HHS Public Access

Author manuscript

Psychopharmacology (Berl). Author manuscript; available in PMC 2026 April 16.

(R)-Ketamine reduces alcohol intake and alcohol seeking induced by reconsolidation of alcohol-related memories in female Marchigian Sardinian alcohol-preferring rats

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Abstract

Background: Alcohol use disorder (AUD) represents a significant medical challenge, with available therapeutic approaches having limited efficacy. Emerging data suggest that psychedelic compounds could represent an alternative treatment for AUD. Among this class of molecules, ketamine, a dissociative psychedelic, shows promising properties. It reduces alcohol drinking in rodents and in humans it is used to treat depression, a condition often comorbid with AUD. Unfortunately, the marked dissociative and anesthetic effects of this molecule severely limit its therapeutic application. Ketamine is a racemic mixture of (S, R)-enantiomers, and the

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Elisabetta Ciccocioppo and Sara Massetti contributed equally to this work.

Author contributions Conceptualization, RC, ED, MM, RS, MR, FF, PR; formal analysis, EC, SM, ED; investigation, EC, SM, ED, RC; resources, RC, ED; data curation, EC, SM. ED; draft preparation, EC, ED, RC; review and editing, EC, FF, ED, RC; funding acquisition, RC, MR, RS, FF, ED. We are thankful to Federica Benvenuti for helping with the experiments and Rina Righi, Agostino Marchi and Matteo Valzano for animal breeding and expert technical assistance. All the authors approved the submitted version and agree to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.

This article belongs to a Special Issue on In Memory of Klaus Miczek.

Ethical approval Experimental procedures were carried out in adherence to the European Community Council Directive for the Care and Use of Laboratory Animals, as well as the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Protocol 1D 58,032).

Competing interests On behalf of all authors, the corresponding authors state that there is no conflict of interest.

R-enantiomer possesses lower dissociative and anesthetic properties compared to the racemic mixture.

Methods: Here, using male and female genetically selected Marchigian Sardinian alcohol-preferring (msP) rats, we evaluated the potential efficacy of (R)-ketamine, in alcohol-related behaviours including home cage voluntary drinking and alcohol self-administration. We also evaluated whether R ketamine may affect the reconsolidation process of alcohol-related memories.

Results: In the two-bottle free choice (free choice between 10% alcohol and water) 24-h drinking paradigm R-ketamine given orally (10, 20 and 40 mg/kg) significantly reduced alcohol consumption in female but not in male rats. No effect was observed on alcohol self-administration. We subsequently tested (R)-ketamine on alcohol memory consolidation/retrieval task and found a significant attenuation in the retrieval of alcohol-related memories in female but not in male msP rats.

Conclusions: Taken together, our findings indicate that (R)-ketamine attenuates alcohol-related behaviors in a sex-dependent manner with females showing a higher sensitivity to its effects. Given its more favorable safety profile compared to the racemic mixture, these results support the clinical investigation of (R)-ketamine as a therapeutic intervention in patients with AUD. Particular attention should be paid to sex-specific effects observed with (R)-ketamine, as they may have important clinical implications.

Keywords

Alcohol use disorder (AUD); Psychedelics; (R)-ketamine; Memory retrieval

Introduction

Excessive alcohol consumption represents a major global health concern and is one of the leading risk factors for disease burden, accounting for approximately 4.7% of all deaths worldwide (WHO 2024). Harmful patterns of alcohol use significantly contribute to the development of alcohol use disorder (AUD), a chronic psychiatric condition associated with substantial societal and economic costs.

Once established, AUD is a complex psychiatric condition characterized by clinically significant distress resulting from harmful alcohol use and extending to a chronic relapsing profile even after prolonged abstinence (MacKillop et al. 2022). Around 70% of patients return to abuse alcohol within one year of sobriety (Durazzo and Meyerhoff 2017) making a major clinical challenge relapse. At the cognitive level, individuals affected by AUD acquire and consolidate maladaptive memories of environmental cues associated to the drug (Milton and Everitt 2012), which contribute to preserve vulnerability to relapse even after long periods of abstinence (Blanco-Gandia and Rodriguez-Arias 2018).

At present the only three drugs for AUD approved by the Food and Drug Administration and the European Medicine Agency are naltrexone, acamprosate and disulfiram (Soyka and Muller 2017). Despite the fact that these drugs have demonstrated clinical efficacy, long-term relapse rates among treated individuals remain high, ranging from 20% to 80%, indicating that treatment responses are still largely unsatisfactory (Moos and Moos 2006).

