

Research report

Lysergic acid diethylamide and [–]-2,5-dimethoxy-4-methylamphetamine increase extracellular glutamate in rat prefrontal cortex

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Abstract

The ability of hallucinogens to increase extracellular glutamate in the prefrontal cortex (PFC) was assessed by *in vivo* microdialysis. The hallucinogen lysergic acid diethylamide (LSD; 0.1 mg/kg, *i.p.*) caused a time-dependent increase in PFC glutamate that was blocked by the 5-HT_{2A} antagonist M100907 (0.05 mg/kg, *i.p.*). Similarly, the 5-HT_{2A/C} agonist [–]-2,5-dimethoxy-4-methylamphetamine (DOM; 0.6 mg/kg, *i.p.*), which is a phenethylamine hallucinogen, increased glutamate to 206% above saline-treated controls. When LSD (10 μM) was directly applied to the PFC by reverse dialysis, a rapid increase in PFC glutamate levels was observed. Glutamate levels in the PFC remained elevated after the drug infusion was discontinued. These data provide direct evidence *in vivo* for the hypothesis that an enhanced release of glutamate is a common mechanism in the action of hallucinogens.

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1. Introduction

Administration of lysergic acid diethylamide (LSD) has profound behavioral effects in humans, including distortion of perception as well as alterations in affective state and cognition. As a widely abused drug, elucidating the cellular and molecular mechanisms for the CNS effects of LSD has clinical relevance. In addition, as some of the clinical manifestations of schizophrenia are similar to the behavioral effects of hallucinogens, studies into the mechanism of action of these compounds also may help illuminate the neurobiological and neurochemical alterations associated

with this disorder. The development of drug-induced stimulus control as a powerful tool for the study of behaviorally active compounds [11,36,54,56] has permitted pharmacological characterization in intact animals of a variety of psychoactive drugs including LSD [22,28]. Furthermore, there is a strong correlation between discriminative stimuli in nonverbal species and subjective effects reported by humans [11,12,45,47]. Early studies using drug-induced stimulus control demonstrated that the serotonergic neurotransmitter system mediates the stimulus effects of the indoleamine and phenethylamine hallucinogens [14,55,56]. Furthermore, by integrating behavioral and biochemical techniques, activation of 5-HT_{2A} receptors was shown to play a critical role in the CNS effects of these hallucinogens [24,26,27]. This conclusion has been confirmed in both rodents and humans using selective receptor antagonists [46,52,60].

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Although stimulation of 5-HT_{2A} receptors is necessary for the behavioral effects of these psychoactive drugs, the glutamatergic neurotransmitter system also appears to play a prominent role. Thus, administration of noncompetitive NMDA receptor antagonists, such as phencyclidine (PCP), enhanced the discriminative stimulus effects of both LSD and the phenethylamine hallucinogen, [–]-2,5-dimethoxy-4-methylamphetamine (DOM; [59,60]). Similarly, head twitches induced by 5-HT₂ agonists were enhanced in the presence of NMDA antagonists [20,31]. Interestingly, schizophrenia appears to involve, at least in part, a dysfunction in the interaction of the serotonergic and glutamatergic neurotransmitter systems [7,13,16].

In slices from the medial prefrontal cortex, activation of 5-HT_{2A} receptors caused a calcium-dependent increase in the frequency of excitatory postsynaptic potentials (EPSPs) and currents (EPSCs; [4,6]). Because these effects can be blocked by the application of AMPA receptor antagonists or the activation of group II mGluRs that function in an autoreceptor capacity [4,6,10], these results have led to the hypothesis that glutamate release represents a common pathway for the actions of serotonergic hallucinogens [6,7]. Similarly, Martín-Ruiz et al. [35] suggested that the 5-HT_{2A} agonist, 4-iodo-2,5-dimethoxyamphetamine (DOI), increased 5-HT release in the medial prefrontal cortex secondary to stimulation of glutamate release. In agreement with a proposed central role for glutamate release in the effects of serotonergic hallucinogens, we recently showed that the stimulus effects of LSD were enhanced by the mGlu2/3 antagonist LY341495, which stimulates glutamate release [60].

