

Sigma-1 antagonism inhibits binge ethanol drinking at adolescence

Leandro Ruiz Leyva,^c Agustín Salguero^{a,b}, Ignacio Morón^d, Enrique Portillo-Salido^e, Cruz

Miguel Cendán^{c, *} and Ricardo Marcos Pautassi^{a,b, *}

^aInstituto de Investigación Médica M. y M. Ferreyra (INIMEC–CONICET-Universidad Nacional de Córdoba), Córdoba, C.P. 5000, Argentina

^bFacultad de Psicología, Universidad Nacional de Córdoba, Córdoba, C.P. 5000 Argentina

^cDepartment of Pharmacology, Faculty of Medicine, University of Granada, Spain; Institute of Neuroscience, Biomedical Research Center (CIBM), University of Granada, Spain; Instituto de Investigación Biosanitaria (IBS), Granada, Spain.

^dDepartment of Psychobiology and Centre of Investigation of Mind, Brain, and Behaviour (CIMCYC), University of Granada, Spain

^eDrug Discovery and Preclinical Development, Esteve Pharmaceuticals, Parc Científic de Barcelona, Barcelona, Spain

*Corresponding authors: Ricardo Marcos Pautassi (rpautassi@gmail.com) and Cruz Miguel Cendán (cmcendan@ugr.es)

ABSTRACT

Background: Ethanol use during adolescence is a significant health problem, yet the pharmacological treatments to reduce adolescent binge drinking are scarce. The present study assessed, in male and female adolescent Wistar rats, if the sigma-1 receptor (S1-R) antagonists S1RA or BD-1063 disrupted ethanol drinking. **Methods:** Three times a week, for two weeks, the rats received the S1-R antagonists. Thirty min later they were exposed, for 2h, to a bottle of 8% or 10% v/v ethanol. A 24h, two-bottle, ethanol intake test was conducted after termination of these procedures. A subset of these rats was tested for recognition memory via the novel object recognition test. **Results:** The rats given 64 mg/kg S1RA drank, in each binge session, significantly less than vehicle counterparts. Male rats given 4 or 16 mg/kg S1RA drank significantly less than those given 0 mg/kg in session 3 or in session 1 and 2, respectively; whereas female rats given 4 or 16 mg/kg drank significantly less than females given 0 mg/kg in session 2-5 or in sessions 2-6, respectively. Administration of 32 mg/kg, but not of 2 or 8 mg/kg, BD-1063 suppressed, across sessions, ethanol drinking. S1-R antagonism reduced absolute ethanol drinking at the two-bottle choice post-test. Recognition memory was not affected by the ethanol exposure. **Conclusions:** The results indicate that S1-R antagonists may be promising targets to prevent increases in ethanol intake at adolescence. The persistent effect of S1-R antagonism in free-choice drinking suggests that modulation of the S1-R is altering plastic effects associated with ethanol exposure.

KEYWORDS: ethanol; sigma-1 receptor; rat; adolescence; binge drinking; sex differences

1. INTRODUCTION

Recent studies have warned on the high prevalence of teenage drinking, the closing of the sex gap in ethanol consumption (Thibaut, 2018), and the rising occurrence of adolescent binge drinking (Pilatti et al., 2017; Spear, 2018). The latter is associated with immediate (Wicki et al., 2018) or long-term negative consequences, including greater likelihood of exhibiting an alcohol use disorder (AUD) (Rial Boubeta et al., 2018).

Sigma receptors, of which two subtypes (sigma-1 and sigma-2) have been described, were first proposed as new opioid receptors, and they were also confused with PCP/NMDA receptors (Maurice and Su, 2009). The endogenous ligand of sigma receptors is still unknown, although some neurosteroids have been suggested as likely candidates (Hayashi and Su, 2005). The sigma-1 receptor (S1-R) is an intracellular chaperone with an aminoacidic sequence well conserved across species (Alon et al., 2017; Maurice and Su, 2009); and is concentrated in brain areas related to motivation, learning and sensorimotor processes, including hippocampus, olfactory bulb, and substantia nigra (Cobos et al., 2008). The S1-R ligands have long been expected to mitigate neurodegenerative and mood disorders, chronic pain or drug abuse, among other diseases (Merlos et al., 2017; Romero and Portillo-Salido, 2019). Systemic or intracerebroventricular administration of a S1-R agonist (Bhutada et al., 2012; Maurice et al., 2003) enhances preference for a place associated with the effects of ethanol (Quadir et al., 2019). Moreover, S1-R agonism via DTG exacerbated operant responding for ethanol (Sabino et al., 2011; Valenza et al., 2020), an effect disrupted by BD-1063 (Sabino et al., 2011). On the other hand, it has been also shown that antagonism of S1-R, via administration of BD-1063/1047 or NE-100, dose-dependently inhibited ethanol-induced behavioral activation in an open field (Maurice et al., 2003) or ethanol self-administration (Blasio et al., 2015; Sabino et al., 2009b). Altogether, these studies suggest that antagonism of S1-R reduces ethanol seeking and intake, and hence is a promising strategy for the development of medications to treat risky drinking.

