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ORIGINAL INVESTIGATION

Effects of the 5-HT_{2A} receptor antagonist volinanserin on head-twitch response and intracranial self-stimulation depression induced by different structural classes of psychedelics in rodents

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Abstract

Background Clinical studies suggest that psychedelics exert robust therapeutic benefits in a number of psychiatric conditions including substance use disorder. Preclinical studies focused on safety and efficacy of these compounds are necessary to determine the full range of psychedelics' effects.

Objectives The present study explores the behavioral pharmacology of structurally distinct psychedelics in paradigms associated with serotonin 2A receptor (5-HT_{2A}R) activation and behavioral disruption in two rodent models. Utilizing the selective 5-HT_{2A}R antagonist volinanserin, we aimed to provide further pharmacological assessment of psychedelic effects in rodents.

Methods We compared volinanserin (0.0001–0.1 mg/kg) antagonism of the phenethylamine 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI, 1.0 mg/kg) and the ergoline lysergic acid diethylamide (LSD, 0.32 mg/kg) in preclinical assays predictive of hallucinations (head-twitch response or HTR in mice) and behavioral disruption (intracranial self-stimulation or ICSS in rats). Volinanserin antagonism of the phenethylamine mescaline, the tryptamine psilocybin, and the k-opioid receptor agonist salvinorin A was also evaluated in the rat ICSS assay.

Results Volinanserin had similar potency, effectiveness, and time-course to attenuate DOI–induced HTR in mice and ICSS depression in rats. Volinanserin completely blocked LSD–induced HTR in mice, but not LSD–induced ICSS depression in rats. Volinanserin also reversed ICSS depression by mescaline, but it was only partially effective to reduce the effects of psilocybin, and it exacerbated ICSS depression by salvinorin A.

Conclusion Although hallucination-related HTR behavior induced by phenethylamine, ergoline, and tryptamine psychedelics appears to be 5-HT_{2A}R-mediated, the receptor(s) responsible for behavioral disruptive effects may differ among these three structural classes.

Keywords Serotonin 5-HT_{2A} receptor · G protein-coupled receptor (GPCR) · Psychedelics · Hallucinogens · Head twitch response (HTR) · Intracranial self-stimulation (ICSS) · Phenethylamines · Tryptamines · Ergolines · Volinanserin · Psychopharmacology

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Introduction

Serotonergic psychedelics constitute a class of compounds that act primarily through activation of the serotonin (5-hydroxytryptamine) 2A (5-HT_{2A}R) receptor (González-Maeso et al. 2007; Halberstadt and Geyer 2011; López-Giménez and González-Maeso, 2018; Kometer et al. 2013; Preller et al. 2018). Typically, these compounds are categorized into two main structural categories: phenethylamines such as mescaline and 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), and tryptamines which can be subdivided into simple tryptamines such as psilocybin and ergolines such as lysergic acid diethylamide (LSD). Although they present distinct molecular scaffolds, these different structural classes of psychedelics produce similar effects on cognition, perception, and sensory processing mostly via 5-HT_{2A}R activation (Nichols 2016; Glennon et al. 1984; Glennon 1994).

Although serotonergic psychedelics are not currently approved for clinical use, several lines of evidence have suggested that this class of psychoactive compounds may be useful for treatment of certain psychiatric conditions, such as mood disorders (Holze et al. 2021; Carhart-Harris et al. 2021, 2016; Gasser et al. 2014; Griffiths et al. 2016) and substance use disorder (Bogenschutz et al. 2015; Johnson et al. 2014, 2017; Krebs and Johansen 2012). Similar findings have been reported in preclinical rodent models of these psychiatric conditions (Cui et al. 2018; Alper et al. 2018; Oppong-Damoah et al. 2019). Even with the growing evidence for their therapeutic potential, the behavioral-disruptive effects associated with the use of these drugs may limit their future success in clinical practice. Furthermore, while the hallucinogenic and related effects of psychedelics are 5-HT_{2A}R-dependent as shown in healthy volunteers (Vollenweider et al. 1998; Schmid et al. 2015) and in rodent models (González-Maeso et al. 2007; Halberstadt et al. 2020), whether the clinically relevant effects of these compounds are exclusively 5-HT_{2A}R-dependent (Ly et al. 2018; Cameron et al. 2021; de la Fuente Revenga et al. 2021*)* or via a mixture of molecular targets (Hesselgrave et al. 2021; Dong et al. 2021; Shao et al. 2021) is still controversial.

Head-twitch response (HTR) is a behavior naturally present in rodents, whose manifestation greatly increases in frequency upon psychedelic administration. This psychedelic-induced side-to-side movement of the head bears a high degree of specificity as it is not seen with other psychoactive drugs such as cocaine, phencyclidine, or amphetamine (de la Fuente Revenga et al. 2020; Halberstadt and Geyer 2011). The potency of psychedelics determined via mouse HTR is highly correlated with potencies to elicit hallucinations in humans (Halberstadt et al. 2020), and several previous findings based on pharmacological blockade with potent 5-HT_2R and 5-HT_{2A}R antagonists such as ketanserin and volinanserin (also known as M100907), respectively, or deletion of the *5-HT_{2A}R* (*Htr2a*) gene in knock-out mice demonstrated HTR to be 5-HT_{2A}R-mediated (González-Maeso et al. 2007; Shao et al. 2021; de la Fuente Revenga et al. 2020; Hanks and González-Maeso 2013). The predictive validity and dependency on 5-HT_{2A}R make HTR a reliable functional readout of psychedelic drug action in mice.

Intracranial self-stimulation (ICSS) is a commonly used rodent behavioral model to assess how psychoactive drugs, including psychedelics, opioids, and novel compounds of interest, affect the brain reward system (Fiorino et al. 1993; Carlezon and Chartoff 2007; Negus and Miller 2014). In ICSS procedures, subjects are equipped with intracranial microelectrodes and trained to engage in operant responding to earn pulses of electrical brain stimulation. Drug-induced increases in ICSS are often interpreted as evidence of abuse liability, whereas drug-induced decreases in ICSS provide a measure of drug-induced behavioral disruption. We reported previously that the psychedelics LSD, mescaline, and psilocybin primarily decrease ICSS in rats (Sakloth et al. 2019), suggesting that these compounds have low abuse potential but can produce robust behavioral disruption that may be related to the behavioral perturbations induced upon acute administration of these drugs in humans.

To further understand the pharmacological target(s) responsible for psychedelic effects in preclinical models of potentially therapeutic hallucinogen drug action and undesirable behavioral disruption, here we compared how a potent and highly selective 5-HT_{2A}R antagonist, volinanserin, can alter HTR and ICSS effects elicited by four members of the three groups of psychedelics: the phenethylamines DOI and mescaline, and the more popularized ergoline LSD and tryptamine psilocybin.