In this study, *in vivo* microdialysis was used to test directly the hypothesis that the dosing regimens used in drug-induced stimulus control with indoleamine and phenethylamine hallucinogens cause release of glutamate. These studies were carried out in the prefrontal cortex, because this area contains a high density of 5-HT_{2A} receptors and has been suggested to play a major role in the actions of serotonergic hallucinogens, as well as in the pathogenesis of schizophrenia [7,21,38].

2. Material and methods

2.1. Surgery and microdialysis

Animals used in these studies were maintained in accordance with U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals as amended August 2002. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the University at Buffalo. The minimal number of animals needed to achieve statistical significance was used. Male Fischer 344 rats (300–350 g) were anesthetized with 1.0 g/kg urethane (*i.p.*). Plastic guide cannulae (CMA Microdialysis, North Chelmsford, MA) were stereotactically

directed to the prefrontal cortex (from bregma, AP +3.2; ML +0.1; DV –3.1 below dura) according to the atlas of Paxinos and Watson [43]. The cannulae were fixed to the skull with jeweler's screws and dental cement, and microdialysis probes (CMA11 microdialysis probe; 3 mm active dialyzing surface; 6 kDa MW cutoff; *in vitro* glutamate recovery approximately 18%) were seated in the cannulae. Immediately after surgery and probe installation, probes were perfused with Dulbecco's phosphate-buffered saline (PBS; in mM: 138 NaCl, 2.7 KCl, 0.5 MgCl₂, 1.5 KH₂PO₄, 1.2 CaCl₂, pH 7.4, filtered and degassed before use) at a rate of 0.5 µl/min. At least 3 h were allowed for recovery and baseline stabilization before the administration of drugs and the collection of dialysis samples. Animals that failed to show stable baseline values were excluded from statistical analyses. The sampling rate for all experiments was 15 min. Immediately after collection into 600-µl sample vials, dialysates were frozen and kept at –80 °C until high-pressure liquid chromatography (HPLC) analysis. Following collection of samples, animals were sacrificed and probe placements verified histologically in 40-µm cryostat sections (Fig. 1). Only animals with the proper probe placement were used for the statistical analysis. For experiment 1, sample size of the group receiving LSD alone and with M100907 was seven and five, respectively. For experiment 2, sample size of the DOM group was five and three for the saline group. For experiment 3, sample size of the group receiving intracortical infusion of LSD was eight.

2.2. Chromatography

Separation and quantification of glutamate were accomplished using HPLC with electrochemical detection. Dialysate was subjected to precolumn derivatization with *o*-phthalaldehyde (OPA) reagent before injection. Briefly, an OPA stock solution (27 mg OPA in 1 ml ethanol diluted with 9 ml 0.1 M Na₂B₄O₇ at pH 9.4) was charged with 5 µl β-mercaptoethanol and stored at –80 °C. For each daily

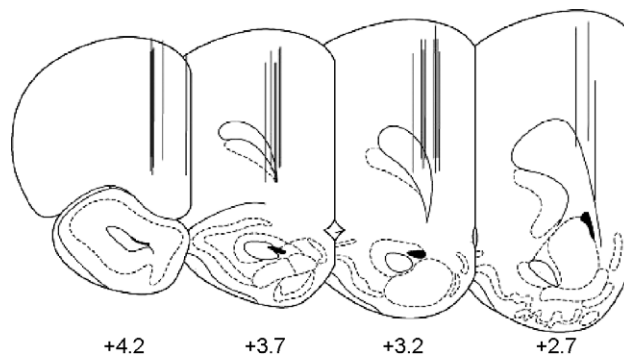


Fig. 1. Representative histology of the prefrontal cortex verifying placement of *in vivo* microdialysis probes. Coronal sections marked with their distance from bregma (in millimeters) were taken from Paxinos and Watson [43]. Vertical lines represent microdialysis probes with 3 mm active membrane length and are to scale.

