Effects of 5-HT_{2A} receptor agonist 2,5-dimethoxy-4-iodoamphetamine on alcohol consumption in Long–Evans rats

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The objectives of this study were to determine alcohol consumption after administration of (*R*)(-)-2,5-dimethoxy-4-iodoamphetamine (DOI) or naltrexone in Long–Evans rats, and to assess the effectiveness of these treatments based on individual differences in alcohol consumption. Adult male Long–Evans rats (*N* = 16) were given opportunities to orally self-administer a 20% (v/v) ethanol (EtOH) solution using an intermittent access, two-bottle (vs. tap water) choice procedure in their home cages. EtOH consumption and preference, total fluid consumption and food intake were measured. Last, we assessed the effects of naltrexone (1 mg/kg; subcutaneous) and (*R*)(-)-DOI (0.1–1 mg/kg; subcutaneous) on EtOH intake and preference using a quartile analysis. Rats showed stable EtOH (20%) intake and preference after 15 EtOH access sessions. Naltrexone produced a transient decrease in EtOH intake, but an inconsistent effect on EtOH preference, whereas DOI dose-dependently reduced EtOH intake and preference for at least 24 h. Subsequent quartile analyses revealed that rats with the highest EtOH intake during the first 60 min of access to EtOH showed greater reductions in EtOH intake and preference after DOI treatment. This is the first report to show that DOI-elicited reductions in EtOH intake and preference in rats depend on baseline EtOH intake, perhaps supporting a 'baseline dependency' hypothesis of effectiveness with phenethylamine psychedelics on EtOH consumption. If so, individuals with greater potential to develop severe AUDs may be particularly responsive to the positive motivational changes produced by treatment with psychedelics that target the 5-HT2 receptor family. *Behavioural Pharmacology* 32: 382–391

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Introduction

Over 14 million adults aged 18 and older have an alcohol use disorder (AUD) in the United States (SAMHSA, 2018), and over 90 000 people die annually from alcohol-related events (CDC, 2019). Earlier studies have implicated serotonergic dysfunction in mediating the pathophysiology of AUDs (reviewed in Sari *et al.*, 2011), but current FDA-approved medications for AUDs, such as naltrexone, disulfiram and acamprosate, target a wide range of nonserotonergic systems (e.g. naltrexone is a μ-opioid receptor antagonist). Human studies have evaluated the effects of some serotonergics (e.g. selective serotonin reuptake inhibitors, such as fluoxetine and sertraline, and the 5-HT_{1A} agonist buspirone) as adjunct treatments for AUDs (Bruno, 1989; Kranzler *et al.*, 1995; Pettinati *et al.*, 2001), but their clinical effectiveness varies, perhaps because of the relatively high rate of comorbid mood disorders, such as depression and posttraumatic stress disorder. Furthermore, approximately 25% of people with an AUD in the United States will relapse within 3 years of remission (Dawson *et al.*, 2007). Together, the high prevalence and persistence of AUDs imply that novel treatments to attenuate alcohol intake and preference are urgently needed.

There is a renewed interest in using psychedelics for treating AUDs (Bogenschutz *et al.*, 2015). Psychedelics may represent a unique treatment option for AUDs as their mind- and perception-altering effects seem to change the motivational properties of alcohol (i.e. cravings and drug-seeking behaviors), rather than directly alter its rewarding or aversive properties (e.g. disulfiram increases the aversive effects of alcohol use by inhibiting aldehyde dehydrogenase, which leads to an accumulation of acetaldehyde and a dysphoric state in the drinker). Psychedelics may persistently attenuate alcohol abuse with a reduced frequency of administration, as compared to other pharmacotherapies for AUDs. A meta-analysis of six randomized controlled trials comprising a total of 536 participants reported that a single administration of lysergic acid diethylamide (LSD) is

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associated with a decrease in alcohol abuse for at least 6 months after administration (Krebs and Johansen, 2012). These findings are supported by rodent studies in which male C57BL/6J mice injected with a single administration of 50 μg/kg LSD show reduced alcohol consumption and preference compared to saline-treated mice for at least 46 days after drug administration (Alper *et al.*, 2018). Pharmacotherapeutics that require few administrations to induce lasting effects may offer many benefits to clients with AUDs, such as improved treatment adherence, significantly less risk of developing dependence and in-person monitoring of any adverse reactions to the drug.

Earlier findings support a major role of the serotonin 5-HT2 receptor family in mediating the unique neuropsychological effects of psychedelics (reviewed in Nichols, 2016), and agonist activity at 5-HT2A has a critical role in producing the mind- and perception-altering effects of psychedelics. Tryptamine psychedelics, such as LSD, psilocybin (prodrug for psilocin) and dimethyltryptamine, have garnered much interest for the treatment of substance use disorders. However, phenethylamine psychedelics, such as 3,4-methylenedioxymethamphetamine (MDMA) [and possibly 2,5-dimethoxy-4-iodoamphetamine (DOI)] may represent valuable tools to understand how manipulation of the serotonergic system may treat AUDs. Earlier studies show reductions in alcohol intake and preference in Wistar rats that received 0.1–0.3 mg/kg DOI (Maurel *et al.*, 1999a), alcohol-preferring cAA rats that received 0.3–3 mg/kg DOI (Maurel *et al.*, 1999b), and Lewis rats that received 0.18–3.2 mg/kg DOI under a multiple or concurrent schedule of ethanol (EtOH) and food reinforcement (Ginsburg and Lamb, 2013). One study in Swiss–Webster mice showed that 3 mg/kg DOI reduced EtOH intake and preference for at least 24 h after the session began in high drinking mice (defined by median split), but DOI did not reduce EtOH intake or preference in the low drinking mice group (Oppong-Damoah *et al.*, 2019). However, no studies in rats have investigated how individual differences in EtOH consumption may affect the apparent therapeutic effects of DOI. Moreover, no studies have examined the effects of DOI on EtOH consumption in the Long–Evans strain, which is a valuable model of alcohol use in two-bottle choice studies (e.g., Simms et al., 2008; 2010). Therefore, we investigated the relationship between the effectiveness of DOI administration on alcohol consumption in Long–Evans rats with varying baseline levels of EtOH intake and preference.

Methods

Drugs

Ethyl alcohol 200 proof (Pharmco-AAPER, Brookfield, Connecticut, USA) was purchased from the pharmacy at UAMS. All EtOH concentrations are expressed as percentage volume/volume (v/v) and were dissolved in tap water. Naltrexone hydrochloride and DOI were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Naltrexone and DOI doses are expressed as weight of the salt, were dissolved in 0.9% physiological saline (naltrexone) or sterile water (DOI), and were administered via subcutaneous injection (subcutaneous) in a 1 ml/kg injection volume.