Methods

Animals

For HTR assays, adult (8–20 weeks old) C57BL/6 male mice (Jackson Laboratory) were housed in cages with up to 5 littermates. For ICSS assays, 11 adult male Sprague–Dawley rats (Envigo) that weighed approximately 300 g at the time of surgery were individually housed. Mice and rats had ad libitum access to food and water in the home cage, and were kept under a 12-h light/dark cycle (lights on 6 a.m. to 6 p.m.) in a temperature- and humidity-controlled facility accredited by Association for the Assessment and Accreditation of Laboratory Animal Care. The animal use protocol was approved by the Virginia Commonwealth University

Institutional Animal Care and Use Committee in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Drugs

For HTR assays, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ((±)-DOI) hydrochloride and (*R*)-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl) ethyl]-4-piperinemethanol (volinanserin, or M100907) were purchased from Sigma-Aldrich. Lysergic acid diethylamide (LSD) free base was purchased from Lipomed AG. (±)-DOI hydrochloride and volinanserin were dissolved in saline (0.9% NaCl), and LSD free base was dissolved in 0.9% saline + DMSO to the appropriate volume and dose for intraperitoneal (i.p.) administration. Vehicle-treated condition denotes injection of saline (i.p.) to the equivalent volume of the drug administered.

For ICSS assays, *R*(–)-DOI hydrochloride, psilocybin, mescaline hydrochloride, LSD tartrate salt (2:1), and salvinorin A were provided by the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD). Volinanserin was also purchased from Sigma-Aldrich. All drugs except salvinorin A were dissolved in sterile saline. Salinvorin A was dissolved in 75% DMSO in sterile water, as we have described previously (Negus et al. 2012).

For simplicity purposes, the racemic ((±)-DOI) and enantiomeric (*R*(–)-DOI) forms of DOI used in HTR and ICSS, respectively, are both named DOI throughout the rest of the manuscript. Drug doses were not adjusted based on their salt form. All drugs were administered i.p., and dissolved in 1-ml/kg vehicle.

Head-twitch behavior (HTR)

Detection of HTR in mice was performed as previously reported (de la Fuente Revenga et al. 2019; de la Fuente Revenga et al. 2020) with additional visual inspection. Neodymium magnets (N50, 3 mm diameter × 1 mm height, 50 mg) were glued with cyanoacrylate to the top surface of aluminum ear tags for rodents (Las Pias Ear Tag, Stoelting Co.) with the magnetic south of the magnet in contact with the tag. Mice were ear-tagged bilaterally through the pinna antihelix under ketamine/xylazine anesthesia (120 mg/kg and 12 mg/kg, respectively) or isoflurane (2%). Following ear-tagging, animals were placed back into their home cages for at least 1 week before initiation of testing. Data acquisition and processing were performed as previously described (de la Fuente Revenga et al. 2019; de la Fuente Revenga et al. 2020). Briefly, HTR was evaluated by placing mice individually into a monitoring chamber (inner dimensions, 11 cm diameter × 14 cm tall) surrounded by a coil (~500 turns 30 AWG enameled wire), and changes in electrical current elicited in the coil by movement of the ear-tag magnets were amplified (Pyle PP444 phono amplifier) and recorded at 1000 Hz using a NI USB-6001 (National Instruments) data acquisition system. To refine HTR detection, the signal was also processed using a deep learning-based protocol based on scalograms (Halberstadt 2020) , and mismatches between both detection methods were inspected visually without clues relative to the timestamp of the event or treatment group. After classification of dubious events, the final values of HTR counts were corrected with a custom script. The |V| threshold for the initial filter using the *findpeaks()* function in MATLAB (Mathworks, R2019a) was set to a more permissive 1/5 of the previously reported value (de la Fuente Revenga et al. 2020). The convolutional neural network employed was trained using HTR ($n = \sim 3000$) and non-HTR ($n = \sim 1000$) events from our previous studies (de la Fuente Revenga et al. 2020) and unpublished from magnet ear-tagged mice.

Testing occurred no more than once per week with at least 7 days between test sessions. On test days, mice were placed individually into the monitoring chamber for 30 min to acclimate to the environment and determine baseline HTR (data not shown). Subsequently, the animals received the corresponding treatment, and HTRs were recorded. In experiments in which animals had a 15-min pretreatment with volinanserin (0.0001–0.1 mg/kg), acclimation was followed first by volinanserin administration and a 15-min HTR recording period and then by DOI or LSD administration (1.0 mg/kg or 0.32 mg/kg i.p., respectively) and recording for an additional 90 min. In experiments where the pretreatment time for 0.032 mg/kg volinanserin was 1, 4, or 24 h, mice received their volinanserin treatment and were returned to their home cage until 15 min prior to DOI or LSD injection. Mice were then placed into the monitoring chamber and HTR recorded for 15 min, followed by administration of DOI or LSD and further HTR recording for an additional 90 min (Fig. 1). Doses of DOI and LSD were based on prior published studies (de la Fuente Revenga et al. 2020; de la Fuente Revenga et al. 2021; Vohra et al. 2021; Halberstadt and Geyer 2014; Halberstadt et al. 2020).

Intracranial self-stimulation (ICSS)

ICSS assays in male rats were performed as previously reported with minor changes (Negus and Miller, 2014; Sakloth et al. 2019). Briefly, prior to initiation of training, all rats were surgically implanted with a microelectrode targeting the medial forebrain bundle (coordinates: 2.8 mm posterior to bregma, 1.7 mm lateral to the midsagittal suture, and 8.8 mm ventral to the skull) using procedures described previously (Negus and Miller 2014; Sakloth et al. 2019). All experiments were conducted in operant conditioning chambers housed in sound-attenuating boxes and equipped

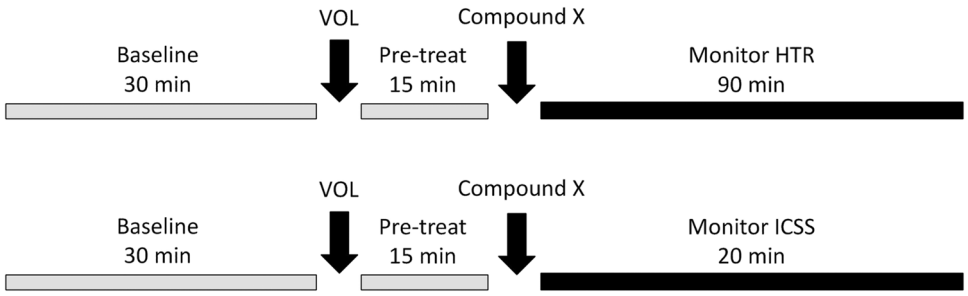

Fig. 1 Schematic of mouse HTR and rat ICSS experimental design. A 30-min baseline period was followed first by administration of volinanserin or vehicle and then by a pretreatment interval before administration of one of the psychedelics, or vehicle. HTR was then monitored for 90 min, with the first 30 min being used for dose–effect and time-course analysis, and ICSS was monitored for 20 min. In dose–effect studies, the dose of volinanserin was varied across experiments and the pretreatment interval was held constant at 15 min as shown in the figure. In time-course studies, the volinanserin dose was held constant at 0.032 mg/kg and the pretreatment interval was varied

with a response lever, three stimulus lights above the lever, a house light, and an ICSS stimulator (Med Associates). The intracranial electrode was connected to the stimulator via bipolar cables routed through a swivel-commutator (Model SL2C, Plastics One). Stimulus deliveries were controlled and lever-press responses were recorded with a computer and interface operated by MED-PC IV Computer software (Med Associates).