Accordingly, more effective pharmacotherapies represent an urgent medical need (Carvalho et al. 2019).

Recent findings on psychedelics, have sparked growing interest in their potential therapeutic applications for treating AUD (Bogenschutz et al. 2015, 2022; Garel et al. 2022). Among them, ketamine, a dissociative anesthetic with antidepressant properties is a promising candidate. Preclinical and clinical studies publications demonstrate the efficacy of ketamine administration in reducing alcohol consumption and in reducing relapse risks in patients with AUD with, or without comorbid depression (Bertholomey et al. 2025; Dakwar et al. 2020; Grabski et al. 2022; Rezvani et al. 2017; Sabino et al. 2013). Initial clinical data demonstrate the efficacy of ketamine administration in reducing alcohol consumption and in reducing relapse risks in alcohol abusers with or without comorbid depression (Dakwar et al. 2020; Grabski et al. 2022). However, a recent metanalysis led to unconclusive results, and the efficacy of ketamine in AUD is still debated (Garel et al. 2022).

Ketamine is a racemic mixture consisting in equal amounts of the two enantiomers R-ketamine and S-ketamine differing in several pharmacological properties (Jelen et al. 2021). S-ketamine has approximately four times higher affinity for NMDA receptors compared to (R)-ketamine (Ebert et al. 1997). Due to its higher affinity for NMDA receptors S-ketamine has long been considered the active enantiomer of ketamine and research has concentrated to its development as a new fast acting antidepressant, culminating in the approval of intranasal esketamine (Spravato) for treatment-resistant depression (Jelen et al. 2021).

However, the use of S-ketamine is complicated by important side effects, such as dissociative effects, exacerbation of psychotic reactions, vestibular problems. In addition, both clinical and preclinical data suggest that S-ketamine has relevant abuse liability (Chang et al. 2019; Jelen et al. 2021; Le et al. 2022; Yang et al. 2015) which is of critical concern for patients with AUD. Preclinical research suggests that R-ketamine may have a more favorable safety profile including a decreased abuse liability (Jelen et al. 2021; Yang et al. 2015). In a recent preclinical multi-center randomized controlled trial (pre-RCT) – a new module in the drug development process that strictly follows the guidelines for clinical RCT - (R)-ketamine reduced relapse-like drinking with more pronounced and long-lasting effects in female rats (Meinhardt et al. 2025).

Considering these promising results, we decided to further explore the therapeutic potential of (R)-ketamine for AUD. Here we tested the effect of (R)-ketamine on alcohol drinking and seeking in genetically selected Marchigian Sardinian alcohol-preferring (msP) rats. In this rat line genetically encoded high alcohol intake has been cosegregated with innate high stress sensitivity, pathological anxiety and depressive-like behaviors (Ciccocioppo 2013). In earlier work we demonstrated that the excessive alcohol drinking of msP rats depends, at least in part, on the need to alleviate these negative affective states. Considering that (R)-ketamine possesses antidepressant properties, attenuates anxiety and post-traumatic stress symptoms we thought that this rat line, mimicking a subset of AUD patients in which excessive drinking is associated with comorbid negative mood disorders, would represent an ideal model to further validate the therapeutic potential of this compound (Borruto et al. 2021). We thus evaluated the effects of (R)-ketamine on voluntary alcohol drinking and

alcohol self-administration. Classical psychedelics have been shown to induce molecular and cellular adaptations associated with enhanced neuroplasticity (Ly et al. 2018). In a similar manner, non-classical psychedelics such as ketamine can trigger comparable neuroadaptive processes in both humans and rodents (Agnorelli et al. 2025). Given that maladaptive reconsolidation of alcohol-related memories represents a key mechanism underlying relapse, we considered it of particular interest to investigate the effects of (R)-ketamine in a validated model of expression and reconsolidation of alcohol-related memories (Barak and Goltseker 2023; Benvenuti et al. 2023; Johnson et al. 2015; Lee et al. 2006; von der Goltz et al. 2009; Wouda et al. 2010).

To determine possible sex-related effects, experiments were conducted both in male and female rats.

Materials and methods

Animals

Male (N = 10) and female (N = 10) msP rats were used to assess effects of (R)-ketamine on alcohol intake with a two-bottle free-choice (2BC) paradigm. Male and female msP rats (N = 10/sex for each group) were used for the alcohol self-administration.