Subjects

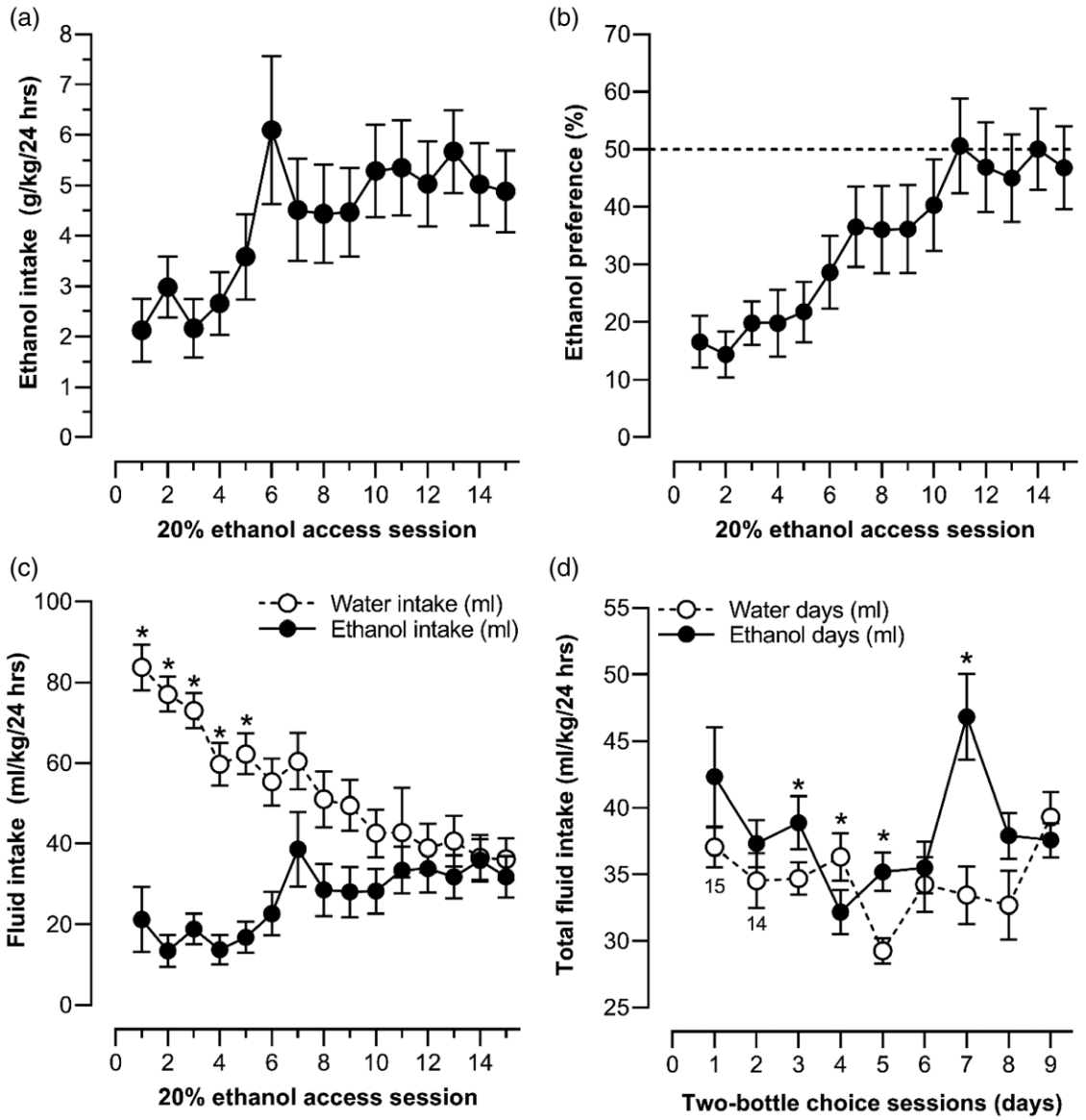
Sixteen male Long–Evans rats (Charles River Laboratories Inc., Wilmington, Massachusetts, USA) aged 10–11 weeks old (346–436 g on arrival) were singly housed in polycarbonate cages (23.75 × 45.40 × 17.78 cm) in corncob bedding in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. The animal colony room was maintained at 22 ± 2 °C and 45–50% humidity with lights set to a 10:14 h light–dark cycle (lights on at 0600 h). Each cage contained a plastic tunnel and cotton nestlet for enrichment. While in their home cages, rats were given ad libitum access to food and water from two water bottles (except on EtOH access sessions, during which only one bottle contained water) that were each equipped with a spout containing a single metal ball to reduce spillage. Rats were given at least one week to acclimate to the housing conditions and handling procedures (e.g. daily weighing) upon arrival before experimental procedures began. All injections, cage changes and replacement of water bottles with EtOH bottles occurred during the animals' light cycle. All experimental protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011) and approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences.

Two-bottle choice procedure

Training procedures and drug administration

Rats were given opportunities to drink 20% EtOH using an intermittent access two-bottle choice procedure in their home cages as described in earlier studies (Simms et al., 2008; 2010; Carnicella *et al.*, 2009). EtOH access sessions occurred every Monday, Wednesday and Friday, and two water bottles (containing tap water) were available on intervening days and over the weekends. Water bottles were swapped with EtOH bottles during 0800–0900 h every MWF, and the EtOH bottle position alternated (either left or right) across access sessions to reduce the influence of a position bias. Rats always had ad libitum access to food. EtOH consumption (ml), total fluid consumption (ml) and food intake (g) were measured before bottle access and 1, 4 and 24 h after access. Two empty cages with one water bottle and one EtOH bottle were included in all measurement assessments to estimate liquid spillage due to handling. The average amount of liquid lost from these two control bottles was subtracted from each of the two bottles