Procedures for training and testing were similar to those used previously to examine effects of psilocybin, mescaline, and LSD administered alone (Sakloth et al. 2019). Briefly, each behavioral session commenced with illumination of the house light, and rats could respond under a fixed-ratio 1 (FR 1) schedule for brain stimulation delivered via the intracranial electrode. Each stimulation consisted of a 0.5-s train of square wave cathodal pulses (0.1 ms per pulse) at a designated frequency and amplitude accompanied by illumination of the stimulus lights over the lever. Under the terminal schedule of reinforcement, 30-min behavioral sessions consisted of three 10-min components, and each component consisted of ten 1-min frequency trials. During each component, the frequency of brain stimulation decreased across the 10 trials in 0.05 log increments from 158 to 56 Hz (2.2 to 1.75 log Hz). The frequency then resets to 158 Hz at the start of the next component. Each trial began with an initial 10-s time out, during which the house light was off, responding had no scheduled consequences, and five noncontingent stimulations at the designated frequency were delivered. For the remaining 50 s of each trial, the house light was illuminated, and responding produced both brain stimulation under an FR 1 schedule and illumination of the three stimulus lights as described above. ICSS training was considered complete when frequency-rate curves were not statistically different over 3 consecutive days of training as indicated by lack of a significant effect of day in a two-way analysis of variance (ANOVA) with day and frequency as the main effect variables (see the "Data analysis" section later). All training was completed within 7 weeks after surgery.

Testing was conducted in two cohorts of rats to evaluate effectiveness of volinanserin to antagonize the ICSS rate-decreasing effects of DOI (1.0 mg/kg), psilocybin (1.0 mg/kg), mescaline (32 mg/kg), and LSD (0.32 mg/kg). Effects of volinanserin pretreatment to the k-opioid receptor agonist salvinorin A (3.2 mg/kg) were also evaluated as an internal control. Agonist doses were based on prior unpublished (DOI) or published (psilocybin, mescaline, LSD, salvinorin A) (Negus et al. 2012; Sakloth et al. 2019) data that identified the lowest agonist dose to decrease ICSS to < 50% of baseline. The first cohort ($N = 5$) had a prior history of studies with methcathinone analogs (Davies et al. 2020), and was used for studies of volinanserin in combination with DOI, psilocybin, and mescaline, and there was a 2-week washout period before initiation of the present study. The electrode in one rat became displaced during studies with mescaline, so effects of volinanserin in combination with mescaline were studied in only four rats, and a new cohort of six drug-naive rats was used for studies of volinanserin in combination with LSD and salvinorin A.

The potency of volinanserin was determined in combination with DOI by manipulating the volinanserin dose (vehicle, 0.001–0.032 mg/kg) administered 15 min before DOI. The time-course of volinanserin was determined by manipulating the pretreatment time (15 min–24 h) for 0.032 mg/kg volinanserin administered before DOI. Based on these results and the preliminary findings that volinanserin doses > 0.032 mg/kg significantly decreased ICSS when administered alone (unpublished), other studies examined the effects of vehicle or 0.032 mg/kg volinanserin administered 15 min before psilocybin, mescaline, LSD, or salvinorin A. Additionally, the effects of two vehicle injections and of 0.032 mg/kg volinanserin administered 15 min before a vehicle injection were also determined as controls in both cohorts. For all studies in both cohorts, test sessions

were conducted twice per week at least 48 h apart and consisted of three "baseline components" followed by a time-out period and then by two "test" components. For most studies, the time-out period was 30 min long. Rats received an i.p. injection of saline or volinanserin at the start of the time-out period followed 15 min later by a second i.p. injection of saline or an agonist (DOI, psilocybin, mescaline, LSD, or salvinorin A), and test components began 15 min after the second injection. For time-course studies, the time-out period was lengthened to accommodate volinanserin pretreatment times of 15 min to 24 h before DOI injection, and test components again began 15 min after DOI (Fig. 1). In each cohort and with each agonist, the order of saline and volinanserin doses was randomized across rats using a Latin square design, and three-component baseline training sessions were conducted on non-testing weekdays to maintain responding.

Data analysis

For HTR, the number of events during sequential 15-min blocks and time-course data were analyzed by repeated measure (RM) two-way ANOVA, with treatment and time as two within-subject independent variables. A secondary dependent measure focused on total HTR from 0 to 30 min after DOI or LSD administration, because this is the time of peak HTR after both drugs. These data were analyzed by both one-way ANOVA with treatment doses as a within-subject independent variable and two-way ANOVA with volinanserin/vehicle and psilocybin/vehicle as two within-subject independent variables. A significant ANOVA for all statistics was followed by the Holm-Šidak post hoc test. Data for % antagonism in experiments with volinanserin and DOI were also analyzed by log-linear regression to determine a volinanserin AD_{50} (i.e., the antagonist dose sufficient to produce 50% antagonism of DOI after a 15-min pretreatment) and half-life for antagonist effects of 0.032 mg/kg volinanserin (i.e., the time until antagonism by this dose decreased to 50%).

For ICSS, the first component of each test session was considered to be an acclimation component, and these data were not used for analysis. The primary dependent variable was the total number of stimulations received per component across all 10 frequency trials. The average number of total stimulations per test component was expressed as a percentage of the average number of stimulations during each baseline component within each rat on each test day: % Baseline Stimulations per Component = (average stimulations per test component ÷ average stimulations per baseline component) × 100. Results were averaged across rats and compared by one-way ANOVA. The criterion for significance was $p < 0.05$, and a significant ANOVA was followed by a Holm-Šidak post hoc test. Data for % Baseline Stimulations per Component in experiments with volinanserin and DOI were also analyzed by log-linear regression to determine a volinanserin AD_{50} and half-life.

A secondary and more granular measure of ICSS performance was the reinforcement rate in stimulations per frequency trial. Raw reinforcement rates for each rat from each trial were converted to percent maximum control rate (%MCR), with MCR defined as the mean of the maximal rates observed at any trial during the baseline components. Thus, %MCR values for each trial were calculated as [(reinforcement rate during a frequency trial ÷ MCR) × 100]. %MCR values were then averaged across rats and analyzed by RM two-way ANOVA, with ICSS frequency and volinanserin dose as the two independent variables. A significant ANOVA was followed by the Holm-Šidak post-hoc test to compare test drug alone with volinanserin + test drug.