For the memory retrieval experiment, a new cohort of male (N = 19) and female (N = 20) msP rats were used. For alcohol drinking experiments using the two-bottle choice paradigm, rats were individually housed, while for self-administration experiments animals were housed in groups of three per cage. msP rats were bred at the School of Pharmacy of the University of Camerino, Italy. All animals were housed under controlled temperature (20–22 °C) and humidity (45–50%) and with a 12 h reverse light/dark cycle (lights off at 9am). Rats were handled by the operator prior testing to familiarize them with the experimental procedures.

Drugs

Alcohol solution 10% (v/v) was prepared by dilution with tap water from 96% alcohol stock solution (F.L. Carsetti S.N.C. Camerino, Italy. Pure orange essential oil (ARS Aromatica Dott. Gino Carsetti, Camerino) was used as a cue in the operant conditioning paradigm. (R)-ketamine (10 mg/kg, 20 mg/kg and 40 mg/kg) was dissolved in sterile saline solution. Alcohol was voluntarily consumed orally and (R)-ketamine was administered intraperitoneally (i.p.) in a volume of 1 ml/kg given 30 min prior each test, except for the memory retrieval test, where the drug was given 5-min following memory reactivation.

Two bottle-choice paradigm

The two-bottle free-choice (2BC) paradigm (choice between water and 10% alcohol) was applied to test alcohol drinking and preference. Male and female msP rats were given continuous access to 10% alcohol and water under free access conditions. Solutions were delivered in two graduated drinking burettes with metallic drinking spouts. Absolute alcohol, water, and food intake expressed in grams per body weight (g/kg) was recorded at 2, 8 and

24 h after alcohol was made available to the animals. Burettes position was switched every day during training, and test days to avoid the development of side preference.

Self-administration apparatus

Self-Administration (SA) sessions were conducted in standard operant conditioning chambers (Med Associates, St Albans, VT, USA) enclosed in ventilated sound-attenuating cubicles. Each chamber measured 30.5 cm × 24.1 cm × 29.2 cm and was equipped with two metal retractable levers: one “active” lever, which pressing led to the delivery of alcohol solution; and the “inactive” control lever. The levers were located in the front panel of the chamber with a drinking reservoir in between (positioned 4 cm above the grid floor), connected with a motorized syringe pump (Med Associates, Inc. Fairfax, VT). A house light was located on the wall opposite to the levers. Behavioral sessions were controlled through a Windows compatible PC equipped with Med-PC-5 software (Med Associates).

Self-administration training

Animals were trained to self-administer a 10% (v/v) alcohol solution five days per week in daily 30-minute sessions using a fixed-ratio 1 (FR1) reinforcement schedule. Prior to operant training, rats were given one week of intermittent access to 10% (v/v) alcohol solution in their home cage, alongside water, to facilitate familiarization.

On the first day of operant training, rats underwent a 15-hour session with access to a single lever (right lever) that delivered 0.1 ml of water under an FR1 reinforcement schedule. During this period, food was freely available on the floor of the operant chamber. Following this initial phase, animals were trained to self-administer the 10% alcohol solution during daily 30-minute operant sessions, under an FR1 schedule. Each session started with lever insertion and concluded with lever retraction. Responses on the right (active) lever resulted in the delivery of 0.1 ml of alcohol into the drinking reservoir, followed by a 5-second time-out (TO) period, during which responses were recorded but not reinforced.

To facilitate ethanol-associated cue learning, an orange flavor extract (S) was placed on the bedding of the operant chamber before each session as a discriminative stimulus. Additionally, each active lever press that led to ethanol delivery was paired with the illumination of a house light (CS). Responses on the inactive lever were recorded but had no programmed consequences. All training sessions were conducted during the dark phase of the animals’ light/dark cycle, five days per week for four weeks (Benvenuti et al. 2023).

Experiment 1: Effect of (R)-ketamine on a two-bottle free-choice drinking test in male and female msP rats

Adult male ($N = 10$) and female ($N = 10$) msP rats had continuous access to 10% alcohol, water and food, under free access conditions. Once a stable baseline of drinking was reached, the effect of (R)-ketamine (0, 10, 20, 40 mg/kg) was tested in counterbalanced within-subject design with 3 days of baseline between each test. (R)-ketamine or its vehicle was administered i.p 30 min prior to the alcohol drinking monitoring phase (at the start of the dark phase of the light/dark cycle). Alcohol, water and food intake (g/kg) was recorded after 2, 8 and 24-hours.