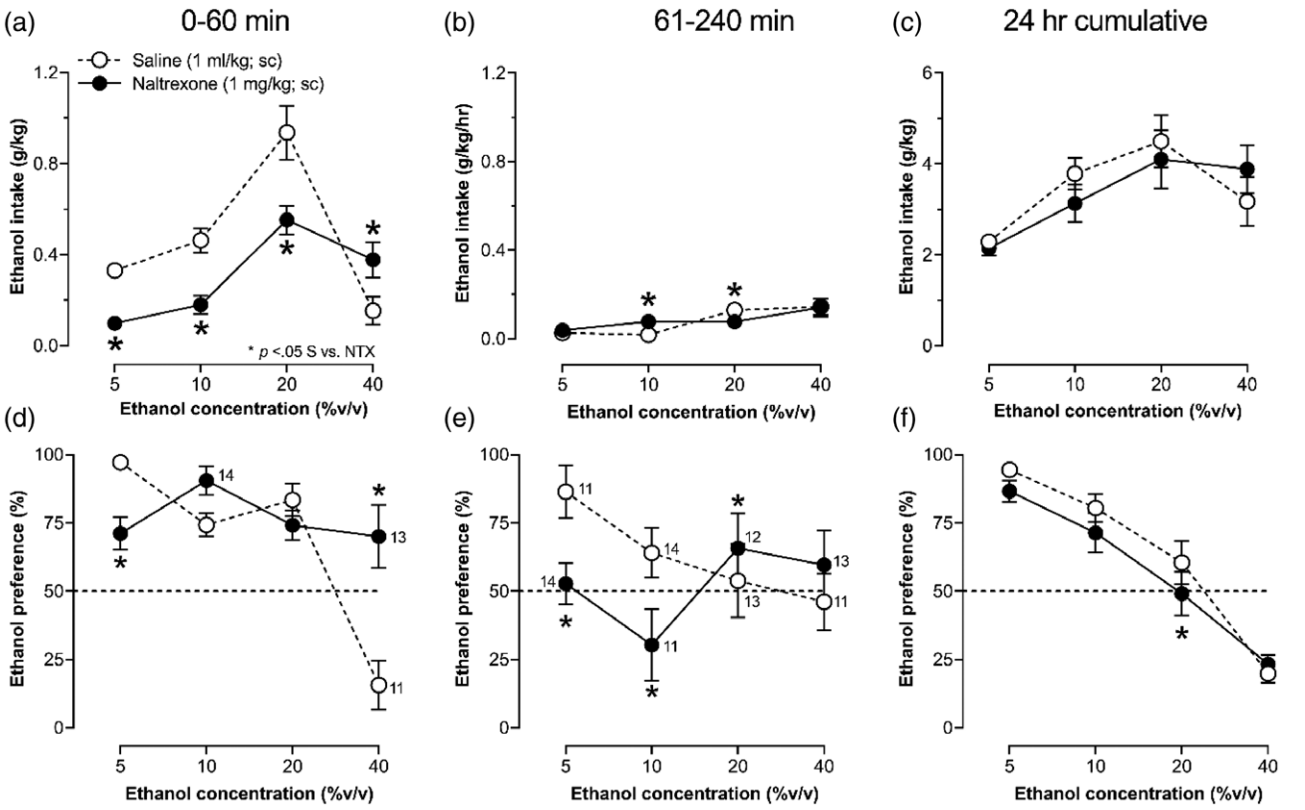
Fig. 1



Intermittent access to 20% ethanol (EtOH) in Long–Evans rats lead to an escalation in (a) EtOH intake (g/kg/24 h), reaching a stable, average EtOH intake of 5.19 g/kg/24 h, and (b) EtOH preference, reaching a stable, average EtOH preference of 47.89%. There was a statistically significant decrease in water intake (open symbols) across EtOH access sessions, while EtOH intake (filled symbols) increased over time (c). Aside from a few instances in which total fluid intake differed between water days (open symbols) compared to EtOH days (filled symbols), there was no consistent difference in total fluid intake across two-bottle choice sessions (d). The values are expressed as mean EtOH intake (g/kg/24 h), preference (ratio of EtOH over total fluid intake), fluid intake (ml/kg/24 h), or total fluid intake (ml/kg/24 h) ± SE at each access session. Error bars are contained within the point for some data. *$P < 0.05$ compared to EtOH intake (c) or between the type of fluid at a specified two-bottle choice session (d) according to Holm–Šídák multiple comparisons tests. $n = 14–16$; on water days 1 and 2, the water bottles spilled water in 1–2 of the animals' home cages, so their data on these days are omitted from (d). Numbers near symbols indicate the sample size.

given to each rat (unless the difference between measurements was zero or the subtracted amount would produce a negative value). After reaching a stable level of EtOH intake (defined as five consecutive EtOH access sessions in which the absolute percent change in EtOH intake across each session was ≤20%, with no increasing or decreasing trend), rats were injected with saline (1 ml/kg; sc) 30 min before EtOH access and then given 24 h to drink one of several concentrations of EtOH (5, 10, 20 and 40% v/v) that were provided in an ascending sequence across access days to generate an EtOH concentration-effect curve. The training EtOH concentration (20% v/v) was reintroduced in between each of the other EtOH concentrations, according to the intermittent access schedule. Next, the effects of naltrexone (1 mg/kg; subcutaneous; 30 min before EtOH access) on EtOH

Fig. 2



Naltrexone (1 mg/kg; subcutaneous) generally decreases EtOH intake during 0–60 min of EtOH access (a), but shows differential effects that varied by EtOH concentration during the 61–240 min (b) and 24 h cumulative (c) measurement periods using an intermittent access to EtOH (5–40%; v/v) drinking procedure in Long–Evans rats. Naltrexone administration also interacted with EtOH concentration to alter EtOH preference during the 0–60 min (d), 61–240 min (e) and 24 h (not cumulative) (f) measurement periods. The values are expressed as mean EtOH intake (g/kg) or (g/kg/h), or preference (ratio of EtOH over total fluid intake). *$P < 0.05$ naltrexone (filled symbols) compared to saline (open symbols) at each EtOH concentration according to Holm–Šídák multiple comparisons tests. $n = 11–16$; some subjects did not consume EtOH or water during an EtOH access measurement period, precluding computation of an EtOH preference. Numbers near symbols indicate the sample size. EtOH, ethanol.

intake and preference were assessed over the complete EtOH concentration-effect curve. After naltrexone, the effects of DOI (0.1, 0.32 and 1 mg/kg; subcutaneous; 30 min before EtOH access) on 20% EtOH intake and preference were assessed. This initial dose-effect determination with DOI on 20% EtOH was intended to determine an effective DOI dose for additional study. However, during the phase of the study in which DOI was administered, over 90% of the rats showed a progressive loss of fur along their dorsal surface and dermatitis. Rapid weight loss and polyuria were observed in the days that followed, and the study was therefore ended because of the apparent decline in animal welfare. A timeline of experimental and adverse events can be found in the Supplementary File, Supplemental digital content 1, *http://links.lww.com/BPHARM/A65*. It is noteworthy that one rat already showed signs of dermatitis upon arrival (which resolved after a few weeks of topical steroid treatment) and that similar DOI doses (0.3–3 mg/kg; subcutaneous) from the same stock solution did not produce any adverse effects in a separate cohort of EtOH-naïve, male Sprague-Dawley rats tested in our laboratory (unpublished findings).

Data analysis

All data are presented as means (±SE). Acquisition of oral self-administration of EtOH was quantified as the number of EtOH access sessions required for all rats to show a group mean EtOH intake (g/kg/24 h) that varied ≤20% for five consecutive days with no increasing or decreasing trend. The effects of saline and naltrexone on the EtOH concentration-effect curves for EtOH intake (g/kg/24 h), EtOH preference (%), food intake (g/kg/24 h), and total fluid intake (water + EtOH or water +water; ml/kg/24 h) were statistically analyzed using a two-factor repeated-measures analysis of variance (ANOVA) with EtOH concentration and drug treatment as the two factors. The naltrexone dose was chosen based on a previous two-bottle choice study in Long–Evans rats that showed that 1 mg/kg naltrexone reduced EtOH intake compared to