All statistical analyses were performed with GraphPad Prism software version 9, and the criterion for statistical significance was $p < 0.05$ for all analyses. All data are represented as mean ± standard error of the mean (S.E.M.).

Results

Volinanserin antagonism of DOI–induced HTR and ICSS depression

In the mouse HTR, DOI alone (1.0 mg/kg) produced a robust and long-lasting increase on HTR counts, typically 200–400 total counts over 90 min, which peaked during the first 30 min post injection before decreasing steadily across time points (Fig. 2A and Fig. S1). In dose–effect studies, volinanserin (0.001–0.1 mg/kg) produced a dose-dependent and complete blockade of the DOI–induced HTR (Fig. 2A, B and Fig. S1). As expected (Fantegrossi et al. 2010), volinanserin (0.01 mg/kg) alone did not affect HTR (Fig. 2C). The volinanserin AD_{50} (95% CL) to produce 50% antagonism of 1.0 mg/kg DOI–induced HTR was 0.0062 mg/kg (0.0040–0.0098). In time-course experiments, peak antagonism produced by 0.032 mg/kg volinanserin was observed after 15 min and was still significant after 1 h but not 4 or 24 h (Fig. S2 and Table 1). The half-life (95% CL) of volinanserin (0.032 mg/kg) antagonism in DOI–induced HTR was 1.85 h (1.48–2.34).

In ICSS, under vehicle control conditions, electrical brain stimulation maintained a frequency-dependent increase in reinforcement rates and acute DOI administration (1.0 mg/kg) eliminated ICSS responding (Fig. 2D). In dose–effect studies, volinanserin (0.001–0.032 mg/kg) produced a dose-dependent and complete blockade of DOI–induced ICSS depression, with significant antagonism at volinanserin doses of 0.01 and 0.032 mg/

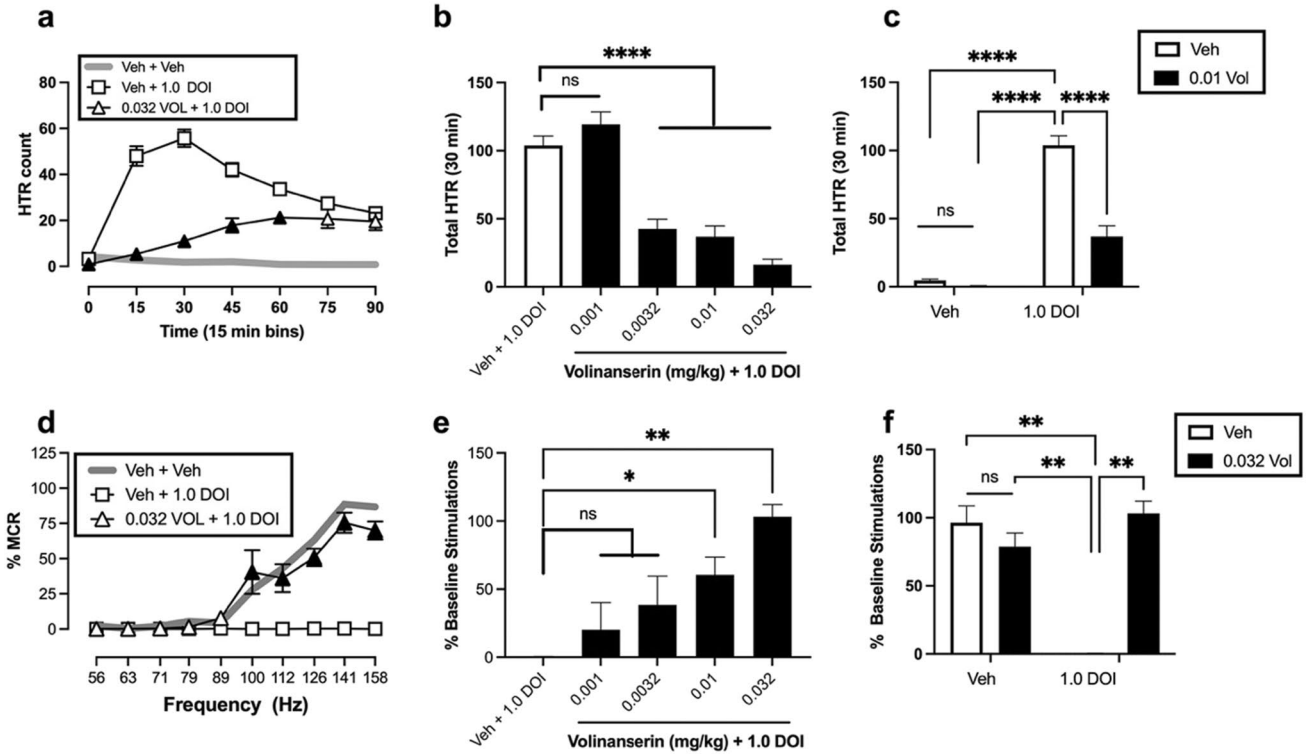

Fig. 2 Effects of volinanserin on DOI–induced HTR in mice (**a**, **b**, **c**) and ICSS depression in rats (**d**, **e**, **f**). **a** Time-course of HTR as head twitch response (frequency of head twitches) over time split into 15-min bins. RM two-way ANOVA followed by Holm-Šidak post hoc: effect of time (F [3.166, 227.9] = 6.911; $p < 0.001$), treatment (F [6, 72] = 37.38; $p < 0.0001$), and time × treatment (F [30, 360] = 14.67; $p < 0.0001$). Filled symbols indicate different from VEH + DOI, $p < 0.05$. **b** Total HTR collapsed into the 30-min peak effect following different doses (mg/kg) of volinanserin + 1.0 DOI (mg/kg). One-way ANOVA: treatment effect (F [5, 6] = 55.02; $p < 0.01$). **c** Total HTR collapsed into the 30-min peak following treatments. Two-way ANOVA analysis followed by Holm-Šidak post hoc: effect of DOI (F [1, 37) = 137.9; $p < 0.0001$), VOL (F [1, 37] = 37.80; $p < 0.0001$), and DOI × VOL (F [1, 37] = 29.47; $p < 0.0001$). **d** Full frequency rate curves for treatments as % Maximum Control Rate (%MCR) and frequency of electrical brain stimulation in Hz (log scale). Two-way ANOVA analysis followed by Holm-Šidak post hoc: effect of frequency (F [9, 36] = 20.57; $p < 0.0001$), treatment (F [1, 4] = 80.05; $p < 0.001$), and frequency × treatment interaction (F [9, 36] = 20.78; $p < 0.0001$). Filled symbols indicate different from VEH + DOI at the designated brain-stimulation frequency, $p < 0.05$. **e** % Baseline Stimulations per Component on the ordinate as a function of treatment with Vehicle/Volinanserin + DOI (doses in mg/kg). RM one-way ANOVA: treatment effect (F [2.038, 8.154] = 9.650; $p < 0.01$). **f** % Baseline Stimulations per Component. Two-way ANOVA followed by Holm-Šidak post hoc: effect of DOI (F [1, 4) = 11.09; $p < 0.05$), VOL (F [1, 4] = 13.32; $p < 0.05$), and interaction between DOI × VOL (F [1, 4] = 396.1; $p < 0.0001$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$; n.s., not significant. All points show mean ± SEM for $N = 8$–12 mice (**a**–**c**) and mean ± SEM for $N = 5$ rats (**d**–**f**)