Experiment 2: Effect of (R)-ketamine on alcohol self-administration in male and female msP rats

Male (N = 10) and female (N = 10) msP rats were trained to self-administer 10% alcohol, under a fixed ratio 1 (FR1) schedule of reinforcement. The house light was illuminated contingently with alcohol delivery and remained on for the time-out duration. Each active lever press resulted in the delivery of 0.1 ml of 10% alcohol. After a stable self-administration baseline, the lower efficacious dose of R-ketamine (20 mg/kg) or its vehicle was administered i.p 30 min prior the test, in a counterbalanced Latin Square design. In between each treatment, rats underwent alcohol self-administration session.

Experiment 3: Effect of (R)-ketamine on reconsolidation of alcohol-related memory in male and female msP rats

Male (N = 19) and female (N = 20) msP rats, were trained to self-administer 10% alcohol (v/v) until a stable self-administration baseline was achieved. Rats were then left undisturbed in their home cages for 3 weeks until the test day to mimic the abstinence phase as previously described (von der Goltz et al. 2009; Wouda et al. 2010).

For the memory retrieval-reconsolidation test male and female rats were divided into two groups to receive i.p. (R)-ketamine (20 mg/kg) or vehicle. For memory retrieval, rats were returned into the self-administration chamber for a 5 min operant task that was identical to that of the training but only the first two lever presses were reinforced with 0.1 ml of 10% alcohol. Subsequent lever responses were recorded but were not reinforced (von der Goltz et al. 2009; Vengeliene et al. 2015). The choice of a 5-min reactivation session was based on prior evidence indicating that such brief exposure induces stronger cue-induced alcohol seeking compared to longer reactivation periods (von der Goltz et al. 2009).

Immediately after this 5-min memory reactivation session, rats received (R)-Ketamine or its vehicle and were returned in their homecages until the next day. Twenty-four hours later rats were subjected to another 30 min self-administration session identical to the 5-min retrieval session of the day before. Responses at both the active and inactive levers were recorded. At the end of this reinstatement session rats were returned in their homecages until the following week when this procedure was repeated. A third memory reactivation test was carried out after one additional week.

Statistical analysis

The effect of (R)-ketamine on alcohol, water and food intake in experiment 1 was analyzed by two-way analysis of variance (ANOVA), with treatment as within- and sex as between-subject factors. The effect of (R)-ketamine on alcohol self-administration and on memory retrieval were analyzed by two-way ANOVA using sex as a between-subject factor and treatment as a within-subject factor.

Dunnet's or Tukey *post-hoc* tests were used for post-hoc analysis when appropriate. For analysis statistical significance was set at $p < 0.05$.

Results

Experiment 1: Effect of (R)-ketamine on a two-bottle choice drinking test in male and female msP rats

In female msP rats, two-way ANOVA on alcohol drinking at the 2-hour time point showed a significant main effect of treatment [$F_{(3,54)} = 4.16$; $p = 0.01$], followed by a significant effect of sex [$F_{(1,18)} = 5$; $p < 0,05$] and a trend close to significance on time × treatment interaction [$F_{(3,54)} = 2.6$; $p = 0.06$]. Dunnettś *post-hoc* test showed that at the 2-hour time point (R)-ketamine significantly reduced alcohol intake at the highest dose (40 mg/kg) compared to vehicle ($p < 0.01$) only in females (Fig. 1A). At the 8-hour time point, two-way ANOVA on alcohol drinking showed a significant main effect of treatment, [$F_{(3,54)} = 11.5$; $p < 0.001$], sex [$F_{(1,18)} = 8.3$; $p < 0.01$], and a significant treatment × sex interaction [$F_{(3,54)} = 6.3$; $p < 0.001$]. *Post-hoc* analysis showed that both 20 mg/kg and 40 mg/kg doses of (R)-ketamine significantly reduced alcohol intake only in females ($p < 0.001$). Tukey *post-hoc* test showed that male vehicle-treated rats showed significantly lower levels of alcohol intake compared to the female vehicle group ($p < 0.01$; Fig. 1B). At 24 h two-way ANOVA showed a significant main effect of treatment, [$F_{(3,54)} = 4.5$; $p < 0.01$], sex [$F_{(1,18)} = 7.7$; $p < 0.05$], and a significant effect of treatment × sex interaction [$F_{(3,54)} = 3.26$; $p < 0.05$]. Dunnettś *post-hoc* test showed that (R)-ketamine significantly reduced alcohol intake only in females at all the tested doses compared to vehicle ($p < 0.05$ for dose 10 and 40; $p < 0.01$ for the dose 20 mg/kg) (Fig. 1C).