The density of S1-R levels is affected by the hormonal changes that take place at adolescence (Moradpour et al., 2016). This suggests that the effects of S1-R agonists can be age-specific. The modulatory effect of the S1-R system on ethanol intake during adolescence remains uncharted and, despite the suggestion for adequate sex representation (Hilderbrand and Lasek, 2018), all of the studies on the role of S1-R in ethanol consumption have employed males (Quadir et al., 2019). This is particularly troubling, as female rats (Varlinskaya et al., 2015; Vetter-O'Hagen et al., 2011) and mice (Lopez et al., 2011; Szumlinski et al., 2019) consistently exhibit greater ethanol intake and preference than males [for a comprehensive review, see (Roth et al., 2004)], and women tend to progress more rapidly from alcohol initiation or first drunkenness to problematic drinking (Brady and Randall, 1999; Erol and Karpyak, 2015). The present study assessed the role of the S1-R system on binge-like ethanol drinking in adolescent Wistar rats, both male and female. We evaluated if pretreatment with the S1-R antagonists S1RA or BD-1063 disrupted drinking in a procedure that induces levels of drinking akin to those found during a binge drinking episode (Salguero et al., 2020). It was possible that the binge exposure altered recognition memory (Marco et al., 2017), and that BD-1063 protected from this effect. This was assessed in Experiment 2, via the novel object recognition (NOR) test.

2. METHODS

2.1 Experimental Design and Subjects

We employed 169 adolescent Wistar rats [80 (40 male) in Experiment 1, 89 (39 male) in Experiment 2], derived from 18 litters reared at the Instituto de Investigación Mercedes y Martín Ferreyra (INIMEC-CONICET-UNC; Córdoba, Argentina). Several studies have described postnatal days (PDs) 27-28 to 42 as corresponding to early/mid-adolescence in humans, and the PD 46-59 period as late adolescence (Burke and Miczek, 2014; Karanikas et al., 2013; Spear, 2000). We conducted the ethanol drinking procedures between PDs 27-44 and the NOR test between PDs 46-48. Testing in the study, thus, spanned all the stages of adolescence.

A 4 (S1RA dose: 0, 4, 16 or 64 mg/kg) × 2 (Sex) factorial design was employed in Experiment 1 ($n = 10$ /group). Experiment 2 employed a 4 (BD-1063 dose: 0, 2, 8 or 32 mg/kg) × 2 (Sex) factorial, with 7-10 rats in each group. Eighteen rats (10 females) were used as non-exposed controls in the NOR. Weaning was conducted at PD21. The procedures complied with the ARRIVE guidelines, the Guide for the Care and Use of Laboratory Animals of NIH and were certified by the Animal Care and Use Committee at INIMEC-CONICET-UNC.

2.2 Drugs

S1RA (4-[2-[[5-methyl-1-(2-naphthalenyl)-1H-pyrazol-3-yl]oxy]ethyl] morpholine hydrochloride) and BD-1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpiperazine dihydrochloride), were supplied by Esteve Pharmaceuticals (Barcelona, Spain) and dissolved in sterile physiological saline or a dPBS (Dulbecco's Phosphate Buffered Saline) 10X solution, respectively. A 5 ml/kg of the drug solution or its solvent was injected subcutaneously (s.c.).

2.3 Ethanol Self-administration and NOR test procedures

Seventy-two hours before the first binge session the rats were habituated to the smell/flavor of ethanol in a 24h, two-bottle (8% ethanol vs water) drinking session. No antagonists were administered at this habituation session. For the next 2 weeks (PDs 30-41) the rats were exposed three times a week (Tuesdays, Thursdays and Saturdays, from 1900 to 2100h; vivarium lights went off at 1845) to a single bottle of 8% (sessions 1 and 2) or 10% v/v ethanol (3rd and following sessions; 96% ethanol, Porta, Cordoba, Argentina). The day before each session the animals received 50% of the water they usually consumed. The S1-R antagonists were administered 30 min before each binge session. A two bottle (8% ethanol vs. water), 24h, intake test was conducted in the homecage, 72h after the last binge session. The ethanol or water bottles were weighed before

and after each session, to calculate ethanol intake (g/kg), percent preference of ethanol intake, overall liquid intake and water intake (ml/100 g of body weight).

In Exp. 2, six rats of each group and 18 naïve rats (i.e., not exposed to ethanol) were tested for short-term memory in the NOR procedure (Salguero et al., 2020). Briefly, on PD46 the rats were habituated for 10 min to a squared-shaped arena (50cm x 50cm x 50cm) and distance travelled was measured via a custom-made behavioral tracking system. On PD47 (familiarization) the rats explored the arena for 5 minutes, which now featured two identical opaque glass flasks. The rats underwent a 5-min testing phase on PD48, in which one of the opaque flasks was replaced by taller and slightly clearly colored flask. The sessions were filmed, and time spent exploring the flasks at the familiarization and time spent in proximity to the new object at the testing (i.e., an indicator of recognition memory) were measured.

2.4 Statistical Analyses

The scores at the intake habituation session, in which no antagonists were given, were analyzed via Student's T test (grouping factor: Sex). Ethanol intake (g/kg) during the binge sessions was assessed using repeated measures ANOVAs, with Sex and Drug dose as between factors and Day of assessment (sessions 1-6) as the within-measure. Ethanol intake scores and water or overall liquid intake at the two-bottle choice ethanol intake test conducted after the binge sessions were analyzed via a factorial ANOVA, with Sex and Dose as between factors. A relative discrimination index (Di) [i.e., time spent exploring the novel object minus time spent exploring the familiar object divided by total exploration time] was calculated at the NOR test and analyzed via ANOVA. The significant main effects and significant interactions ($\alpha \leq 0.05$) were explored via Tukey's *post hoc* tests (for significant effects involving between-subject factors) or planned comparisons (significant main effects or interactions encompassing between-by-within factors).