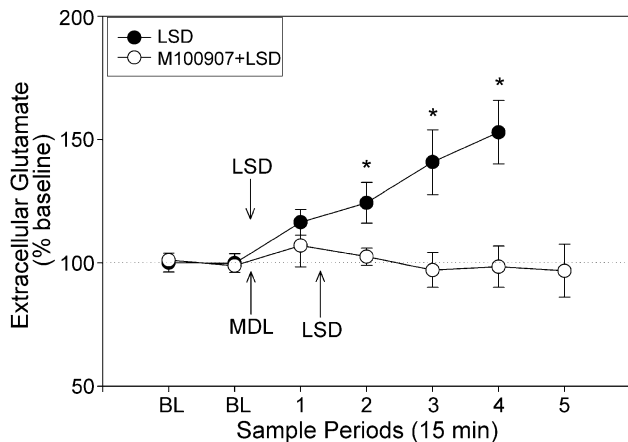


Fig. 2. LSD increases glutamate efflux in the prefrontal cortex via the 5-HT_{2A} receptor. Levels of glutamate in dialysate from the prefrontal cortex were measured in animals receiving LSD (0.1 mg/kg, i.p.) alone or LSD in combination with M100907 (0.05 mg/kg, i.p.). Baseline (BL) represents samples prior to drug administration. Arrows indicate the times of drug administration. Collection times for all samples were 15 min. Data are plotted as mean±S.E.M. ($n=7$ for LSD and 5 for LSD+M100907); * $p<0.05$, compared to baseline (one-way ANOVA and Tukey's post hoc test).

analysis, a new aliquot of the OPA stock solution was thawed and diluted 3:1 with Na₂B₄O₇ buffer to give a fresh working solution. Two volumes of sample was mixed with one volume of OPA reagent and allowed to react for 2 min before injection into the HPLC system using a GBC autosampler (model LC1650, Victoria, Australia) with a Rheodyne injector (model 7010, 20 μ l sample loop, Rohnert Park, CA). Mobile phase (0.085 M Na₂HPO₄, 135 μ M EDTA, 15% MeOH, pH 6.50), pumped at 0.35 ml/min by a Gilson Medical Electronics pump (model 307, Middleton, WI), delivered analytes onto a ThermoQuest (Cheshire, England) reversed-phase minibore column (3 mm inner diameter, 50 cm long, packed with 3 μ m Hypersil C-18 particles). After separation on the column, electrochemical detection was performed with Antec's Decade system (Leiden, The Netherlands). Amino acid derivatives were oxidized in a VT-03 micro flow cell (11 nl) by a 2.74 mm glassy carbon working electrode maintained at +0.8 V relative to a Ag/AgCl reference. Chromatograms were acquired and analyzed on an IBM-compatible computer running Gilson's Unipoint system controller software. Mean basal glutamate in dialysate collected during experiments was 961 ± 221 pg/ μ l ($n=10$).

2.3. Drugs

LSD and DOM were generously provided by the National Institute on Drug Abuse (Rockville, MD) and dissolved in 0.9% saline for i.p. injection or Dulbecco's PBS for reverse-dialysis experiments. *R*-(+)- α -(2,3-dimethoxyphenyl)-1-[2-4-fluorophenyl]ethyl]-4-piperidinemethanol (M100907) was dissolved in a minimal volume of 0.2% w/v tartaric acid and diluted with water.

3. Results

3.1. Experiment 1

As shown in Fig. 2, administration of LSD (0.1 mg/kg, i.p.) produced a time-dependent increase in extracellular glutamate in the frontal cortex [sample \times drug, $F(5,5)=8.76$, $p<0.001$; drug, $F(1,10)=10.62$, $p<0.01$, two-way repeated measures ANOVA]. This dose of LSD is behaviorally relevant, as it is identical to that used in stimulus training and produces maximal LSD-appropriate responding in animals [28,57]. Extracellular glutamate levels were significantly increased 30 min after the injection of LSD and continued to rise for at least an additional 30 min, reaching 156% of baseline [$F(5,30)=14.65$, $p<0.001$, one-way repeated measures ANOVA with Tukey's post hoc test].