kg (Fig. 2D, E). Volinanserin (0.032 mg/kg) alone did not significantly affect ICSS (Fig. 2F). The volinanserin AD_{50} (95%CL) to produce 50% antagonism of DOI–induced ICSS depression was 0.0040 mg/kg (0.0017–0.0095). In time-course studies, peak antagonism produced by 0.032 mg/kg volinanserin was observed after 15 min and was still significant after 1 h, but not 4 or 24 h (Table 1). The half-life (95%CL) of DOI antagonism produced by 0.032 mg/kg volinanserin was 1.35 h (0.95–2.04). Volinanserin AD_{50} values and half-life values for DOI antagonism in the mouse HTR and rat ICSS had 95% CL values that overlapped across procedures.

These data suggest that the 5-HT_{2A}R antagonist volinanserin has a similar potency and time-course to block both DOI–induced HTR in mice and DOI–induced ICSS depression in rats.

Volinanserin antagonism of LSD–induced HTR and ICSS depression.

We next evaluated the effects volinanserin on LSD–induced HTR in mice and ICSS depression in rats.

LSD (0.32 mg/kg)–induced HTR peaked during the first 30 min post injection before decreasing (Fig. 3A and Fig. S3). LSD alone (0.32 mg/kg) produced robust and long-lasting increase on HTR counts, though typically lower than DOI–induced HTR counts (~ 100 counts/90 min). Administration of a range of volinanserin doses (0.0001–0.1 mg/kg) produced a dose-dependent blockade of LSD–induced HTR (Fig. 3A and Fig. S3). At a moderate dose (0.032 mg/

Table 1 Time-course of volinanserin (0.032 mg/kg) antagonism on DOI (1.0 mg/kg)–induced HTR and ICSS depression. HTR data shown as % antagonism ± SEM of the collapsed 30-min peak effect ($N = 10–12$). One-way ANOVA with min Holm-Šidak post hoc: pretreatment effect (F [5, 61] = 99.26; $p < 0.0001$). ICSS data show the mean ± SEM % Baseline Stimulations during each ICSS component ($N = 5$). One-way ANOVA min Holm-Šidak post hoc: pretreatment effect (F [5, 24] = 20.73; $p < 0.0001$). Note that DOI augments HTR and volinanserin antagonism which is seen as a decrease in collapsed HTR, whereas DOI depresses ICSS and volinanserin antagonism which is seen as a reduction of ICSS depression. Asterisks indicate significantly different from VEH + DOI at 15 min (****$p < 0.0001$, ***$p < 0.001$)

Pretreatment	Time before 1.0 DOI	Collapsed HTR	% Baseline Stimulations in ICSS
VEH	15 min	100 ± 0	0.3 ± 0.3
0.032 Voli	15 min	2.5 ± 0.6****	103.2 ± 8.9****
0.032 Voli	1 h	18.0 ± 4.9****	57.7 ± 8.6***
0.032 Voli	4 h	87.4 ± 16.1***	17.1 ± 9.6
0.032 Voli	24 h	162.9 ± 30.6****	2.4 ± 2.2

kg), volinanserin was able to produce complete antagonism of LSD–induced HTR (Fig. 3A, B and Fig. S3). The AD_{50} (95% CL) of volinanserin to produce 50% antagonism of 0.32 mg/kg LSD was 0.00047 mg/kg (0.00018–0.0010).

As previously reported (Sakloth et al. 2019), acutely administered LSD (0.32 mg/kg) also produced significant depression of ICSS (Fig. 3C). There was a trend for 0.032 mg/kg volinanserin to attenuate effects of LSD, but this antagonism did not meet the criterion for statistical significance (Figs. 3C, D).

Volinanserin antagonism of psilocybin- and mescaline-induced ICSS depression

To further examine serotonin antagonistic properties with additional psychedelic compounds, we assessed the effect of volinanserin on ICSS depression produced by the phenethylamine mescaline and the tryptamine psilocybin.

Mescaline (32 mg/kg) produced significant depression of ICSS (Fig. 4A), consistent with previous results (Sakloth et al. 2019). Mescaline-induced ICSS depression was significantly attenuated by 0.032 mg/kg volinanserin in analysis of both full frequency-rate curves (Fig. 4A) and total stimulations per component (Fig. 4B). Moreover, in the latter analysis, the effects of volinanserin + mescaline were not different from vehicle + vehicle treatment (Fig. 4B). As expected, based on previous findings (Sakloth et al. 2019), psilocybin (1.0 mg/kg) also produced significant depression of ICSS (Fig. 4C). Volinanserin showed a modest, yet statistically significant, effect on attenuation of psilocybin-induced ICSS depression in the frequency-rate curve analysis (Fig. 4C), as well as in the analysis of total stimulations per component (Fig. 4D). However, the effect of volinanserin + psilocybin on ICSS was still statistically different from the vehicle + vehicle group (Fig. 4B).

Volinanserin antagonism of salvinorin A-induced ICSS depression

As an internal control, we also assessed volinanserin effectiveness to antagonize ICSS depression by a k-opioid receptor agonist, salvinorin A, which has been reported to induce hallucinogenic effects in humans (Roth et al. 2002; Listos et al. 2011).

When administered alone, salvinorin A (3.2 mg/kg) significantly depressed ICSS (Fig. 5A), consistent with previous literature (Ebner et al. 2010; Negus et al. 2012; Negus et al. 2012). Volinanserin pretreatment did not antagonize ICSS depression by salvinorin A in analysis of either frequency-rate curves (Fig. 5A) or total stimulations per component (Fig. 5B). Rather, there was a trend for volinanserin pretreatment to exacerbate salvinorin A-induced ICSS depression, and this effect was significant at brain-stimulation frequencies of 112 and 126 Hz in the frequency-rate curve analysis (Fig. 5A).