vehicle, but was not statistically different from the effects of 3 mg/kg naltrexone (Simms *et al*., 2008). The effects of DOI on these measures were only assessed with 20% EtOH. As such, a one-factor repeated-measures ANOVA where drug treatment served as the factor (saline and 0.1, 0.32 and 1 mg/kg DOI) was conducted to compare drug treatment conditions. Naltrexone data were plotted in the DOI dose-response curves to serve as a visual point of reference but were not included in the statistical analysis. DOI doses were chosen based on previous studies that reported DOI-induced reductions in EtOH intake and preference in rats (Maurel et al., 1999a; 1999b; Ginsburg and Lamb, 2013). Ordinary repeated-measures ANOVA cannot analyze data when values are missing, so in cases where repeated-measures data were missing (e.g. due to insufficient intake data to compute an EtOH preference), a mixed-effects model was used as implemented in GraphPad Prism 9.0. The mixed model uses a compound symmetry covariance matrix and is fit using restricted maximum likelihood (GraphPad Software Inc.). The results of the mixed model analysis are interpreted like an ordinary repeated-measures ANOVA with no missing values. To assess individual differences, rats were assigned to quartiles (n = 4/quartile) based on EtOH intake (g/kg) during the first 60 min ('loading phase') of a two-bottle choice session prior to which they received a saline injection. Quartile assignments were kept constant for all subsequent analyses with naltrexone and DOI pretreatments. Quartile (treated as a pseudo-independent variable) and drug treatment (saline, 1 mg/kg naltrexone, and 0.1, 0.32 and 1 mg/kg DOI) served as factors in a two-factor ANOVA with one between-subjects factor (quartile) and one within-subjects factor (drug treatment). Ethanol, food and water consumption data were compared after naltrexone and DOI administration in the quartile analyses to determine if these treatments affected EtOH, food and water consumption based on the rats' baseline level of EtOH intake. The Geisser–Greenhouse correction was applied to all one- and two-factor ANOVAs with a repeated-measures factor. Following a statistically significant one- or two-factor ANOVA, Tukey's or Holm–Šídák multiple comparisons tests were conducted to perform all pairwise comparisons or simple effects and simple main effects (in case of statistically significant interaction), respectively. Complete results for all statistical analyses can be found in the Supplemental File, Supplemental digital content 1, *http://links.lww.com/BPHARM/A65*. The creation of graphs and statistical analysis was achieved using GraphPad (version 9, La Jolla, California, USA). Statistical significance was declared at $P < 0.05$ for all analyses.

Results

Rats met the acquisition criterion after 15 EtOH access sessions or 5 weeks of intermittent EtOH access (Fig. 1a). Baseline EtOH intake computed as the average of the 5 days in which the acquisition criteria were met 5.19 g/kg/24 h. Fig. 1b shows that rats preferred water to EtOH in early access sessions but shifted to no preference for one fluid over the other by the 11th access session (average baseline EtOH preference = 47.89%). Fig. 1c presents the water and EtOH intake (ml) across EtOH access sessions. There was a statistically significant interaction between fluid type (water and EtOH) and EtOH access session [$F(4.73, 70.93) = 14.73$, $P < 0.001$, $\eta^2_{partial} = 0.50$], with a difference between water and EtOH intake on EtOH access sessions 1–5 (Fig. 1c). Fig. 1d shows the total fluid intake (water + water, or water + EtOH) across two-bottle choice session days. There was a statistically significant interaction between access day (water and EtOH) and choice session days [$F(3.80, 55.54) = 6.06$, $P < 0.001$], although there was no consistent pattern in total fluid intake as a function of two-bottle choice sessions.

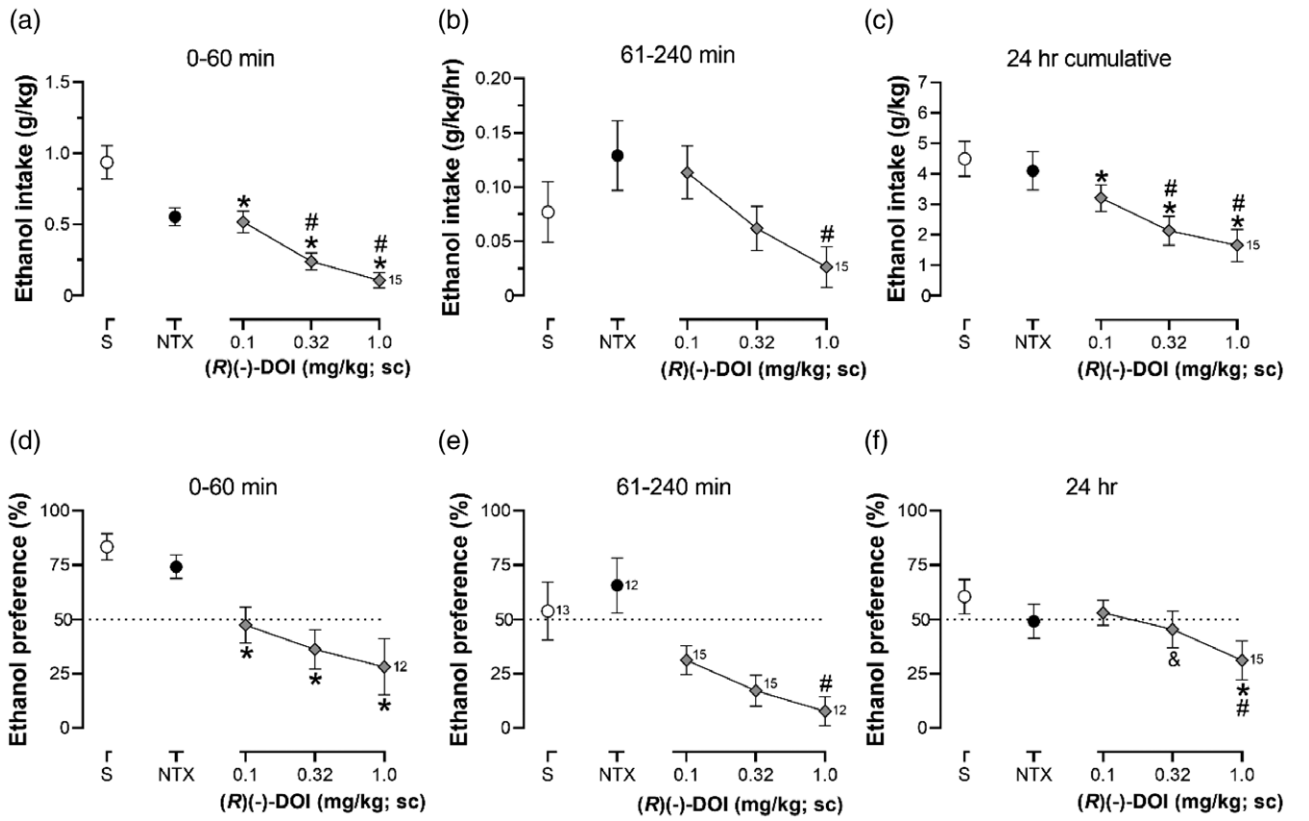
After the acquisition, rats were given access to varying concentrations of EtOH (5-40% v/v) to generate a complete EtOH concentration-effect curve (Fig. 2). Rats first received pretreatment with saline (1 ml/kg; subcutaneous) at each EtOH concentration, and the EtOH concentration curve was then redetermined after pretreatment with naltrexone (1 mg/kg; subcutaneous). Fig. 2a shows that naltrexone pretreatment reduced EtOH intake within the first 60 min of EtOH access compared to the saline main effect of treatment (naltrexone vs. saline): [$F(1, 15) = 12.04$, $P < 0.005$, $\eta^2_{partial} = 0.40$]; a main effect of EtOH concentration: [$F(2.02, 30.34) = 32.49$, $P < 0.001$, $\eta^2_{partial} = 0.80$]; and, a statistically significant interaction between treatment and EtOH concentration: [$F(1.94, 29.15) = 18.98$, $P < 0.001$), $\eta^2_{partial} = 0.56$]. There were no main effects of treatment on EtOH intake during the 61–240 min period (Fig. 2b) or at the 24 h (cumulative) time point (Fig. 2c), but there were statistically significant interactions between treatment and EtOH concentration at both time points (see Supplementary File, Supplemental digital content 1, *http://links.lww.com/BPHARM/A65*). There was a main effect of treatment on EtOH preference at the 61–240 min time point [$F(1, 15) = 6.08$, $P < 0.05$] (Fig. 2e), in which naltrexone reduced EtOH preference compared to saline, but no main effect of treatment on EtOH preference at the 60 min or 24 h time points (Fig. 2d, f). There were statistically significant treatment x EtOH concentration interactions on EtOH preference (Fig. 2d–f; see results in Supplementary File, Supplemental digital content 1, *http://links.lww.com/BPHARM/A65*). The effects of naltrexone on food intake and fluid intake are presented with the DOI pretreatment results below.