Because LSD can interact with various serotonin receptor subtypes as well as other neurotransmitter receptors [15], experiments were undertaken to determine which receptor was responsible for the LSD-induced increase in extracellular glutamate. In studies using drug-induced stimulus control, the selective 5-HT_{2A} receptor antagonist M100907 (0.05 mg/kg, i.p.) blocked drug-appropriate responding in LSD-trained animals [60]. M100907 at the same dose (0.05 mg/kg, i.p.) and pretreatment time (15 min) completely abolished the increase in glutamate release induced by LSD (Fig. 2). Injection of M100907 alone had no significant effects on extracellular glutamate during the 15 min prior to administration of LSD [$F(5,20)=0.217$, n.s.].

3.2. Experiment 2

To confirm that 5-HT_{2A} receptor activation increases glutamate efflux in the frontal cortex, experiments were undertaken using the hallucinogenic 5-HT_{2A/2C} agonist

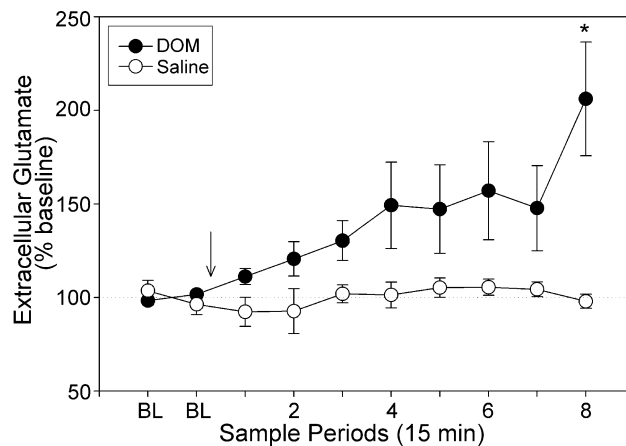


Fig. 3. DOM stimulates glutamate efflux in the prefrontal cortex. Levels of glutamate in dialysate from the prefrontal cortex were measured in animals receiving DOM (0.6 mg/kg, i.p.) or saline (0.9%, 1 ml/kg); arrow indicates time of injection. All sample collection times were 15 min; baseline (BL) represents samples prior to drug administration. Data are plotted as the mean±S.E.M. ($n=5$ for DOM and 3 for saline); * $p<0.05$ compared to baseline (one-way ANOVA and Tukey's post hoc test).

DOM (Fig. 3). Administration of DOM caused a time-dependent increase in extracellular glutamate levels in the prefrontal cortex [sample \times drug, $F(9,9)=2.78$, $p<0.05$; drug, $F(1,5)=6.92$, $p<0.05$, two-way repeated measures ANOVA]. The dose (0.6 mg/kg, i.p.) of DOM used in these studies is comparable to that used for stimulus training and shows complete substitution in LSD-trained subjects [23,58]. Subsequent analysis (one-way ANOVA and Tukey's post hoc test) of the data indicated a statistically significant increase in glutamate levels at the final time point [$F(9,27)=4.44$, $p<0.05$]. Animals injected with saline (1.0 ml/kg, i.p.) failed to show any statistically significant change in extracellular glutamate levels over the course of sample collection [$F(9,18)=0.733$, n.s., one-way repeated measures ANOVA; Fig. 3].