Discussion

This study evaluated effectiveness of the 5-HT_{2A}R antagonist volinanserin to block behavioral effects induced by phenethylamine (DOI and mescaline), ergoline (LSD), and tryptamine (psilocybin) psychedelic compounds in two preclinical behavioral procedures. The mouse HTR is commonly used as a behavioral proxy to measure hallucinogenic activity in rodents (González-Maeso et al. 2007; Halberstadt et al. 2018; Hanks and González-Maeso 2013; de la Fuente Revenga et al. 2019), whereas the ICSS procedure has been used to assess a broader array of drug effects, including undesirable behavioral disruption (Negus and Miller 2014; Moerke and Negus 2019). Our data provide three main insights into the effects of psychedelics. First, volinanserin antagonism of HTR elicited by DOI and LSD corroborates that hallucinogenic-related effect of psychedelics is mediated via the 5-HT_{2A}R. Second, volinanserin at the dose of 0.032 mg/kg had graded effectiveness to block ICSS depression induced by different psychedelics, with greater antagonism of the phenethylamines DOI and mescaline than of the tryptamine psilocybin and ergoline LSD. Lastly, LSD produced a lower HTR maximal effect than DOI, and LSD–induced HTR was more sensitive than DOI–induced HTR to volinanserin antagonism.

The relative effectiveness of DOI and LSD alone to produce HTR is consistent with previous findings. HTR

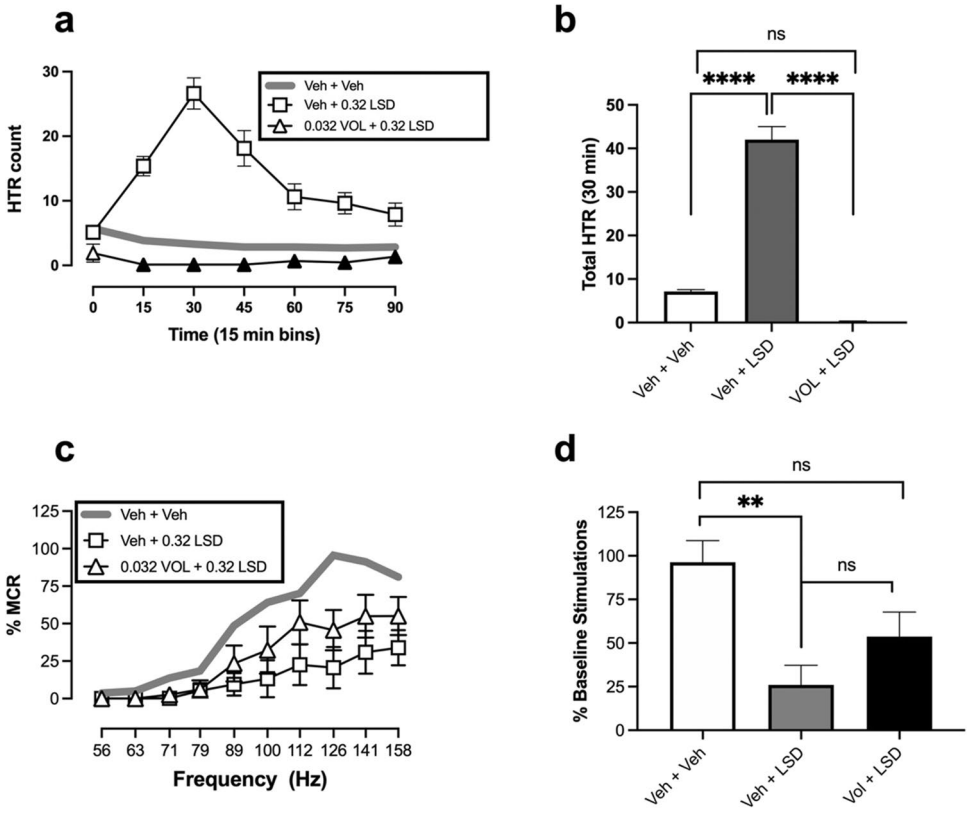

Fig. 3 Effects of volinanserin on LSD–induced HTR (**a**, **b**) and ICSS depression in rats (**c**, **d**). **a** Time-course of HTR as head twitch response (frequency of head twitches) over time split into 15-min bins. RM two-way ANOVA followed Holm-Šidak post hoc: effect of time (F [2.469, 49.39] = 18.06; $p < 0.0001$), treatment (F [2, 20] = 58.96; $p < 0.0001$), and time × treatment (F [10, 100] = 20.53; $p < 0.0001$). Filled symbols indicate difference from VEH + LSD, $p < 0.05$. **b** Total HTR collapsed into the 30-min peak effect following 0.032 mg/kg volinanserin + 0.32 mg/kg LSD. One-way ANOVA: treatment effect (F [2, 21] = 165.4; $p < 0.0001$) followed Holm-Šidak post hoc. **c** Full frequency-rate curves for treatments shown as % Maximum Control Rate (%MCR) and frequency of electrical brain stimulation in Hz (log scale). RM two-way ANOVA: effect of frequency (F [9, 45] = 8.144; $p < 0.0001$), treatment (F [1, 5] = 2.455; $p > 0.05$), and frequency × treatment (F [9, 45] = 1.747; $p > 0.05$). Filled symbols indicate significant difference from VEH + LSD at the designated brain-stimulation frequency, $p < 0.05$. **d** % Baseline Stimulations per Component on the ordinates as a function of treatment with Vehicle/0.032 mg/kg Volinanserin + Vehicle/0.32 mg/kg LSD. RM one-way ANOVA: treatment effect (F [1.511, 7.554] = 11.12, $p < 0.01$). $^{**}p < 0.01$, $^{****}p < 0.0001$; n.s., not significant. All points show mean ± SEM for $N = 8$ mice (**a**, **b**) and mean ± SEM for $N = 6$ rats (**c**, **d**)

is representative of 5-HT_{2A}R-mediated effects specific to psychedelic compounds. Antagonists have also been previously shown to attenuate and block psychedelic-induced HTR, but this study is the first to report differences in antagonist effectiveness against two distinct classes of psychedelics. Similarly, in ICSS, the rate-decreasing effects of LSD, psilocybin, and mescaline are consistent with those previously reported (Sakloth et al. 2019). Using a fixed agonist dose of DOI (1.0 mg/kg), volinanserin produced a dose-dependent blockade in both HTR and ICSS, with an AD_{50} at 0.0062 mg/kg in HTR and 0.0040 mg/kg in ICSS. Conversely, when volinanserin was given before a fixed agonist dose of LSD, it displayed an increased potency in the dose-dependent blockade of LSD–induced HTR. The AD_{50} of volinanserin against LSD was 0.00047 mg/kg in HTR. In ICSS, however, volinanserin only produced a non-significant trend for LSD antagonism.

An intriguing observation is that volinanserin (0.032 mg/kg) was able to completely abolish LSD–induced HTR for up to 90 min, whereas during these time-course studies, DOI–induced HTR was statistically similar in the vehicle + DOI and volinanserin + DOI groups at the 75- and 90-min time-points. These findings correlate with the higher potency (i.e., lower AD_{50} value) of volinanserin reducing LSD–induced HTR, and may be explained by pharmacokinetic processes and bioavailability of these two psychedelics throughout the time-courses.