Regarding water consumption, at the 2-hour time point, two-way ANOVA showed a significant main effect of treatment, [$F_{(3,54)} = 5.7$; $p < 0.01$], sex [$F_{(1,18)} = 9.6$; $p < 0.01$], with no significant treatment × sex interaction [$F_{(3,54)} = 1.39$; $p = 0.25$]. Dunnettś *post-hoc* analysis indicated that (R)-ketamine at 40 mg/kg significantly increased water intake ($p < 0.01$) in both sexes, while Tukey's test confirmed higher water intake in females compared to males ($p < 0.01$;Figure 1D). At the 8-hour time point, water intake analysis showed significant main effects of treatment [$F_{(3,54)} = 3.6$; $p < 0.05$], sex [$F_{(1,18)} = 11.4$; $p < 0.01$], without showing an overall effect of treatment × sex interaction [$F_{(3,54)} = 1.37$; $p = 0.26$]. (R)-ketamine at 40 mg/kg significantly increased water intake ($p < 0.01$) in both sexes, with females showing overall higher intake levels than males ($p < 0.01$; Fig. 1E). At the 24-hour time point there was no significant effect of treatment [$F_{(3,54)} = 2.5$; $p = 0.07$]; however, there was a significant main effect of sex [$F_{(1,18)} = 7.7$; $p < 0.05$] with female rats consuming more water than males ($p < 0.05$). ANOVA did not show a significant treatment × sex interaction [$F_{(3,54)} = 1.67$; $p = 0.2$], (Fig. 1F).

Analysis of food consumption did not show significant effects of treatment, sex, or treatment × sex interaction at any time point (Figs. 1G–I).

Experiment 2: Effect of (R)-ketamine on alcohol self-administration in male and female msp rats

The mean ± SEM values of operant responding of the last 3 days self-administration training days were 82 ± 6 for female and 110 ± 18 for male rats. Rats were divided into two balanced groups, one receiving i.p. saline solution as vehicle, the other one receiving i.p. 20 mg/kg

(R)-ketamine. Two-way ANOVA did not show an effect of sex [$F_{(1,18)}$ = 0.37; p = 0.55], treatment [$F_{(1,18)}$ = 0. 33; p = 0.57] or sex × treatment interaction [$F_{(1,18)}$ = 0. 08; p = 0.77] on the number of alcohol rewards Fig. 2A. Due to major differences in body weight between males (≈ 600 g) and females (≈ 300 g) alcohol reinforces were also converted in g/kg of pure alcohol consumed. When alcohol intake was expressed in g/kg, there was a main effect of sex [$F_{(1,18)}$ = 23,3; p < 0.001], with females showing a significantly higher alcohol intake (g/kg). However, there was no effect of treatment [$F_{(1,18)}$ = 0,33; p = 0.57] or sex × treatment interaction [$F_{(1,18)}$ = 0,01; p = 0.9], Fig. 2B. Active [$F_{(1,18)}$ = 0,99; p = 0.07], and inactive [$F_{(1,18)}$ = 1.37; p = 0.25] lever responding was also not affected by treatment with (R) ketamine.

Experiment 3: Effect of (R)-ketamine on reconsolidation of alcohol-related memory in male and female msP rats