3. RESULTS

3.1 Experiment 1

Males and females showed similar ethanol intake (g/kg or %) or overall fluid intake at the habituation session ($p > 0.05$; see Table 1). The ANOVA for g/kg ingested during the binge sessions (Fig. 1) yielded significant main effects of Dose ($F_{3,70} = 34.08$, $p < .001$, $\eta^2p = .59$), Day of Assessment ($F_{5,350} = 58.17$, $p < .001$, $\eta^2p = .45$) and Sex ($F_{1,70} = 28.2$, $p < .001$, $\eta^2p = .29$), and significant two-way interactions between Day of Assessment and Sex ($F_{5,350} = 4.59$, $p < .001$, $\eta^2p = .06$), and between Day of Assessment and Dose ($F_{15,350} = 3.13$, $p < .001$, $\eta^2p = .13$). The interaction Day of Assessment x Sex x Dose was also significant ($F_{15,350} = 3.38$, $p < .001$, $\eta^2p = .13$). In each binge session, male and female rats given 64 mg/kg S1RA drank significantly less than their sex-matched vehicle counterparts. Male rats given 4 or 16 mg/kg S1RA drank significantly less than those given 0 mg/kg in session 3 or in session 1 and 2, respectively; whereas female rats given 4 or 16 mg/kg drank significantly less than females given 0 mg/kg in session 2-5 or in sessions 2-6, respectively.

FIGURE 1 AND TABLE 1

Post-test ethanol drinking scores were significantly lower in males or female rats treated with 16 or 64 mg/kg S1RA, and in females treated with 4 mg/kg, than in vehicle-treated controls (significant Sex x treatment interaction, $F_{3,70} = 3.50$, $p < .05$, $\eta^2p = .13$ and $F_{3,69} = 4.49$, $p < .01$, $\eta^2p = .16$, g/kg and percent ethanol intake, respectively). Liquid ingestion was lower in rats treated with either dose of S1RA than in vehicle-treated rats ($F_{3,69} = 7.80$, $p < .001$, $\eta^2p = .85$). The ANOVA for water ingestion indicated that females given 64 mg/kg S1RA and males given 4 mg/kg S1RA drank significantly more and less water, respectively, than their sex-matched vehicle controls (significant Sex x treatment interaction, $F_{3,70} = 4.01$, $p < .05$, $\eta^2p = .15$). These scores are in the supplemental figure and in Table 1.

3.2 Experiment 2

Males and females exhibited similar ethanol (g/kg) or liquid intake at the habituation (Table 1), yet females exhibited significantly greater percent ethanol intake than did males ($t = 2.58$, $p < 0.001$; 14.66 vs 6.53). The ANOVA for g/kg ingested during the binge sessions revealed significant main effects of Dose and Day of Assessment ($F_{3,62} = 8.88$, $p < .001$, $\eta^2 p = .30$ and $F_{5,310} = 32.63$, $p < .001$, $\eta^2 p = .34$). As confirmed by the *post-hoc* tests and shown in Fig. 1 D-E, ethanol drinking (g/kg) was significantly higher in binge days 1 and 2 than in day 3 and significantly greater in binge days 1, 2 or 3 compared to days 4-6. Across days (Fig. 1F) the rats treated with 32 mg/kg BD-1063, males or females, drank significantly less than the other groups. The variables measured at the post-test (Table 1 and supp. figure) were not significantly affected by BD-1063 nor by sex. Guided by a priori hypothesis, we conducted a planned comparison on g/kg scores between the 0 mg/kg and the 32 mg/kg group, which revealed significantly less ethanol consumed in the 32 vs. the 0 mg/kg group ($F_{1,62} = 4.05$, $p < .005$). The ANOVAs for NOR scores, including Di scores at test, did not reveal significant main effects or significant interactions (Table 1).

4. DISCUSSION

The heightened vulnerability of adolescents to AUD is a significant concern. Thus, it is important to understand its neurobiological underpinnings and develop preventive and treatment strategies (Waller et al., 2019). There are only a few pharmacological treatments approved to maintain abstinence, reduce drinking, or prevent reinstatement; and these have been rarely studied in models of adolescent alcohol drinking (Sable et al., 2006). The present study found that antagonism of S1-R may prevent increases in adolescent ethanol intake, prior to the development of dependence. Future work, however, should evaluate if this antagonism reduces binge drinking after a protracted prior history of ethanol exposure (i.e., longer than the single habituation day used in this study).

The antagonism of S1-R, via administration of S1RA or BD-1063, blocked ethanol binge drinking in both male and female adolescent Wistar rats. Adolescents given 64 mg/kg S1RA

exhibited, relative to vehicle-treated counterparts, an overall two-fold reduction in ethanol drinking. The S1-R antagonists were given, similar to other medications to treat chronic alcoholism or relapse [e.g., disulfiram (Bharadwaj et al., 2018; Mutschler et al., 2016)], acutely prior to drinking. The influence of both treatments largely surpassed the threshold of a large effect (i.e., $\eta^2p \geq 0.14$). To our knowledge, this is the first report of S1-R modulating ethanol consumption at adolescence, and the first to analyze interaction between the S1-R system and ethanol intake in females, which usually drink more ethanol than their male counterparts (Roth et al., 2004).