After the assessment of naltrexone pretreatment on EtOH intake and preference, rats received ascending doses of (*R*)(-)-DOI (0.1–1 mg/kg; subcutaneous) prior to each 20% EtOH access session. Several rats presented with a dermatitis condition during this time of the study. However, bodyweights, food intake, and water intake were unchanged for at least up to 24 h after the

1 mg/kg DOI pretreatment session (see Supplementary File, Supplemental digital content 1, *http://links.lww.com/BPHARM/A65*), showing that the dermatitis condition did not significantly impact appetitive responding. One rat died within 24 h after receiving 1 mg/kg DOI, so its intake data after this dose are excluded from all analyses and plots. Fig. 3 shows that all three doses of DOI reduced EtOH intake by ~50% or greater compared to saline during the 0–60 min (Fig. 3a) [$F(2.58, 37.84) = 24.43$, $P < 0.001$] and 24 h cumulative (Fig. 3c) time points [$F(1.74, 25.47) = 19.45$, $P < 0.001$], while pretreatment with 1 mg/kg DOI reduced EtOH intake compared to 0.1 mg/kg DOI during the 61–240 min period [$F(2.30, 33.70) = 4.09$, $P = < 0.025$]. DOI reduced EtOH preference compared to saline at the 60 min (Fig. 3d) [$F(2.48, 33.95) = 11.70$, $P < 0.001$] and 24 h (not cumulative) time points (Fig. 3f) [$F(2.15, 31.56) = 6.44$, $P < 0.005$]. All three DOI doses shifted rats' preference away from EtOH to water during the 60 min period, and 1 mg/kg DOI produced a shift in preference for water for at least 24 h after EtOH access. There were no differences in EtOH preference between saline and any DOI dose during the 61–240 min period (Fig. 3e); however, 0.1 mg/kg DOI differed from 1.0 mg/kg DOI [$F(1.34, 16.12) = 5.10$, $P < 0.05$].

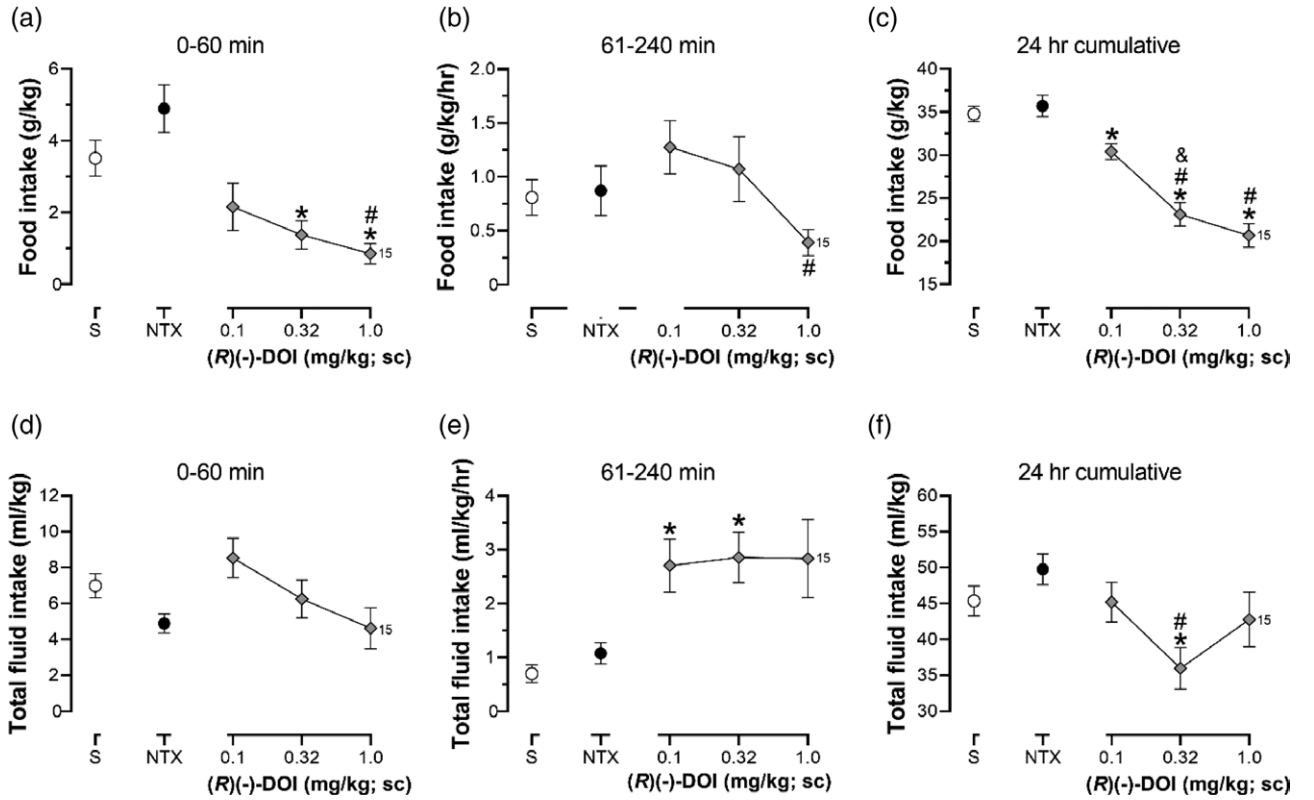
DOI pretreatment reduced food intake at both the 60 min (Fig. 4a) [$F(2.58, 37.88) = 6.23$, $P < 0.005$] and 24 h cumulative (Fig. 4c) time points [$F(2.12, 31.03) = 57.09$, $P < 0.001$], while there were no differences in food intake between saline and any DOI dose during the 61–240 min period (Fig. 4b); although, 1.0 mg/kg DOI reduced food intake compared to 0.1 mg/kg DOI [$F(2.18, 31.93) = 4.11$, $P< 0.025$]. Last, no difference in total fluid intake between any doses of DOI and saline occurred at the 60 min time point (Fig. 4d), but 0.32 mg/kg DOI reduced total fluid intake compared to saline and 0.1 mg/kg DOI at the 24 h cumulative point (Fig. 4f) [$F(1.82, 26.70) = 3.76$, $P < 0.05$]. Aside from the 0.32 mg/kg DOI dose at the 24 h cumulative time point, DOI did not appear to reduce all