3.3. Experiment 3

The observed 5-HT_{2A} receptor-mediated increase in extracellular glutamate could involve activation of 5-HT_{2A} receptors in the prefrontal cortex or in other brain areas that send projections to the prefrontal cortex [18,39]. To determine if the increased extracellular glutamate efflux was due to activation of a 5-HT_{2A} receptor population in the prefrontal cortex, LSD (10 μ M) was reverse-dialyzed for 30 min into the prefrontal cortex, after which drug-free Dulbecco's PBS was perfused for an additional 45 min. Dialysate was not collected for the time immediately after changing to or from the LSD-containing perfusion medium to exclude artifacts from the momentarily decreased flow rate. Collection of the dialysate was initiated when the perfusate exiting the probe showed LSD fluorescence under

UV light. Similarly, collection of postdrug sample was begun when LSD fluorescence was no longer detected in the probe. In both cases, approximately 15 min was required for LSD to fill or be flushed from the probe.

As shown in Fig. 4, reverse dialysis of LSD directly into the prefrontal cortex resulted in a statistically significant increase in extracellular glutamate levels [$F(6,36)=8.90$, $p<0.001$, one-way repeated measures ANOVA and Tukey's post hoc test]. Interestingly, glutamate levels remained elevated for at least 45 min after LSD was withdrawn from the perfusion medium.

4. Discussion

This study provides direct evidence in vivo for the hypothesis that an enhanced release of glutamate is a common mechanism in the action of hallucinogens [4,9,10]. In this study, the indoleamine hallucinogen LSD caused an increase in extracellular glutamate levels in the prefrontal cortex. Furthermore, this increase in glutamate efflux appears to involve stimulation of the 5-HT_{2A} receptor. Thus, the effects of LSD were abolished by the selective 5-HT_{2A} antagonist M100907, and the 5-HT_{2A/C} agonist DOM, which is a phenethylamine hallucinogen, also increased glutamate efflux. These results supply in vivo confirmation for the in vitro electrophysiological studies suggesting that stimulation of 5-HT_{2A} receptors induce glutamate efflux based upon the observed increase in the frequency of EPSPs and EPSCs [4,9,10].

The hallucinogen-induced increase in glutamate efflux appears to play a significant role in the behavioral effects of the phenethylamine and indoleamine hallucinogens. Ligands for the mGlu2/3 metabotropic glutamate receptor, a putative presynaptic autoreceptor on glutamatergic neurons [17], modulated LSD-appropriate responding in subjects trained to discriminate this compound from saline [60]. Thus, administration of the mGlu2/3 receptor antagonist LY341495, which would be expected to increase glutamate efflux [61], enhanced LSD-appropriate responding, while LY379268, a mGlu2/3 receptor agonist, which would be expected to decrease glutamate efflux [34], reduced LSD-appropriate responding [60]. Similarly, head shakes in rats [25] and head twitches in mice [32] induced by DOI, a congener of DOM, were found to be enhanced and suppressed by the administration of mGlu2/3 receptor antagonists and agonist, respectively. We have previously reported that phencyclidine (PCP), ketamine, and dizocilpine potentiated the stimulus effects of DOM [59]. Although these compounds function as antagonists at the NMDA receptor, PCP and ketamine also have been shown to increase glutamate efflux in the prefrontal cortex [2,40,41]. Thus, the behavioral data support a role for glutamate efflux in the actions of hallucinogens.

The observed time courses for the increase in extracellular glutamate after LSD and DOM administration appear