In Experiment 1, S1-R antagonism diminished ethanol intake of females in a dose-dependent manner, whereas males displayed a suppressing effect mainly after the highest S1RA dose. Studies have shown that ethanol drinking of female rats is more affected by stress, than that of males (Varlinskaya and Spear, 2015; Wille-Bille et al., 2017), suggesting that females are more like to drink ethanol to reduce anxiety. Our drinking model involves brief, yet significant, social isolation and a mild liquid deprivation that can serve as stressors. It is possible that low doses of S1RA are selectively effective to reduce ethanol drinking driven by the drug's anxiolytic effects. This is just a hypothesis, yet it is intriguing that the selective S1-R antagonist NE-100 dose dependently blocks ethanol intake in the stress-sensitive and anxiety-prone, Sardinian alcohol preferring rats (Sabino et al., 2009b). Moreover, S1-R binds compounds effective for treating anxiety at adolescence (Cheer and Figgitt, 2002) and S1-R activation mediates stress effects in animal models of gastrointestinal tract disorders seen in anxiety (Gue et al., 1992).

S1RA had a lingering effect, reducing absolute ethanol drinking vs. vehicle-treated controls at the two-bottle post-test, long after its clearance. The effect was seen across S1RA doses for females, and there was some evidence of this effect after 32 mg/kg BD-1063. S1RA achieves, in rodents, maximum plasma concentration shortly after its administration. Specifically, S1RA plasma levels are undetectable 6 h after its administration, its metabolites are inactive and it does not accumulate in the brain (Gris et al., 2016). The pattern resembles findings in which naloxone prevented binge exposure and the promoting effect of such exposure upon later drinking (Salguero

et al., 2020), as well as studies in which S1-R antagonists blocked cocaine-induced behavioral sensitization (Ujike et al., 1996). This molecule has shown efficacy and safety in Phase I/II trials (Bruna et al., 2018).

Limitations of this report are the lack of neurobiological measurements or of a reversal of the effects via sigma agonism. Moreover, we did not measure if acute treatment with the S1-R antagonists affected voluntary ingestion of water. The binge drinking procedure employed did not feature access to water, thus precluding these measurements. Yet, it is important that, in the two-bottle intake tests conducted after the last binge session, prior treatment with S1RA was associated with a significant decrease in overall liquid ingestion, whereas BD-1063 altered ethanol ingestion without significantly altering water or overall liquid ingestion. The latter result is consistent with a study indicating that BD-1063 altered ethanol self-administration but did not inhibit water or saccharin self-administration (Sabino et al., 2009a). Another limitation is that it was not possible to assess the effects of S1-R antagonism in ethanol-induced memory impairments. Similar to a previous report (Salguero et al., 2020), the binge-like exposure did not alter recognition memory in the NOR test.

Despite these limitations, the results indicate that S1-R antagonists may be promising targets to prevent increases in ethanol intake at adolescence. The persistent effect of S1-R antagonism in free-choice drinking suggests that modulation of the S1-R is altering plastic effects associated with ethanol exposure.

Role of Funding Source: This work was supported by PICT 2015-0325 of Agencia Nacional de Promoción Científica y Tecnológica (FONCyT), to RMP; by the Junta de Andalucía (grant CTS 109), and by funding from Esteve Pharmaceuticals and the European Regional Development Fund (ERDF). This research was done in partial fulfillment of the requirements for the doctoral thesis of LR-L, who was supported by a predoctoral grant from the Programa de Movilidad Internacional para Estudiantes de Doctorado 2019/20 of the University of Granada. The funders had no further role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Author contribution: Conceptualization, Visualization, Writing, Original draft preparation: Leandro Ruiz Leyva, Agustín Salguero, Ignacio Morón, Cruz Miguel Cendán, Ricardo Pautassi. Funding acquisition: Ricardo Pautassi, Cruz Miguel Cendán, Ignacio Morón, Enrique Portillo-Salido. Data curation, Project administration, Investigation: Leandro Ruiz Leyva, Agustín Salguero, Ricardo Pautassi. Project administration, Resources: Leandro Ruiz Leyva, Agustín Salguero. Writing, Original draft preparation, Resources: Enrique Portillo-Salido

Conflict of interest: We declare having no competing interest nor conflict of interest related to our MS or its results. EPS is an employee of the international pharmaceutical company ESTEVE. The latter company, however, had no role in study design, in the collection, analysis, and interpretation of data nor in the writing of the report.

FIGURES LEGENDS.

Figure 1: Panels A-B and D-E. Ethanol intake (g/kg) in adolescent Wistar rats as a function of sex, binge intake session (i.e., Day of Assessment 1-6) and treatment with S1RA (0, 4, 16 or 64 mg/kg; Exp. 1) or BD-1063 (0, 2, 8 or 32 mg/kg; Exp. 2). The rats were given S1RA or BD-1063 s.c. and, 30 min later, self-administered 8% (first two sessions) or 10% ethanol (third and subsequent session) during the first 2 hours of the dark cycle, three times a week (Monday, Wednesday, and Friday) during postnatal days 30-41. **Panels C and F.** Same data as in A-B and D-E, respectively,

collapsed across day of assessment. In Exp. 1 the ANOVA yielded a significant Treatment x Sex x Day interaction. The female rats given 64 mg/kg or 4 mg/kg S1RA drank significantly less than the 0 mg/kg group on days 1-6 or 2-5, respectively. These differences are indicated by the * and # signs. Among females, the 0 mg/kg group and the 16 mg/kg S1RA group differed significantly on days 2-6, as indicated by the @ sign. The male rats given 64 mg/kg or 16 mg/kg S1RA drank significantly less than the 0 mg/kg group on days 1-6 or 1-2, respectively. These differences are indicated by the * and @ signs. Males treated 0 mg/kg or 4 mg/kg S1RA differed significantly only on day 3, as indicated by the # sign. In Exp. 2 the ANOVA indicated a main effect of treatment: the rats treated with 32 mg/kg BD-1063, males or females, drank significantly less than the other groups, an effect indicated by the * sign in panel F. The data are expressed as mean \pm SEM.