The effectiveness of salvinorin A to depress ICSS responding is consistent with previous studies (Ebner et al. 2010; Negus et al. 2012). Other groups have also described the effectiveness of salvinorin A to dose-dependently

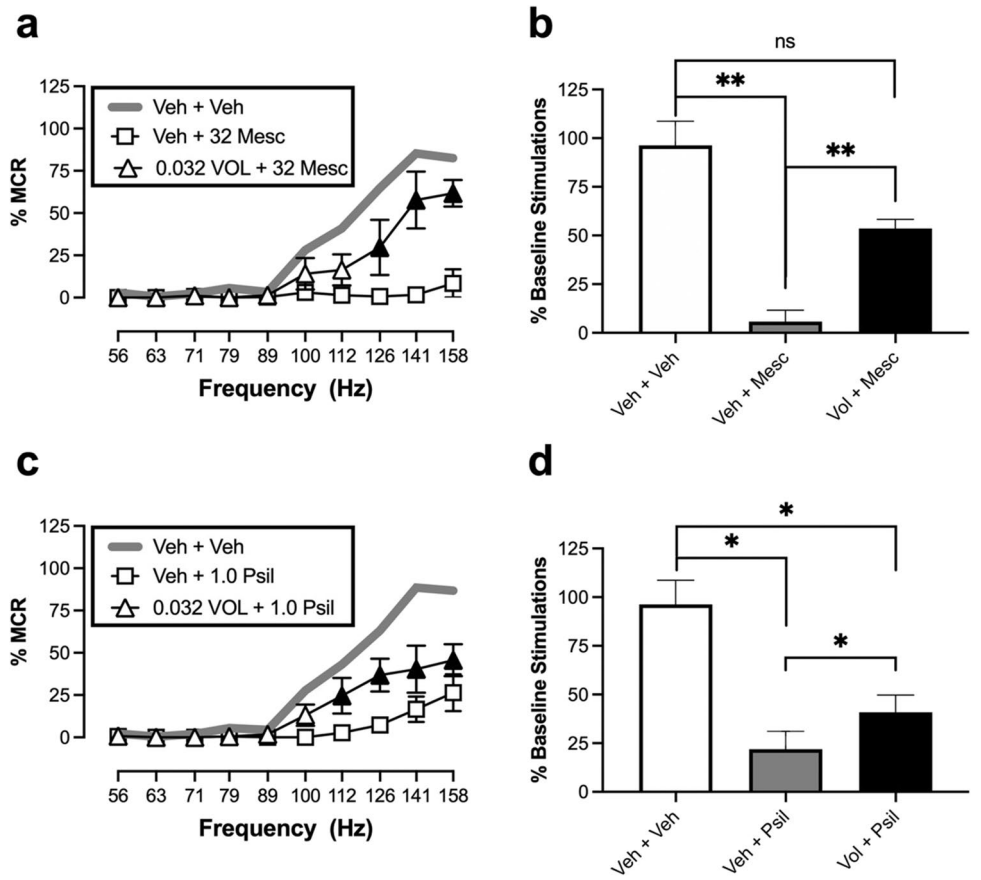

Fig. 4 Effects of volinanserin on mescaline- (**a**, **b**) or psilocybin-(**c**, **d**)induced ICSS depression in rats. **a** Full frequency-rate curves for treatments shown as % Maximum Control Rate (%MCR) and frequency of electrical brain stimulation in Hz (log scale). RM two-way ANOVA: effect of frequency (F [9, 27] = 9.635; $p < 0.0001$), treatment (F [1, 3] = 14.49; $p < 0.05$), and frequency × treatment (F [9, 27] = 5.859, $p < 0.001$). Filled symbols indicate significant difference from VEH + MESC at the designated brain-stimulation frequency, $p < 0.05$. **b** % Baseline Stimulations per Component on the ordinates as a function of treatment with Vehicle/0.032 mg/kg Volinanserin + Vehicle/32.0 mg/kg mescaline. RM one-way ANOVA: treatment effect (F [1.321, 3.962] = 41.15, $p < 0.01$). **c** Full frequency-rate curves for treatments shown as % Maximum Control Rate (%MCR) and frequency of electrical brain stimulation in Hz (log scale). RM two-way ANOVA: effect of frequency (F [9, 36] = 9.729; $p < 0.0001$), treatment (F [1, 4] = 18.80; $p < 0.05$), and frequency × treatment (F [9, 36] = 3.412, $p < 0.01$). Filled symbols indicate significant difference from VEH + PSIL at the designated brain-stimulation frequency, $p < 0.05$. **d** % Baseline Stimulations per Component on the ordinates as a function of treatment with Vehicle/0.032 mg/kg Volinanserin + Vehicle/1.0 mg/kg Psilocybin. RM one-way ANOVA: treatment effect (F [1,120, 4.480] = 16.29, $p < 0.05$). (*$p < 0.05$, **$p < 0.01$; n.s., not significant). All points show mean ± SEM for $N = 5$ mice (**a**, **b**) or $N = 4$–5 rats (**c**, **d**)

increase the baseline threshold of ICSS (Béguin et al. 2008; Potter et al. 2011). Using a fixed dose of salvinorin A, volinanserin produced an increased depression of ICSS responding at brain-stimulation frequencies of 112 and 126 Hz compared to salvinorin A alone. Thus, it is unsurprising that volinanserin was unable to block the effect of the k-opioid receptor agonist salvinorin A on ICSS given their unrelated mechanism of action.

Psychedelic tryptamines, ergolines, and phenethylamines converge in their subjective effect in humans, interoceptive stimulus in rodents and involvement of 5-HT_{2A}R in these actions. However, structural differences—even within chemical families—define their divergence in their ability to interact with a broader range of G protein-coupled receptors (GPCRs), including several dopamine receptors and other receptors in the serotonin family (Ray 2010; Pierce and Peroutka 1989). As an example, the scaffold underlying the structure of LSD has produced several promiscuous dopamine receptor agonists with high affinity for D2 receptors (Burt et al. 1976; Giacomelli et al. 1998; Seeman et al. 2005; De Gregorio et al. 2016), which are known to play a significant role in nucleus accumbens-mediated reward and reinforcement. Furthermore, the involvement of dopamine receptors in ICSS has been described previously. Thus, it was reported that high-efficacy D1 agonists produced dose-dependent abuse-related facilitation of ICSS, whereas lower efficacy D1 agonists and all D2/3 ligands failed to facilitate ICSS at any dose or pretreatment time (Lazenka et al. 2016).

