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Short communication

D-serine deficiency attenuates the behavioral and cellular effects induced by the hallucinogenic 5-HT_{2A} receptor agonist DOI

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HIGHLIGHTS

- D-serine deficiency diminishes head twitch response to a 5-HT_{2A} receptor agonist.
- D-serine deficiency diminishes *c-fos* induction to a 5-HT_{2A} receptor agonist.
- D-serine deficiency does not alter 5-HT_{2A} and mGlu2 receptor expression.

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ABSTRACT

Both the serotonin and glutamate systems have been implicated in the pathophysiology of schizophrenia, as well as in the mechanism of action of antipsychotic drugs. Psychedelic drugs act through the serotonin 2A receptor (5-HT_{2A}R), and elicit a head-twitch response (HTR) in mice, which directly correlates to 5-HT_{2A}R activation and is absent in 5-HT_{2A}R knockout mice. The precise mechanism of this response remains unclear, but both an intrinsic cortico-cortical pathway and a thalamo-cortical pathway involving glutamate release have been proposed. Here, we used a genetic model of NMDAR hypofunction, the serine racemase knockout (SRKO) mouse, to explore the role of glutamatergic transmission in regulating 5-HT_{2A}R-mediated cellular and behavioral responses. SRKO mice treated with the 5-HT_{2A}R agonist (±)-2,5-dimethoxy-4-iodoamphetamine (DOI) showed a clearly diminished HTR and lower induction of *c-fos* mRNA. These altered functional responses in SRKO mice were not associated with changes in cortical or hippocampal 5-HT levels or in 5-HT_{2A}R and metabotropic glutamate-2 receptor (mGluR2) mRNA and protein expression. Together, these findings suggest that D-serine-dependent NMDAR activity is involved in mediating the cellular and behavioral effects of 5-HT_{2A}R activation.

Abbreviations: (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride, DOI; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; serotonin 2A receptors, 5-HT2ARs; head twitch response, HTR; mGluR2, metabotropic glutamate receptor 2/3; M2, motor cortex; CG1, cingulate cortex; PrL, prelimbic cortex NMDA receptor, NMDAR; prefrontal cortex, PFC; phencyclidine, PCP; S1 cortex, primary somatosensory cortex; serine racemase knockout, SRKO

Activation of serotonin 2A receptors (5-HT_{2A}Rs) by psychedelic drugs mediate their psychotomimetic effects, which include alterations of human consciousness, emotion, and cognition [1]. In rodents, administration of hallucinogens results in an induction of immediate-early genes (IEGs), such as *Arc*, *c-fos*, and *Egr-2* in cortical brain regions and a characteristic head-twitch response (HTR) [2,3]. These cellular and behavioral outputs are blocked by selective 5-HT_{2A}R antagonists [4] and eliminated in 5-HT_{2A} R deficient mice, but can be rescued by genetic restoration of 5-HT_{2A}R to cortical pyramidal neurons [5]. Furthermore, only compounds, which cause hallucinations in humans induce HTR, suggesting that this response is an indicator of hallucinogenic action [5]. Thus, both the induction of cortical IEG expression and HTR can be utilized as a 5-HT_{2A}R-specific measure of receptor efficiency.

Several studies suggest an interaction between 5-HT_{2A}Rs and the glutamate system. 5-HT_{2A}Rs are primarily expressed on apical dendrites of pyramidal neurons, particularly in cortical layer V [6].

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Glutamate serves as the principal neurotransmitter of the pyramidal cells, and it is thought that cortico-cortical connections, which are mostly comprised of synaptic contacts at apical dendrites [7], are important in generating and shaping the neural activity that underlies consciousness [8].