Suppl. Figure 1: Ethanol intake (g/kg) in adolescent Wistar rats at a two-bottle, 24h, intake test conducted on postnatal day (PD) 44. The data is depicted as a function of sex and treatment with S1RA (0, 4, 16 or 64 mg/kg; Experiment 1, panel A) or BD-1063 (0, 2, 8 or 32 mg/kg; Experiment 2, panel B). The rats were given S1RA or BD-1063 and, 30 min later, self-administered 8% or 10% ethanol for 2 hours, three times a week during PDs 30-41. The statistical analysis indicated that, in Exp. 1, males and females treated with 16 or 64 S1RA mg/kg, or females treated with 4 mg/kg S1RA, drank significantly less than vehicle-treated counterparts. In Exp. 2 the male rats given 32 mg/kg BD-1063 drank significantly less ethanol than controls. These significant differences are indicated by the asterisks. The data are expressed as mean \pm SEM.

REFERENCES

- Alon, A., Schmidt, H., Zheng, S., Kruse, A.C., 2017. Structural Perspectives on Sigma-1 Receptor Function. *Advances in experimental medicine and biology* 964, 5-13.
- Bhutada, P.S., Mundhada, Y.R., Ghodki, Y.R., Chaware, P., Dixit, P.V., Jain, K.S., Umathe, S.N., 2012. Influence of sigma-1 receptor modulators on ethanol-induced conditioned place preference in the extinction-reinstatement model. *Behavioural pharmacology* 23(1), 25-33.
- Blasio, A., Valenza, M., Iyer, M.R., Rice, K.C., Steardo, L., Hayashi, T., Cottone, P., Sabino, V., 2015. Sigma-1 receptor mediates acquisition of alcohol drinking and seeking behavior in alcohol-preferring rats. *Behavioural brain research* 287, 315-322.
- Brady, K.T., Randall, C.L., 1999. Gender differences in substance use disorders. *The Psychiatric clinics of North America* 22(2), 241-252.

Bruna, J., Videla, S., Argyriou, A.A., Velasco, R., Villoria, J., Santos, C., Nadal, C., Cavaletti, G., Alberti, P., Briani, C., Kalofonos, H.P., Cortinovis, D., Sust, M., Vaque, A., Klein, T., Plata-Salaman, C., 2018. Efficacy of a Novel Sigma-1 Receptor Antagonist for Oxaliplatin-Induced Neuropathy: A Randomized, Double-Blind, Placebo-Controlled Phase IIa Clinical Trial. *Neurotherapeutics* 15(1), 178-189.

Burke, A.R., Miczek, K.A., 2014. Stress in adolescence and drugs of abuse in rodent models: role of dopamine, CRF, and HPA axis. *Psychopharmacology* 231(8), 1557-1580.

Cheer, S.M., Figgitt, D.P., 2002. Spotlight on fluvoxamine in anxiety disorders in children and adolescents. *CNS Drugs* 16(2), 139-144.

Cobos, E.J., Entrena, J.M., Nieto, F.R., Cendan, C.M., Del Pozo, E., 2008. Pharmacology and therapeutic potential of sigma(1) receptor ligands. *Curr Neuropharmacol* 6(4), 344-366.

Erol, A., Karpyak, V.M., 2015. Sex and gender-related differences in alcohol use and its consequences: Contemporary knowledge and future research considerations. *Drug and alcohol dependence* 156, 1-13.

Gris, G., Portillo-Salido, E., Aubel, B., Darbaky, Y., Deseure, K., Vela, J.M., Merlos, M., Zamanillo, D., 2016. The selective sigma-1 receptor antagonist E-52862 attenuates neuropathic pain of different aetiology in rats. *Scientific reports* 6, 24591.

Gue, M., Junien, J.L., Del Rio, C., Bueno, L., 1992. Neuropeptide Y and sigma ligand (JO 1784) suppress stress-induced colonic motor disturbances in rats through sigma and cholecystinin receptors. *The Journal of pharmacology and experimental therapeutics* 261(3), 850-855.

Hayashi, T., Su, T., 2005. The sigma receptor: evolution of the concept in neuropsychopharmacology. *Curr Neuropharmacol* 3(4), 267-280.

Hilderbrand, E.R., Lasek, A.W., 2018. Studying Sex Differences in Animal Models of Addiction: An Emphasis on Alcohol-Related Behaviors. *ACS Chem Neurosci* 9(8), 1907-1916.

Karanikas, C.A., Lu, Y.L., Richardson, H.N., 2013. Adolescent drinking targets corticotropin-releasing factor peptide-labeled cells in the central amygdala of male and female rats. *Neuroscience* 249, 98-105.

Lopez, M.F., Doremus-Fitzwater, T.L., Becker, H.C., 2011. Chronic social isolation and chronic variable stress during early development induce later elevated ethanol intake in adult C57BL/6J mice. *Alcohol* 45(4), 355-364.

Marco, E.M., Penasco, S., Hernandez, M.D., Gil, A., Borcel, E., Moya, M., Gine, E., Lopez-Moreno, J.A., Guerri, C., Lopez-Gallardo, M., Rodriguez de Fonseca, F., 2017. Long-Term Effects of Intermittent Adolescent Alcohol Exposure in Male and Female Rats. *Frontiers in behavioral neuroscience* 11, 233.

Maurice, T., Casalino, M., Lacroix, M., Romieu, P., 2003. Involvement of the sigma 1 receptor in the motivational effects of ethanol in mice. *Pharmacology, biochemistry, and behavior* 74(4), 869-876.