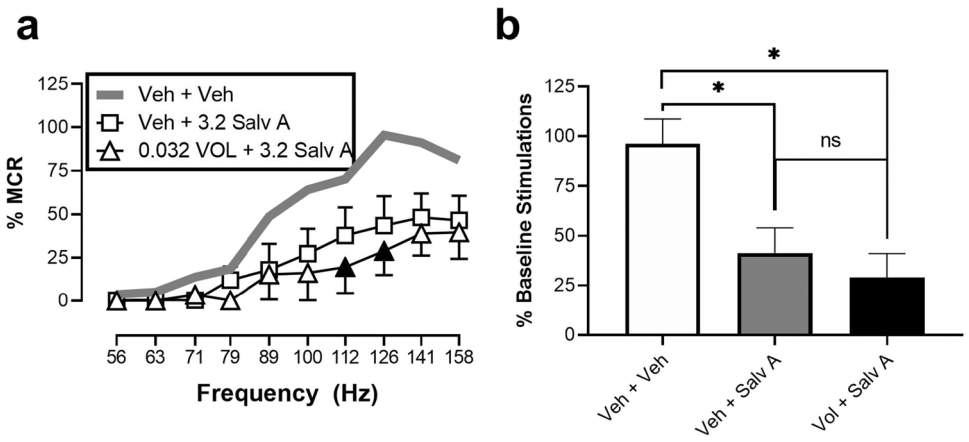

Fig. 5 Effects of volinanserin on salvinorin A-induced ICSS depression in rats. **a** Full frequency-rate curves for treatments shown as % Maximum Control Rate (%MCR) and frequency of electrical brain stimulation in Hz (log scale). RM two-way ANOVA: effect of frequency (F [9, 45] = 5.043; $p < 0.001$), effect of treatment (F [1, 5] = 5.412; $p > 0.05$), and frequency × treatment. Filled symbols indicate significant difference from VEH + Salvinorin A at the designated brain-stimulation frequency, $p < 0.05$. **b** % Baseline Stimulations per Component on the ordinates as a function of treatment with Vehicle/0.032 mg/kg Volinanserin + Vehicle/3.2 mg/kg Salvinorin A. RM one-way ANOVA: treatment effect (F [1.161, 5.805] = 14.49, $p < 0.01$) (*$p < 0.05$; n.s., not significant). All points show mean ± SEM for $N = 6$ rats

Similarly, the high affinity of psilocybin for a broad panel of serotonergic receptors is unsurprising considering its close structural resemblance to 5-HT. Psilocin, the active metabolite of psilocybin, is a very potent ligand of the 5-HT_{1A}R, another target known to affect behavioral reinforcement (Watts et al, 1995; Borroto-Escuela et al. 2014; Schindler et al. 2012; Passie et al. 2002).

Our data reporting a nonsignificant trend toward volinanserin-induced antagonism on LSD or psilocybin as compared to the robust antagonism on DOI or mescaline in ICSS responding, together with the antagonistic properties of volinanserin on psychedelic-induced HTR, suggest off-target LSD and/or psilocybin effects resulting from the involvement of these non-5-HT_{2A}R types. This is further supported by some evidence from other behavioral procedures, such as drug-discrimination, that LSD and phenylethylamine cues evoke different stimulus effects (Fiorella et al. 1995). In such studies, it was found that higher doses of antagonists are necessary to block the discriminative stimulus effects of LSD than of phenylethylamines (Meert et al.1996; Fiorella et al. 1995; Winter et al. 2005). Moreover, other reports have recently suggested that the 5-$HT_{2A/2C}$R antagonist ketanserin prevents potential therapeutic-related effects of DOI, but not psilocybin, on compulsive- and anxiety-like behaviors in mice (Odland et al. 2021).

Thus, our current findings suggest that ICSS depression induced by phenethylamines is 5-HT_{2A}R dependent, but the effects of ergolines and tryptamines on the same ICSS depression model may be not. Nevertheless, an alternative, but not mutually exclusive, possibility to explain differences in the antagonistic properties of volinanserin on DOI– vs LSD–induced HTR in mice and ICSS depression in rats could be related to the well-known phenomenon of receptor reserve with which certain agonists do not need to occupy all receptors to drive a maximum response (Kenakin 2008). Thus, with a large receptor reserve, it could be possible that the fraction of the population of 5-HT_{2A}Rs blocked by the dose of volinanserin tested in our assays was sufficient to block LSD–induced HTR in mice but not LSD–induced ICSS depression in rats. As mentioned in the "Methods" section, our pilot assays suggested that higher doses of volinanserin by themselves were able to induce ICSS depression. Testing the effect of alternative 5-HT_{2A}R antagonists/inverse agonists such as pimavanserin or altanserin may provide additional information about the potential role of 5-HT_{2A}R-dependent signaling on ICSS depression induced by psychedelics.

The present data taken together could have important implications for future investigations of psychedelics, specifically in understanding their potential therapeutic (or lack thereof) mechanisms. As mentioned above, psilocybin and LSD have been investigated for their treatment of psychiatric disorders, as well as substance use disorders (Carhart-Harris et al. 2021, 2016; Gasser et al. 2014; Griffiths et al. 2016; Nutt 2016; Ly et al. 2018; Bogenschutz et al. 2015; Johnson et al. 2014, 2017; Krebs and Johansen 2012; Alper et al. 2018; Zamarripa et al. 2020). Our data highlight a clear difference in receptor involvement between the structural classes in behavioral disruption as assessed by ICSS depression in rats, but ergolines such as LSD and tryptamines such as psilocybin are prioritized in clinical research. It is important to note that these experiments were done in only

male mice and should also be completed in females. Other caveats of this study include differences in the enantiomeric vs racemic (DOI) and salt form (LSD) which may influence the administered drug pharmacodynamics and dosage equivalence.

Our data suggest that both HTR and ICSS depression induced by the phenethylamine psychedelic DOI are prevented upon volinanserin administration, whereas the same pharmacological blockade of 5-HT_{2A}R reduced LSD–induced HTR but lacked an antagonistic effect on LSD–induced ICSS depression. If such divergences can be extrapolated to other animal models of therapeutic value, classic psychedelics can pave the way for new chemotypes devoid of the limiting actions of the parent drugs on 5-HT_{2A}R-induced hallucinations.

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Author contribution Conceived and designed experiments, analyzed the data, and wrote the manuscript: A.M.J., H.E., S.S.N, and J.G.-M. Performed experiments: A.M.J., H.E., and S.A.M. Supervised the research and obtained funding: J.G.-M. and S.S.N. Provided advice on behavioral assays and editorial suggestions on early drafts of the report: M.d.l.F.R. All authors discussed the results and commented on the manuscript prior to submission for publication consideration.

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Declarations

Ethics approval Experiments were conducted in accord with NIH guidelines and were approved by the Virginia Commonwealth University Animal Care and Use Committee.

Conflict of interest J.G.-M. has a sponsored research contract with *NeuRistic*, and M.d.l.F.R. has a consulting agreement with *Noetic Fund*. The remaining authors declare that they have no conflict of interest.

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