The mean ± SEM number of active lever presses of the last three self-administration training days were 68 ± 9 for females Veh and 70 ± 6 for females (R)-ketamine. The values for males were 110 ± 7 for the Veh group and 107 ± 7 for the (R)-ketamine group. When in the reinstatement test the effect of (R)-ketamine given after the memory recall was analyzed in female rats ANOVA showed an overall effect of treatment [$F_{(1,18)}$ = 5.28; p < 0.05] and a significant effect of time [$F_{(2,36)}$ = 13.96; p < 0.001] without showing a significant effect of treatment × time interaction [$F_{(2,36)}$ = 0.43; p = 0.63]. Dunnettś *post-hoc* test showed a significant (p < 0.05), reduction of alcohol seeking in the (R)-Ketamine treated group compared to the vehicle control (Fig. 3A). ANOVA applied to inactive lever responding showed no effect of treatment [$F_{(1,18)}$ = 0.79; p = 0.38], time [$F_{(2,36)}$ = 0.36; p = 0.6] or treatment × time interaction [$F_{(2,36)}$ = 0.36; p = 0.69], (Fig. 3B). In male msP rats ANOVA showed no significant effect of treatment [$F_{(1,17)}$ = 0.27; p = 0.61], a significant effect of time [$F_{(2,34)}$ = 23.38; p < 0.001] and no effect of treatment × time interaction [$F_{(2,34)}$ = 0.67; p = 0.5], (Fig. 3C). ANOVA applied to inactive lever responding showed no effect of treatment [$F_{(1,17)}$ = 0.08; p = 0.76], time [$F_{(2,34)}$ = 1.1; p = 0.34] or treatment × time interaction [$F_{(2,34)}$ = 0.64; p = 0.53], (Fig. 3D).

Discussion

The present study investigated the effect of (R)-ketamine in preclinical paradigms of alcohol drinking and relapse in msP rats. This rat line was chosen because their excessive drinking is, at least in part, dependent upon the attempt to alleviate negative affective states.

from their stress coping difficulties and innate propensity to pathological anxiety and depression (Borruto et al. 2021; Ciccocioppo et al. 2006). As shown in earlier studies, in fact, msP rats show enhanced anxiety, depression and post-traumatic stress disorder-like traits that are attenuated after voluntary alcohol drinking (Borruto et al. 2021; Ciccocioppo 2013; Ciccocioppo et al. 2006; Cippitelli et al. 2015; Hansson et al. 2007). A growing body of evidence from laboratory animal studies has demonstrated that (R)-ketamine possesses remarkable antidepressant properties (Johnston et al. 2024; Yang et al. 2015). In a recent multicentric preclinical study mimicking a phase II clinical trial (R)-ketamine significantly reduced alcohol deprivation-induced increases of drinking (i.e., relapse-like

behaviour) in Wistar rats (Meinhardt et al. 2025). Based on this background we postulated that (R)-ketamine should have been particularly efficacious in reducing alcohol drinking and relapse to alcohol seeking in msP rats.

Results of the 2BC drinking test demonstrated that (R)-ketamine significantly reduced alcohol intake at 2, 8 and 24 h in female rats, with a trend in efficacy observed starting from a sub-anesthetic dose of 10 mg/kg. The effect was increased when the drug was given at 20 and 40 mg/kg. (R)-ketamine appeared to be more effective in reducing alcohol intake at delayed time points following administration. This temporal profile is consistent with recent findings demonstrating that (R)-ketamine, but not racemic ketamine, significantly reduced alcohol consumption up to four weeks post-treatment, highlighting its sustained efficacy (Meinhardt et al. 2025). The prolonged therapeutic effect may be attributable to the action of stable, bioactive metabolites such as dehydronorketamine which has shown stable peak plasma concentrations following three hours of intravenous (R)-ketamine administration in humans (Kamp et al. 2020). Alternatively, (R)-ketamine may act through well-characterized metaplasticity mechanisms involving glutamatergic and GABAergic transmission, modulating subsequent neuronal plasticity responses (Brown and Gould 2024).

