binding

Twenty hours after the last treatment, rats were decapitated and frontal cortices were dissected. With slight modifications, receptor binding assays were performed as earlier described [41,42]. Tissue was homogenised, pelleted by centrifugation (10 min, 50,000 × *g*,