Maurice, T., Su, T.P., 2009. The pharmacology of sigma-1 receptors. *Pharmacology & therapeutics* 124(2), 195-206.

Merlos, M., Burgueno, J., Portillo-Salido, E., Plata-Salaman, C.R., Vela, J.M., 2017. Pharmacological Modulation of the Sigma 1 Receptor and the Treatment of Pain. *Advances in experimental medicine and biology* 964, 85-107.

Moradpour, F., Fathollahi, Y., Naghdi, N., Hosseinmardi, N., Javan, M., 2016. Prepubertal castration-associated developmental changes in sigma-1 receptor gene expression levels regulate hippocampus area CA1 activity during adolescence. *Hippocampus* 26(7), 933-946.

Pilatti, A., Read, J.P., Pautassi, R.M., 2017. ELSA 2016 Cohort: Alcohol, Tobacco, and Marijuana Use and Their Association with Age of Drug Use Onset, Risk Perception, and Social Norms in Argentinean College Freshmen. *Frontiers in psychology* 8, 1452.

Quadir, S.G., Cottone, P., Sabino, V., 2019. Role of Sigma Receptors in Alcohol Addiction. *Frontiers in pharmacology* 10, 687.

Rial Boubeta, A., Golpe, S., Barreiro, C., Gomez, P., Isorna Folgar, M., 2018. The age of onset for alcohol consumption among adolescents: Implications and related variables. *Adicciones* 0(0), 1266.

Romero, L., Portillo-Salido, E., 2019. Trends in Sigma-1 Receptor Research: A 25-Year Bibliometric Analysis. *Frontiers in pharmacology* 10, 564.

Roth, M.E., Cosgrove, K.P., Carroll, M.E., 2004. Sex differences in the vulnerability to drug abuse: a review of preclinical studies. *Neuroscience and biobehavioral reviews* 28(6), 533-546.

Sabino, V., Cottone, P., Blasio, A., Iyer, M.R., Steardo, L., Rice, K.C., Conti, B., Koob, G.F., Zorrilla, E.P., 2011. Activation of sigma-receptors induces binge-like drinking in Sardinian alcohol-preferring rats. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 36(6), 1207-1218.

Sabino, V., Cottone, P., Zhao, Y., Iyer, M.R., Steardo, L., Jr., Steardo, L., Rice, K.C., Conti, B., Koob, G.F., Zorrilla, E.P., 2009a. The sigma-receptor antagonist BD-1063 decreases ethanol intake and reinforcement in animal models of excessive drinking. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 34(6), 1482-1493.

Sabino, V., Cottone, P., Zhao, Y., Steardo, L., Koob, G.F., Zorrilla, E.P., 2009b. Selective reduction of alcohol drinking in Sardinian alcohol-preferring rats by a sigma-1 receptor antagonist. *Psychopharmacology* 205(2), 327-335.

Sable, H.J., Bell, R.L., Rodd, Z.A., McBride, W.J., 2006. Effects of naltrexone on the acquisition of alcohol intake in male and female periadolescent and adult alcohol-preferring (P) rats. *International journal of adolescent medicine and health* 18(1), 139-149.

Salguero, A., Suarez, A., Luque, M., Ruiz-Leyva, L., Cendán, C.M., Morón, I., Pautassi, R.M., 2020. Binge-Like, Naloxone-Sensitive, Voluntary Ethanol Intake at Adolescence Is Greater Than at Adulthood, but Does Not Exacerbate Subsequent Two-Bottle Choice Drinking. 14(50).

Spear, L.P., 2000. The adolescent brain and age-related behavioral manifestations. *Neuroscience and biobehavioral reviews* 24(4), 417-463.

Spear, L.P., 2018. Effects of adolescent alcohol consumption on the brain and behaviour. *Nature reviews. Neuroscience* 19(4), 197-214.

Szumliński, K.K., Coelho, M.A., Lee, K.M., Tran, T., Sern, K.R., Bernal, A., Kippin, T.E., 2019. DID it or DIDn't it? Exploration of a failure to replicate binge-like alcohol-drinking in C57BL/6J mice. *Pharmacology, biochemistry, and behavior* 178, 3-18.

Thibaut, F., 2018. Alert out on tobacco and alcohol consumption in young European women. *European archives of psychiatry and clinical neuroscience* 268(4), 317-319.

Ujike, H., Kuroda, S., Otsuki, S., 1996. sigma Receptor antagonists block the development of sensitization to cocaine. *European journal of pharmacology* 296(2), 123-128.

Valenza, M., Blasio, A., DiLeo, A., Cottone, P., Sabino, V., 2020. Sigma receptor-induced heavy drinking in rats: Modulation by the opioid receptor system. *Pharmacology, biochemistry, and behavior* 192, 172914.

Varlinskaya, E.I., Spear, L.P., 2015. Social consequences of ethanol: Impact of age, stress, and prior history of ethanol exposure. *Physiology & behavior* 148, 145-150.

Varlinskaya, E.I., Truxell, E.M., Spear, L.P., 2015. Ethanol intake under social circumstances or alone in sprague-dawley rats: impact of age, sex, social activity, and social anxiety-like behavior. *Alcoholism, clinical and experimental research* 39(1), 117-125.

Vetter-O'Hagen, C.S., Sanders, K.W., Spear, L.P., 2011. Evidence for suppressant effects of testosterone on sex-typical ethanol intake in male Sprague-Dawley rats. *Behavioural brain research* 224(2), 403-407.