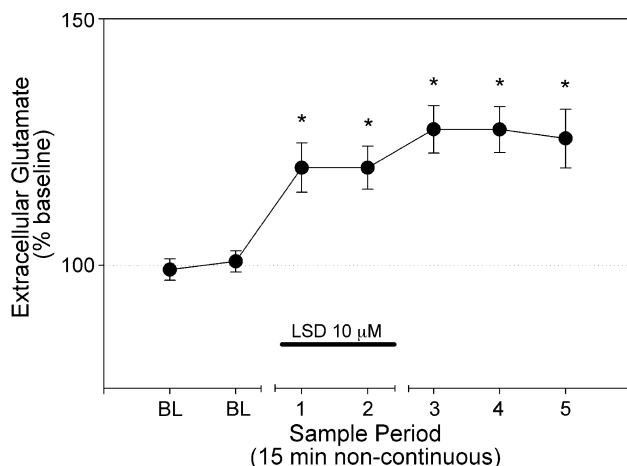


Fig. 4. Reverse dialysis of LSD into the prefrontal cortex increases glutamate efflux. Levels of glutamate in dialysate from the prefrontal cortex were measured in animals receiving 10 μ M LSD by reverse dialysis. All sample collection times were 15 min; baseline (BL) represents samples prior to drug administration. LSD was reverse-dialyzed during the time indicated by the bar; breaks in the x-axis represent periods of transition from PBS to PBS containing LSD and then back to PBS. Data are plotted as the mean \pm S.E.M. ($n=8$); * $p<0.05$ compared to baseline (one-way ANOVA and Tukey's post hoc test).

to be consistent with the behavioral effects of these compounds. In drug-induced stimulus control, a much longer pretreatment time is required for DOM to produce a maximal and stable stimulus effect compared to LSD [23]. Likewise in humans, disruption of performance peaks within 45 min after administration of LSD [3,53], while the effects of DOM reach a maximum only after 3–5 h [29,50,51]. The increase in glutamate efflux caused by these drugs also showed a similar temporal difference, with the effects of LSD occurring much sooner than DOM. Interestingly, glutamate efflux remained elevated even after discontinuation of the intracortical administration of LSD. This however is consistent with the long-lasting hallucinogenic activity of LSD, which can last for 6 to 12 h [1].

Recently, Scruggs et al. [49] also observed an increase in glutamate efflux in the somatosensory cortex after the administration of the phenethylamine hallucinogen DOI. The DOI-induced increase in extracellular glutamate was very slow to develop, and local administration of DOI by reverse dialysis resulted in a transient increase in glutamate efflux. The reason for the temporal differences between these results and this study is unclear. However, these results in conjunction with the present data do suggest that the serotonergic hallucinogens stimulate glutamate efflux in various parts of the cerebral cortex.

The source of the glutamate released by 5-HT_{2A} receptor stimulation is unclear. However, the finding that both systemic and intracortical administration of LSD increased glutamate suggests that the hallucinogens are acting locally within the prefrontal cortex. Although a small amount of 5-HT_{2A} receptor immunoreactivity is associated with presynaptic structures that appear to make asymmetric contact with cortical pyramidal cells, the majority of 5-HT_{2A} receptor protein is located postsynaptically in the pyramidal cells themselves [19,30,39]. Activation of these postsynaptic 5-HT_{2A} receptors by hallucinogens is posited to evoke glutamate release from thalamocortical terminals indirectly via an unidentified retrograde messenger [5]. The role of thalamocortical afferents as the source of the glutamate is suggested by studies in which ablation of thalamic nuclei contributing afferents to the cortex was reported to attenuate both serotonin-induced EPSCs [33,35] and DOI-induced Fos expression [48]. Conversely, Puig et al. [44] reported that extensive thalamic lesions did not alter the effects of DOI on the firing of cortical pyramidal neurons. Stimulation of 5-HT_{2A} receptors can cause glutamate efflux from C6-glioma cells in culture [37], indicating that glia could be the cellular source of glutamate release in native brain tissue. Recently, it has been demonstrated that a small population of glial cells in the prefrontal cortex expresses 5-HT_{2A} receptors [39]. Given the importance of glia in the management of glutamatergic transmission through “tripartite” synapses [8,42], the possibility of these cells contributing to the effect presented here cannot be ruled out.

In conclusion, this study demonstrates that 5-HT_{2A} receptors in the rat prefrontal cortex induce an increase in

glutamate efflux when activated by indoleamine and phenethylamine hallucinogens. This finding is suggestive of glutamate release as an essential element in drug-induced stimulus control and a possible biochemical feature of hallucination.

Acknowledgements

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