Acute administration of the NMDA receptor (NMDAR) antagonists PCP and MK-801 to rodents induces hyperlocomotion and HTR, which are blocked by 5-HT_{2A}R antagonists [9–11]. Also, atypical antipsychotics with 5-HT_{2A}R antagonistic properties are able to reverse acute PCP-induced cellular disruptions in the prefrontal cortex (PFC) [12]. Together, these studies indicate a potential role of circuits working through the 5-HT_{2A}R in mediating the behavioral responses induced by NMDAR antagonists.

Previous studies have proposed that cortical activation by DOI requires the activation of 5-HT_{2A}Rs expressed on thalamo-cortical axon terminals [13]. However, recent evidence suggests that the circuit involved in this effect is a cortico-cortical circuit and does not require activation of a thalamo-cortical pathway [5]. In fact, DOI does not induce *Arc* directly in 5-HT_{2A}R expressing neurons, but in neurons containing AMPA and NMDA receptors [3]. Furthermore, activation of 5-HT_{2A}Rs in the PFC results in a robust increase in glutamate receptor activation, which suggests that certain actions of 5-HT_{2A}Rs in this region may be mediated by the release of glutamate [14].

To study the involvement of the glutamate system in mediating the cellular and behavioral responses to 5-HT_{2A}R activation, we used an animal model of NMDAR hypofunction, the constitutive serine racemase knockout (SRKO) mouse. Activation of NMDARs requires the binding of either glycine or D-serine to the glycine modulatory site (GMS) of the GluN1 subunit. SRKO mice, which lack the ability to convert L-serine to D-serine, have an 85% reduction in cortical D-serine [15]. D-serine is enriched in corticolimbic regions of the brain, where its localization closely parallels that of NMDARs [16]. Thus, SRKO mice exhibit reduced forebrain NMDAR-mediated neurotransmission [15,17] that is associated with cognitive impairments dependent on the PFC [18] and hippocampus [17]. In the present study, we tested whether the functional outputs following 5-HT_{2A}R activation were altered due to decreased NMDAR activity.

SRKO mice were generated as previously described [15]. Mice with a serine racemase null mutation resulting from targeted deletion of the first coding exon were backcrossed for over 10 generations onto a C57BL/6J background. SR heterozygous sires and dams were bred to produce wild-type (WT), as well as SRKO offspring. Adult male and female mice were used for all the experiments in this study. Animals were housed in groups of four in polycarbonate cages and maintained on a 12:12 h light/dark cycle in a temperature (22 °C) and humidity controlled vivarium. Animals were given access to food and water ad libitum. All the animal procedures were approved by the McLean Hospital Institutional Animal Care and Use Committee.

Head-twitch response (HTR) scoring was performed as previously reported [19], with minor modifications. In one cohort, WT and SRKO mice ($n = 6$ per group) were injected with either (±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI: synthesized at the Department of Medicinal Chemistry, Faculty of Pharmaceutical Sciences, Copenhagen University; 2 mg/kg, 10 ml/kg, i.p.) or a corresponding volume of saline (10 ml/kg). In a second cohort, WT and SRKO mice ($n = 5$ to 6 per group) were injected with either DOI (0.5 mg/kg, 10 ml/kg, i.p.) or a corresponding volume of saline (10 ml/kg). After drug administration the mice were immediately moved to a standard cage (15 × 24 cm) without enrichment material and videotaped for 25 min. Subsequently, the total number of head-twitches was scored in the interval between 5 and 25 min after drug administration by an observer not aware of the treatment of the animal. For the first cohort, the experiment was repeated one week later with a crossover design so that animals receiving DOI on the first test day were given saline on the second test day and vice versa. The crossover design was not implemented for the second cohort, as separate groups of WT and SRKO mice received either DOI or saline.