Waller, R., Murray, L., Shaw, D.S., Forbes, E.E., Hyde, L.W., 2019. Accelerated alcohol use across adolescence predicts early adult symptoms of alcohol use disorder via reward-related neural function. *Psychological medicine* 49(4), 675-684.

Wicki, M., Mallett, K.A., Delgrande Jordan, M., Reavy, R., Turrise, R., Archimi, A., Kuntsche, E., 2018. Adolescents who experienced negative alcohol-related consequences are willing to

experience these consequences again in the future. *Experimental and clinical psychopharmacology* 26(2), 132-137.

Wille-Bille, A., Ferreyra, A., Sciangula, M., Chiner, F., Nizhnikov, M.E., Pautassi, R.M., 2017. Restraint stress enhances alcohol intake in adolescent female rats but reduces alcohol intake in adolescent male and adult female rats. *Behavioural brain research* 332, 269-279.

Table

Experiment 1

		Males				Females			
S1RA dose		0 mg	4 mg	16 mg	64 mg	0 mg	4 mg	16 mg	64 mg
Intake habituation session	Ethanol intake (g/kg)	3.92 ±0.61	4.24 ±1.15	1.99 ±0.29	3.49 ±1.09	5.41 ±2.3	2.83 ±0.89	1.82 ±0.29	1.99 ±0.47
	Ethanol intake (%)	19.83 ±1.49	22.42 ±5.12	12.65 ±1.68	22.27 ±7.76	23.43 ±8.82	19.16 ±5.63	11.93 ±1.52	12.97 ±2.81
	Overall fluid intake (ml/100 g of body weight)	24.92 ±1.38	25.89 ±2.77	22.5 ±0.97	21.76 ±1.67	25.19 ±2.2	18.62 ±0.54	19.38 ±0.39	19.27 ±0.86
Post-test	Ethanol intake (g/kg)	4.34 ±0.82	4.92 ±0.69	1.57 ±0.52	1.08 ±0.18	4.83 ±1.14	1.85 ±0.30	0.81 ±0.15	1.39 ±0.71
	Ethanol intake (%)	27.49 ±5.43	44.76 ±6.74	16.58 ±6.08	9.68 ±1.88	35.34 ±6.81	18.85 ±3.48	8.19 ±1.32	8.5± 3.0
	Overall fluid intake (ml/100 g of body weight)	59.09± 6.24	42.17± 2.22	35.91± 2.18	44.10± 1.79	34.8± 2.6	28.72± 4.0	26.74± 1.0	34.06± 2.87
	Water intake (ml/100 g of body weight)	17.28± 1.81	9.96± 1.54	13.04± 1.17	16.08± 0.67	12.67± 0.86	13.68± 1.91	14.40± 0.44	18.63± 0.95