In male msP rats no effect was observed even at the highest dose (40 mg/kg). In pilot experiments (data not shown) we found that at doses higher than 40 mg/kg (R)-ketamine starts to have unspecific effects probably associated with its sedative anesthetic action. Hence, we could not test whether higher doses would have been able to reduce drinking also in males. This sex-related different sensitivity to (R)-ketamine replicates earlier study showing heightened response to the drug in females compared to males (Meinhardt et al. 2025). The reason for the higher sensitivity to (R)-ketamine in females is still a matter of investigation. Previous studies have shown that females exhibit greater sensitivity to ketamine, responding to lower doses than males (Bertholomey et al. 2025; Carrier and Kabbaj 2013; Dossat et al. 2018; Ponton et al. 2022; Saland and Kabbaj 2018; Thelen et al. 2016; Wright et al. 2019). At pharmacological level, this sex difference may stem from pharmacokinetic (i.e., metabolism) or pharmacodynamic factors (ie.- receptor binding profile). (R)- and (S)-ketamine differ markedly in receptor affinity and downstream signaling —(S)-ketamine exhibits greater NMDA receptor affinity and mTOR activation, whereas (R)-ketamine more potently engages the BDNF–TrkB pathway (Jelen et al. 2021; Yang et al. 2019). If pharmacodynamic mechanisms were the main determinant, one would expect sex-specific effects specific to the ketamine enantiomer. However, studies investigating S-ketamine have not reported significant sex differences in response to treatment (Arjmand et al. 2023). Instead, pharmacokinetic differences may play a more prominent role, with recent evidence showing higher plasma levels of ketamine and norketamine in female rats (Meinhardt et al. 2025). An additional hypothesis is that females' increased sensitivity to (R)-ketamine may relate to their alcohol use being more closely linked to negative affect and post-traumatic stress (Goldstein et al. 2012; Karpyak et al. 2016; Lehavot et al. 2018; Toivainen et al. 2024). This hypothesis is consistent with earlier study in which we demonstrated that if male msP rats show traits reminiscent of generalized anxiety, female msPs show a pronounced endophenotype reminding PTSD (Borruto et al. 2021).

Results in operant self-administration experiments showed no drug effect neither in males nor in females. This lack of activity was somehow unexpected, as we would have expected a replication of the results observed with the 2-BC test. On the other hand, earlier studies showing the efficacy (R)-ketamine were always carried out under home cage alcohol drinking and this was the first time the drug was tested on operant alcohol self-administration. At present we can only speculate about this lack of activity. One possibility is that the effect of the drug requires longer daily alcohol exposure to be established, and/or longer interval from drug treatment and alcohol exposure.

(R)-ketamine leads to the activation of multiple post-transcriptional changes that could be responsible for the reduction of alcohol drinking and may vary depending on previous alcohol exposure or could require longer than 30 min to take place. For instance, it is known that (R)-ketamine leads to the activation of brain-derived neurotrophic factor (BDNF) and its receptor tropomyosin kinase B (TrkB) system as well as of the transforming growth factor β (TGF-β) (Choi et al. 2017; Gregorio et al. 2021). Both of these two pathways have been involved in the regulation of alcohol drinking and seeking (Hamada and Lasek 2020; Pandey 2016; Portelli et al. 2020). Another possible explanation for the lack of efficacy of (R)-ketamine in our self-administration study is that, under operant conditions, the motivation to consume alcohol may be particularly high due to the restricted 30-minute daily access. It should also be noted that differences in operant parameters, such as the fixed ratio (FR) schedule employed, the alcohol concentration and the use of a daily (rather than intermittent) access schedule may have limited the sensitivity to the effects of (R)-ketamine. A limitation of the present study is that, based on the 2BC data, only the dose (20 mg/kg) was tested. Therefore, we cannot rule out the possibility that a higher dose of (R)-ketamine (i.e., 40 mg/kg), might also reduce operant alcohol self-administration.

Several studies demonstrated that long-term memory formation and development of substance use disorder share common molecular mechanisms and neuronal circuitries (Hyman et al. 2006; Kelley 2004). At behavioral level it has been demonstrated that learned associations between drug-related environmental stimuli and drug effects play a major role in maintaining drug use and in promoting relapse. It is known that newly acquired memories are initially labile but are rapidly stabilized through memory consolidation processes. Once consolidation is completed, memory is stored in a fixed and stable manner. However, when a memory is retrieved, it enters a temporary labile state before being reconsolidated and stored again (Dudai and Eisenberg 2004; McGaugh 2000). Previous studies demonstrated that following retrieval, drug related memories, including alcohol undergo a reconsolidation process and that administration of NMDA receptor antagonists interfering with this process reduced subsequent drug seeking and relapse (Bernardi et al. 2006; Hellemans et al. 2006; Lee et al. 2005; Miller and Marshall 2005; von der Goltz et al. 2009; Xue et al. 2012). Considering that NMDA antagonism is one of the major molecular mechanisms of action of ketamine and that several studies demonstrated that NMDA receptor antagonists can affect memory acquisition and consolidation (Rezvani 2006) we decided to evaluate whether (R)-ketamine can contribute to extinguish alcohol related memories. This approach is also based on previous reconsolidation literature which demonstrates that brief, non-reinforced retrieval sessions can destabilize original drug-related memories, especially when followed by pharmacological intervention (Tronson and Taylor 2007).