For mRNA expression analysis, animals ($n = 6$–9 per group) were injected with either DOI (2 mg/kg, 10 ml/kg, i.p.) or a corresponding volume of saline and returned to their home-cage. After 60 min, the mice were killed by cervical dislocation, and their brains quickly removed and frozen in powdered dry ice. Total RNA from cortical samples was isolated with Trizol Reagent (Sigma–Aldrich). cDNA for each RNA sample was generated using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Foster City, CA) according to the manufacturer's instructions. The real-time qPCR reactions were performed by adding the sample cDNA to a reaction mixture consisting of 1x Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen, Carlsbad, CA) and 15 pmol of each primer. Specific primers were as follows: *GAPDH* primers, Forward: 5′-CATCAAGAAGGTGGTGAAGCA-3′, Reverse: 5′-CTGTTGAAGTCACAGGAGACA-3′; *c-fos* primers, Forward: 5′-CAAAGTAGAGCAGCTATCTCC-3′, Reverse: 5′- CTCGTCTTCAAGTTGATCTGT-3′; *5-HT_{2A}R* primers, Forward: 5′-CCGCTTCAACTCCAGAAC CAAAGC-3′, Reverse: 5′-CTTCGAATCATCCTGTACCCGAA-3′; *5-mGluR2* primers, Forward: 5′-CCATCTTCTACGTCACCTCC-3′, Reverse: 5′-AGGAACAAGCTGGGATCCAG-3′. Data were collected using a 48-well MJ minioption personal thermal cycler (BioRad; Hercules, CA). Each sample was assayed in triplicate. For relative quantification of mRNA expression, geometric means were calculated using the comparative $2^{-\Delta\Delta Ct}$ method, with the housekeeping gene GAPDH used as the endogenous reference.

To determine receptor binding, animals ($n = 8$ per group) were decapitated and brains were quickly removed and stored at −80 °C until sectioning. Brains were cut in 12 μm coronal sections and mounted on Super frost plus slides and stored at −80 °C until further processing. 5-HT_{2A}R autoradiography was performed using [^{3}H]-MDL100907 [R(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorphenyl)-ethyl]-4-piperidin-methanol] (specific activity; 64 Ci/mmol, a gift from Professor Halldin, Karolinska Institute, Stockholm), and non-specific binding was determined using 10 μM ketanserin tartrate (Sigma). For mGluR2/3 autoradiography, [^{3}H]-LY341495 (GE Healthcare, UK) was used and non-specific binding was measured with 10 μM glutamate (Sigma). Briefly, sections were allowed to thaw for 1 h at RT and then pre-incubated with 50 mM Tris–HCl (Sigma), pH 7.4 for 30 min at RT under constant gentle shaking. Sections were then incubated for 60 min at RT using the same buffer containing either 2 nM [^{3}H]-MDL100907 or 2 nM [^{3}H]-LY341495 with or without the respective cold ligand. Following incubation, slides were washed for 2 × 20 s in ice-cold 50 mM Tris–HCl, pH 7.4, for 20 s in ice-cold H_2O, and dried for 1 h under a gentle stream of air. All sections were placed at 4 °C overnight in a fixator containing paraformaldehyde vapor and then put in an excitator box for 3 h before slides, together with [^{3}H]-microscales (GE Healthcare, UK), were exposed to a BAS-TR2040 imaging plate (Science Imaging Scandinavia AB, Nacka, Sweden) for 3–14 days at 4 °C. Finally, the imaging plate was scanned on a BAS-2500 phosphoimage scanner (Fujifilm Europe GmbH, Düsseldorf, Germany). Specific and non-specific binding were determined at two brain levels where the following regions were analyzed (i) bregma 2.34 mm: total M2/CG1/PrL (motor/cingulate/prelimbic) cortex, (ii) bregma −2.06 mm: hippocampus and S1 cortex.

Head-twitch behavior and *c-fos* mRNA expression in cortical areas are reliably and robustly elicited by hallucinogenic 5-HT_{2A}R agonists in rodents. We first assayed the HTR induced by DOI in wild-type and SRKO mice (Fig. 1A). Two-way ANOVA showed a significant effect of treatment [$F(1,19) = 57.16$; $p < 0.001$] in animals treated with 0.5 mg/kg DOI or saline, as well as a