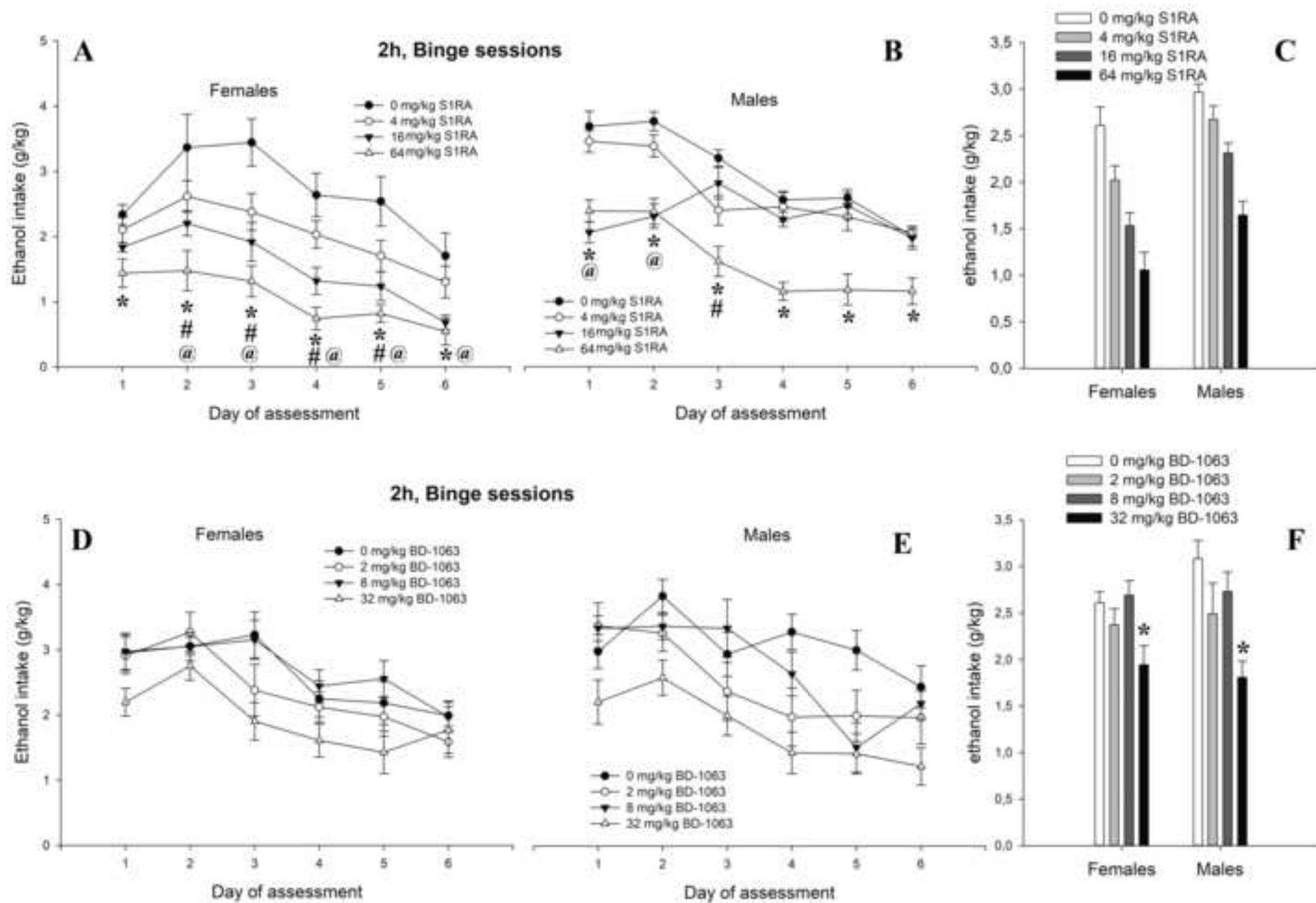
Experiment 2

BD-1063 dose		0 mg	2 mg	8 mg	32 mg	0 mg	2 mg	8 mg	32 mg
Intake habituation session	Ethanol intake (g/kg)	1.76 ±0.2	2.39 ±0.34	1.82 ±0.18	1.45 ±0.11	2.71 ±0.44	2.09 ±0.8	2.22 ±0.65	1.7 ±0.18
	Ethanol intake (%)	10.79 ±1.79	13.42 ±1.88	10.78 ±1.22	8.62 ±0.9	16.36 ±2.34	10.77 ±3.45	11.66 ±3.16	10.48 ±1.36
	Overall fluid intake (ml/100 g of body weight)	21.49 ±1.09	22.57 ±1.11	21.37 ±0.62	21.53 ±0.97	20.33 ±0.95	23.08 ±0.85	23.72 ±0.93	20.82 ±0.67
Post-test	Ethanol intake (g/kg)	3.94 ±1.21	2.81 ±1.29	3.27 ±1.36	0.85 ±0.21	2.25 ±0.59	2.12 ±0.88	2.55 ±1.06	2.5 ±0.85
	Ethanol intake (%)	30.6 ±8.04	24.91 ±9.64	31.34 ±12.12	6.78 ±1.31	25.15 ±4.70	20.36 ±5.37	26.27 ±8.63	21.84 ±8.82
	Overall fluid intake (ml/100 g)	15.50 ±0.73	14.54 ±0.58	14.69 ±0.56	16.48 ±0.66	15.07 ±0.98	17.44 ±1.55	14.57 ±0.47	16.08 ±0.75
	Water intake (ml/100 g of body weight)	10.15 ±1.35	9.66 ±1.64	8.88 ±2.20	15.05 ±0.60	9.85 ±1.27	12.66 ±1.73	9.37 ±1.99	10.98 ±2.33
NOR Habituation Phase	Distance (cm)	3880.06 ±221.57	4547.2 ±287.85	4387.6 ±404.89	3787.4 ±277.73	3193.98 ±238.82	3882.85 ±344.02	3299.16 ±231.47	3115.56 ±214.26
NOR Familiarization Phase	Total exploration time (s)	66.65 ±7.81	54.59 ±6.10	64.22 ±4.39	57.03 ±7.07	64.83 ±8.71	63.88 ±6.95	53.57 ±3.58	66.68 ±6.22

NOR	Discrimination	0.18	0.22	0.21±	0.29	0.25	0.32	0.32	0.26
Test Phase	Index (Di)	±0.07	±0.1	0.08	±0.07	±0.11	±0.1	±0.05	±0.01

Table 1: Intake scores at the habituation and post-test (i.e., post-binge) sessions (Experiments 1 and 2) and distance travelled, total exploration time and discriminative scores at the Novel Object Recognition test (NOR, Experiment 2). The NOR also included rats never exposed to ethanol or BD-1063 (i.e., naïve). Scores for these rats are not shown.

Figure

[Click here to download high resolution image](#)

Supplementary Material

[Click here to download Supplementary Material: Figure SUPP.TIF](#)

*Highlights (for review)

- We assessed effects of the S1-R antagonists S1RA or BD-1063 on ethanol drinking.
- S1RA blocked ethanol binge drinking in male and female adolescent Wistar rats.
- BD-1063 blocked ethanol binge drinking in male and female adolescent Wistar rats.
- S1RA reduced free-choice ethanol drinking long after its clearance.
- S1-R antagonists are promising targets for the development of new medications.

Author Disclosures: One of the authors is an employee of the international pharmaceutical company ESTEVE. The latter company, however, had no role in study design; in the collection, analysis, and interpretation of data nor in the writing of the report.

Author contribution: Conceptualization, Visualization, Writing, Original draft preparation: Leandro Ruiz Leyva, Agustín Salguero, Ignacio Morón, Cruz Miguel Cendán, Ricardo Pautassi. Funding acquisition: Ricardo Pautassi, Cruz Miguel Cendán, Ignacio Morón, Enrique Portillo-Salido. Data curation, Project administration, Investigation: Leandro Ruiz Leyva, Agustín Salguero, Ricardo Pautassi. Project administration, Resources: Leandro Ruiz Leyva, Agustín Salguero. Writing, Original draft preparation, Resources: Enrique Portillo-Salido

*Conflict of interest statement

We declare having no competing interest nor conflict of interest related to our MS or its results. One of the authors is an employee of the international pharmaceutical company ESTEVE. The latter company, however, had no role in study design; in the collection, analysis, and interpretation of data nor in the writing of the report.