Fig. 3



2,5-Dimethoxy-4-iodoamphetamine (DOI; 0.1–1 mg/kg; subcutaneous) decreases 20% EtOH intake during the 0–60 min (a) and 24 h cumulative (c) measurement periods, but not the 61–240 min measurement period (b). DOI administration reduces EtOH preference during both the 0–60 min (d) and 24 h period (f), and the largest dose of DOI reduces EtOH preference for at least 24 h after ethanol access (f). DOI did not affect EtOH preference during the 61–240 min period (e). S, saline (open circles), NTX = 1 mg/kg naltrexone (filled circles), and DOI is indicated by dose (filled diamonds). *$P < 0.05$ compared to saline, #$P < 0.05$ compared to 0.1 mg/kg DOI, and $P < 0.05$ compared to 1 mg/kg DOI according to Tukey's multiple comparisons tests. $n = 12–16$; some subjects did not consume either fluid, precluding computation of an EtOH preference. In addition, one animal died within 24 h after 1.0 mg/kg DOI, so its data are omitted from all plots and analyses. EtOH, ethanol.

Fig. 4



DOI administration produced a persistent decrease in food intake across measurement periods (a, b, c) with effects lasting for at least 24 h. DOI administration did not reduce total fluid intake during the 0–60 min period (d), increased total fluid intake during the 61–240 min period (e), and 0.32 mg/kg DOI reduced total fluid intake at the 24 h cumulative measurement period (f); abbreviations and indications of statistical significance as in Fig. 3.

Table 1 Results of quartile analyses

Ethanol intake (ml) after first 60 min of access					
Quartile	Saline	NTX	0.1 DOI	0.32 DOI	1.0 DOI
1	0.36 (0.09)	0.27 (0.05)	0.26 (0.04)	0.05 (0.05)	0 (0)
2	0.76 (0.06)	0.52 (0.05)	0.63 (0.16)	0.41 (0.18)	0.05 (0.05)
3	1.06 (0.08)	0.68 (0.10)	0.58 (0.04)	0.16 (0.06)	0.35 (0.14)*
4	1.56 (0.06)	0.66 (0.14)	0.60 (0.23)	0.33 (0.08)*	0 (0)*
Cumulative ethanol (ml) intake after 24 h of access					
Quartile	Saline	NTX	0.1 DOI	0.32 DOI	1.0 DOI
1	1.31 (0.36)	0.93 (0.15)	1.35 (0.35)	0.52 (0.10)	0.32 (0.06)
2	5.21 (0.99)	3.88 (1.03)	4.37 (0.69)	3.44 (1.37)	3.09 (1.72)
3	5.09 (0.44)	6.12 (1.02)	2.79 (0.24)	1.86 (0.64)	2.00 (0.52)
4	6.36 (0.73)	5.50 (0.80)*	4.34 (1.07)	2.73 (0.84)*#	1.06 (0.80)*

Data presented as group mean (SE). Rats were assigned to quartiles according to ethanol intake after the first 60 min of EtOH access and saline pretreatment. $n = 4$/quartile except $n = 3$ in quartile 4 for 1.0 DOI.
DOI, 2,5-dimethoxy-4-iodoamphetamine; EtOH, ethanol; NTX, naltrexone.
*$P < 0.05$ vs. saline.
#$P < 0.05$ vs. NTX within a quartile, according to Holm–Šídák multiple comparisons tests.

appetitive responses, as rats still consumed water during DOI administration periods. Total fluid intake increased after 0.1 mg/kg and 0.32 mg/kg DOI compared to saline during the 61–240 min period [$F(2.40, 35.26) = 6.61$, $P < 0.005$) (Fig. 4e).

A quartile analysis revealed that rats with an initially higher level of EtOH intake after saline pretreatment and during the 0–60 min period of EtOH access (rats in the 4th quartile) showed greater reductions after DOI pretreatment compared to their EtOH intake after saline

pretreatment at the 60 min (interaction: [$F(12, 59) = 4.31$, $P < 0.001$]) and 24 h (cumulative) time points (interaction: [$F(12, 47) = 2.53$, $P < 0.025$]) (Table 1; full statistical results in Supplementary File, Supplemental digital content 1, *http://links.lww.com/BPHARM/A65*). Ethanol intake data for the 61–240 min period were not included in any quartile analysis because there were no differences in intake or preference between any DOI dose and saline (Fig. 3b, e). Similarly, there were no statistically significant interactions between quartile and treatment in any other measure (Supplementary File, Supplemental digital content 1, *http://links.lww.com/BPHARM/A65*).

Discussion

This is the first report to demonstrate that the phenethylamine psychedelic DOI reduces EtOH intake and preference for at least 24 h after administration in Long–Evans rats. These findings are consistent with earlier studies that found that 0.3 mg/kg DOI reduced 10% EtOH (v/v) preference in Wistar rats during a 30 min oral EtOH self-administration procedure in operant chambers (Maurel *et al.*, 1999a), and that 0.3 mg/kg and 3 mg/kg DOI reduced 10% EtOH (v/v) intake and preference during a 12 h two-bottle choice procedure in alcohol-preferring cAA rats (Maurel *et al.*, 1999b). However, Maurel *et al.* (1999b) reported that DOI did not affect food intake, whereas we observed a reduction in food intake after all DOI doses that persisted for at least 24 h after DOI administration. This discrepancy may be due to the difference in EtOH concentration (10 vs. 20%), experimental procedures (e.g. duration of EtOH access during training and test sessions), strain of rat, or a combination of these factors. Nevertheless, the Long–Evans rats in this study and the cAA rats used by Maurel *et al.* (1999b) both showed little effect of DOI on fluid intake, which suggests that DOI may specifically reduce EtOH intake and preference (but not all appetitive responses) under different experimental conditions, perhaps suggesting a targeted, 'anti-EtOH' effect of DOI. These findings provide additional support for a possible role of the serotonin 5-HT2 receptor family in reducing drug-maintained responding.

Naltrexone is one of three FDA-approved medications for AUDs, and it is often used in rodent EtOH studies as a positive control for reducing EtOH intake. We found that naltrexone reduced EtOH intake, and at certain EtOH concentrations and time points, naltrexone also reduced preference in Long–Evans rats, which is consistent with an earlier two-bottle choice study that showed that 0.3–3 mg/kg naltrexone reduced EtOH intake in Long–Evans rats when tested 30 min after administration (Simms *et al.*, 2008). We also found a comparable decrease of approximately 40% in EtOH intake from control after 1 mg/kg naltrexone during the first 60 min of 20% EtOH access in this study and the report by Simms *et al.* (2008). Notably, DOI administration reduced 20% EtOH intake compared to saline control by over 80% during the first 60 min of EtOH access and shifted fluid preference toward the water for at least 24 h, whereas naltrexone only had a marginal effect on EtOH preference. These data provide initial evidence that DOI and other psychedelics may alter motivational aspects of alcohol reward and abuse, such as choice (e.g. shifting a preference from EtOH to other, healthier reinforcers).

Direct comparisons between the psychedelics, like DOI, and naltrexone should be made with caution (and may not be particularly helpful, in any case) given the significant pharmacological differences between drugs and their intended uses for treating AUDs. Naltrexone is a μ-opioid receptor antagonist that is well-tolerated and generally safe for at least 6 months of use in liver-healthy individuals for the treatment of AUDs (Balldin *et al.* 2003). The extended-release injectable suspension of naltrexone (Vivitrol; Alkermes Inc., Wilmington, Ohio, USA) may also offer greater medication adherence and prolong time to relapse compared to the standard oral formulation of naltrexone (Leighty and Ansara, 2019). In contrast, the 5-HT_2 receptor family is considered the primary site of pharmacological action for the psychedelics, and their psychological effects are substantially different from those produced by standard AUD pharmacotherapeutics, such as naltrexone. Although clinical data with phenethylamine psychedelics lack for the treatment of AUDs and other substance use disorders, individuals seeking treatment for substance use disorders typically receive <2–3 total administrations of a tryptamine psychedelic, such as LSD, for alcohol dependency problems (reviewed in Krebs and Johansen, 2012) or psilocybin for nicotine smoking cessation (Johnson *et al.*, 2014). As such, it is generally understood that tryptamine psychedelics may be most useful as occasional adjunct medications to add to an existing treatment program for AUDs. Additional research is necessary to determine if phenethylamines produce clinical effects similar to the tryptamines.

A quartile analysis of EtOH intake and preference in this study revealed that rats with the highest EtOH intake during the first 60 min of EtOH access showed the greatest reductions in EtOH intake after DOI (or naltrexone) administration compared to their own baseline levels of EtOH intake. These findings are generally consistent with an earlier study by Oppong-Damoah *et al.* (2019) in which a high alcohol-preferring subset of male Swiss–Webster mice injected with 3 mg/kg DOI and given opportunities to consume 20% EtOH in a two-bottle choice procedure showed reduced EtOH intake and preference 24 h after EtOH access, whereas DOI administration had no effect on EtOH intake or preference in a low alcohol-preferring subset of mice. Increased sensitivity to DOI's effects on EtOH intake and preference among rodents with a high baseline level of EtOH intake could be related to rate dependence (Dews, 1977), which describes a pattern (usually an inverse relationship in

behavioral pharmacological studies) between a baseline rate of behavior and the change in that behavior after some intervention, such as drug administration. However, contrary to an increase in lower baseline levels of responding engendered by contingencies of reinforcement that would be predicted by a rate dependence analysis, rats with an initially lower baseline level of EtOH intake in the present study did not increase their intake after naltrexone or DOI. Rather, rats in quartiles 1–3 either showed a decrease or no change in EtOH intake or preference after DOI administration. Moreover, a more appropriate term to describe the relationship between baseline EtOH intake and subsequent responsivity to DOI observed in the present study may be 'baseline dependency' because there is no clear 'response rate' datum or manipulation of reinforcement contingency with the two-bottle choice procedures. Future oral EtOH self-administration studies conducted in rodent operant conditioning chambers may provide additional support for a baseline dependency explanation, as well as rate dependency, given the added experimental control over reinforcement contingencies and operanda, such as lickometers, possible in the operant conditioning chamber environment. Future studies that provide analyses of individual differences in drug intake before and after some intervention may reveal divergent clusters of phenotypes within a sample of subjects that each may respond better to some treatments over others based on their initial level of response output. Similarly, whether DOI is more potent or more effective in rats with higher baseline, EtOH intake is not easily discerned from the pattern of results here reported.

There are several limitations of the present study. First, it is possible that the differences here highlighted between naltrexone and DOI in the quartile analyses could be dose-limited, in addition to the aforementioned differences in pharmacological mechanisms of action between these drugs. We chose to administer a single dose of naltrexone, as opposed to generating a complete naltrexone dose-effect curve, because the 1 mg/kg naltrexone dose effectively reduced EtOH intake in Long–Evans rats in an earlier two-bottle choice study, and there was no statistical difference between 1 mg/kg and 3 mg/kg naltrexone, the largest tested dose, on EtOH intake (Simms *et al.*, 2010). Nevertheless, it is possible that larger naltrexone doses could produce larger decreases in EtOH intake and preference than what was observed in the present study. Future two-bottle choice studies that include naltrexone as a positive control should consider determining a complete naltrexone dose-effect function for EtOH intake and preference as part of the experimental design.

A second limitation to the present study is that naltrexone and DOI may have reduced EtOH intake and preference by altering the taste of EtOH, rather than the motivation, per se, to consume EtOH. Oral, unadulterated EtOH serves as a relatively 'weak' reinforcer in rodents and its unique taste likely plays an important role in its reinforcing properties. Another important factor to consider for choice studies with oral EtOH is the availability of concurrent reinforcers. For example, Ginsburg and Lamb (2014) reported that a selective effect of DOI for reducing responding for EtOH over food might not be maintained if food access is concurrently available with EtOH in an operant chamber environment. Indeed, we found that DOI administration produced a persistent decrease in both food and EtOH intake over the 24 h of access, but with an increase in water consumption, under a two-bottle choice environment in the animals' home cages. It is noteworthy that Maurel *et al.* (1999b) did not detect an effect of 3 mg/kg DOI on food intake using a two-bottle choice arrangement that is similar to the present study, whereas Ginsburg and Lamb (2014) found an almost complete suppression of responding for food after 3.2 mg/kg DOI in rats responding in an operant conditioning chamber environment. These discrepancies may be due to the aforementioned strain differences (cAA rats, Lewis rats, Long–Evans rats), differences in experimental procedures and response requirements (i.e. two-bottle choice in home cages vs. responding under multiple or concurrent schedules of reinforcement in operant conditioning chambers), or some interaction between these variables. Regardless of these discrepancies, rats in the present study showed no major impact on total fluid intake and a decrease in EtOH intake and preference after DOI administration, despite a persistent decrease in food consumption. Future studies that compare various EtOH self-administration procedures (e.g. two-bottle choice, EtOH-maintained responding in operant chambers, alcohol deprivation effect models) may shed light on the critical experimental variables that predict the specificity of drug treatment on EtOH intake.

A final limitation is the unexpected presentation of a dermatitis condition that appeared within days after DOI administration in the majority of rats that served in the present study, which precluded us from testing a dose of DOI against a full EtOH concentration-effect curve. Accordingly, it remains unknown how the effects of DOI on EtOH consumption vary based on the unit concentration of EtOH. Nevertheless, the skin condition did not appear to affect appetitive responding or bodyweights in rats during testing with DOI (Supplementary File, Supplemental digital content 1, *http://links.lww.com/BPHARM/A65*), suggesting that the condition did not interfere with the accuracy of the EtOH intake data for at least 24 h after 1 mg/kg DOI administration. Moreover, the more substantial decrease in animal welfare was not observed until approximately 10–11 days after the final 24 h measurement.

Conclusion

There is a renewed interest in the use of psychedelics for the treatment of substance use disorders and other mental health conditions. The present study extends the

findings of earlier studies that support a potential role of phenethylamine psychedelics, such as DOI, in reducing the positive motivational properties of alcohol. We are the first to report that a single administration of DOI reduces EtOH intake and preference for at least 24 h after administration in Long–Evans rats. In addition, DOI produced the largest reduction in EtOH intake and preference in Long–Evans rats with the highest baseline levels of EtOH consumption, perhaps supporting a 'baseline dependency' explanation for these findings. Elucidating the mechanisms by which psychedelics reduce alcohol intake and preference and targeting the use of these compounds in subpopulations of individuals with AUDs and comorbid conditions may hold great promise for new treatment strategies for AUDs.

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Conflicts of interest

There are no conflicts of interest.

References

Alper K, Dong B, Shah R, Sershen H, Vinod KY (2018). LSD administered as a single dose reduces alcohol consumption in C57BL/6J mice. *Front Pharmacol* **9**:994.

Bogenschutz MP, Forcehimes AA, Pommy JA, Wilcox CE, Barbosa PC, Strassman RJ (2015). Psilocybin-assisted treatment for alcohol dependence: a proof-of-concept study. *J Psychopharmacol* **29**:289–299.

Bruno F (1989). Buspirone in the treatment of alcoholic patients. *Psychopathology* **22** (Suppl 1):49–59.

Carnicella S, Amamoto R, Ron D (2009). Excessive alcohol consumption is blocked by glial cell line-derived neurotrophic factor. *Alcohol* **43**:35–43.

CDC (2019). Alcohol and Public Health: Alcohol-Related Disease Impact (ARDI). https://nccd.cdc.gov/DPH_ARDI/Default/Report.aspx?T=AAM&P=1A04A664-0244-42C1-91DE-316F3AF6B447&R=557A7447-041F-477C-80D6-54CF5F20A27A&M=32B5FFE7-81D2-43C5-A892-9B9B3C4246C7&F=&D= [Accessed July 8, 20].

Dawson DA, Goldstein RB, Grant BF (2007). Rates and correlates of relapse among individuals in remission from DSM-IV alcohol dependence: a 3-year follow-up. *Alcohol Clin Exp Res* **31**:2036–2045.

Dews PB (1977). Rate-dependency hypothesis. *Science* **198**:1182–1183.

Ginsburg BC, Lamb RJ (2014). Drug effects on multiple and concurrent schedules of ethanol- and food-maintained behaviour: context-dependent selectivity. *Br J Pharmacol* **171**:3499–3510.

GraphPad Software Inc. 1995-2021. How to report the methods used for the mixed model analysis. https://www.graphpad.com/guides/prism/latest/statistics/stat_how-to-report-the-methods-used.htm. [Accessed December 1, 2020].

Kranzler HR, Burleson JA, Korner P, Del Boca FK, Bohn MJ, Brown J, Liebowitz N (1995). Placebo-controlled trial of fluoxetine as an adjunct to relapse prevention in alcoholics. *Am J Psychiatry* **152**:391–397.

Krebs TS, Johansen PØ (2012). Lysergic acid diethylamide (LSD) for alcoholism: meta-analysis of randomized controlled trials. *J Psychopharmacol* **26**:994–1002.

Johnson MW, Garcia-Romeu A, Cosimano MP, Griffiths RR (2014). Pilot study of the 5-HT2AR agonist psilocybin in the treatment of tobacco addiction. *J Psychopharmacol* **28**:983–992.

Leighty AE, Ansara ED (2019). Treatment outcomes of long-acting injectable naltrexone versus oral naltrexone in alcohol use disorder in veterans. *Ment Health Clin* **9**:392–396.

Maurel S, De Vry J, Schreiber R (1999a). 5-HT receptor ligands differentially affect operant oral self-administration of ethanol in the rat. *Eur J Pharmacol* **370**:217–223.

Maurel S, De Vry J, De Beun R, Schreiber R (1999b). 5-HT2A and 5-HT2C/5-HT1B receptors are differentially involved in alcohol preference and consummatory behavior in cAA rats. *Pharmacol Biochem Behav* **62**:89–96.

National Research Council (US) Committee for the Update of Guide for the Care and Use of Laboratory Animals (2011). *Guide for the Care and Use of Laboratory Animals*. 8th ed. Washington, DC: National Academies Press (US).

Nichols DE (2016). Psychedelics. *Pharmacol Rev* **68**:264–355.

Oppong-Damoah A, Curry KE, Blough BE, Rice KC, Murnane KS (2019). Effects of the synthetic psychedelic 2,5-dimethoxy-4-iodoamphetamine (DOI) on ethanol consumption and place conditioning in male mice. *Psychopharmacology (Berl)* **236**:3567–3578.

Pettinati HM, Volpicelli JR, Luck G, Kranzler HR, Rukstalis MR, Cnaan A (2001). Double-blind clinical trial of sertraline treatment for alcohol dependence. *J Clin Psychopharmacol* **21**:143–153.

Sari Y, Johnson VR, Weedman JM (2011). Role of the serotonergic system in alcohol dependence: from animal models to clinics. *Prog Mol Biol Transl Sci* **98**:401–443.

Simms JA, Steensland P, Medina B, Abernathy KE, Chandler LJ, Wise R, Bartlett SE (2008). Intermittent access to 20% ethanol induces high ethanol consumption in Long-Evans and Wistar rats. *Alcohol Clin Exp Res* **32**:1816–1823.

Simms JA, Bito-Onon JJ, Chatterjee S, Bartlett SE (2010). Long-Evans rats acquire operant self-administration of 20% ethanol without sucrose fading. *Neuropsychopharmacology* **35**:1453–1463.

SAMHSA (2018). 2018 National Survey on Drug Use and Health (NSDUH). Table 5.4A–Alcohol Use Disorder in Past Year among Persons Aged 12 or Older, by Age Group and Demographic Characteristics: Numbers in Thousands, 2017 and 2018. https://www.samhsa.gov/data/sites/default/files/cbhsq-reports/NSDUHDetailedTabs2018R2/NSDUHDetTabsSect5pe2018.htm#tab 5-4a. [Accessed July 8, 20].