For instance, it has been shown that in a clinical study in hazardous drinkers, ketamine was able to disrupt maladaptive reward memories when administered immediately after memory retrieval producing beneficial effects (Das et al. 2019). Here, to expand this finding on (R)-ketamine we set up an experimental setting mimicking this clinical study wherein the drug is administered immediately following the retrieval of alcohol-associated memories. Results demonstrated a remarkable effect of (R)-ketamine in female rats while it was not effective in males. Recently published data showed that ketamine reduced alcohol reinstatement in female rats when, following an extinction training, relapse was induced by a combination of alcohol-paired cues and yohimbine, (Bertholomey et al. 2025).

It is important to note that the effects of (R)-ketamine may also, at least theoretically, arise from non-specific actions (e.g., taste aversion or alterations in emotional response) of NMDA receptor antagonists rather than from modulation of memory processes. Previous studies examining ketamine as an aversive stimulus in sucrose consumption reported only weak effects, requiring four conditioning trials to produce a measurable reduction in intake (Aguado et al. 1997). In contrast, in our study, ketamine induced a significant decrease in alcohol-seeking behavior from the first conditioning test. Furthermore, if ketamine (20 mg/kg) had caused malaise, aversion or affected the taste of alcohol, a reduction in intake would also have been observed in the two-bottle choice experiment; however, no such decrease was observed.

A limitation of the present study is the absence of a control group that did not undergo memory retrieval, which would allow us to determine whether the reduction of alcohol relapse occurs specifically when memory retrieval precedes the administration of (R)-ketamine. Nonetheless, it is worth noting that in a number of previous studies using this protocol, the data were interpreted in the context of changes in memory reconsolidation (Aguado et al. 1997; Lee et al. 2006; Vengeliene et al. 2015; von der Goltz et al. 2009; Wouda et al. 2010); Importantly, in an earlier study in which this experimental paradigm was used to test memantine, another NMDA receptor antagonist (Johnson et al. 2015), results showed a decrease in alcohol relapse only following a reactivation session, without altering the extinction process (Vengeliene et al. 2015).

Altogether our findings strengthen previous clinical observation made with ketamine expanding it to the more tolerable (R)-ketamine. In addition, our data confirm that females are more responsive to the effect of these drugs. Altogether these findings open to the possibility of using (R)-ketamine in human laboratory settings in which alcohol-related memories are artificially recalled, and the patient is treated immediately after. It would be interesting to determine whether, reflecting our preclinical data (R)-ketamine would be more efficacious in women than in men.

It could be that the effects of (R)-ketamine described here are secondary to the motor impairment and sedative effect of the drug. However, we exclude this possibility for several reasons: First in the 2BC experiment, the drug reduced drinking in female rats starting from the dose of 20 mg/kg, while even at higher doses 40 mg/kg, it never reduced food and water consumption. Second, in self-administration experiments, in which operanda is highly dependent upon the integrity of the motor performance (R)-ketamine was ineffective. Third,

in the reconsolidation experiment (R)-ketamine was no longer onboard as 24 h passed from it administration and the test.

To summarize, our data suggest that (R)-ketamine is efficacious in reducing alcohol drinking and in preventing alcohol relapse by targeting the recall of associated memories, particularly in female subjects. These findings offer promising insights into potential clinical applications, with the possibility that females patients, possibly with comorbid PTSD traits, may be more responsive to the treatment than other AUD subpopulations.

Funding

This research was supported by grant ERA-NET Neuron Psi-Alc (FKZ: 01EW1908) and by Eva-Maria and Rutger Hetzler Foundation grant Psy-Alc to RC, NIAAA - AA014351 (FW and RC) and NIAAA - AA017447 (MR and RC). PRIN 2022B5RMW9; PRIN PNRR P2022ZXF98 to ED, PRIN-PNRR2022 -P202274WPN (to RC), PRIN-2022 20227HRFPJ (to RC). Project MNESYS (PE0000006)-Project AMSUD 2024 “Animal Models to Study Substance Use Disorders and Cooccurring Psychiatric Conditions”. Financial support for this work was also provided by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) for the TRR 265 (A05; Spanagel et al., 2024) to RS and MWM. MIUR Progetto Eccellenza 2023–2027 to FF.

Data availability

The data that support the findings of this study are available on request from the corresponding authors, R.C and E.D.

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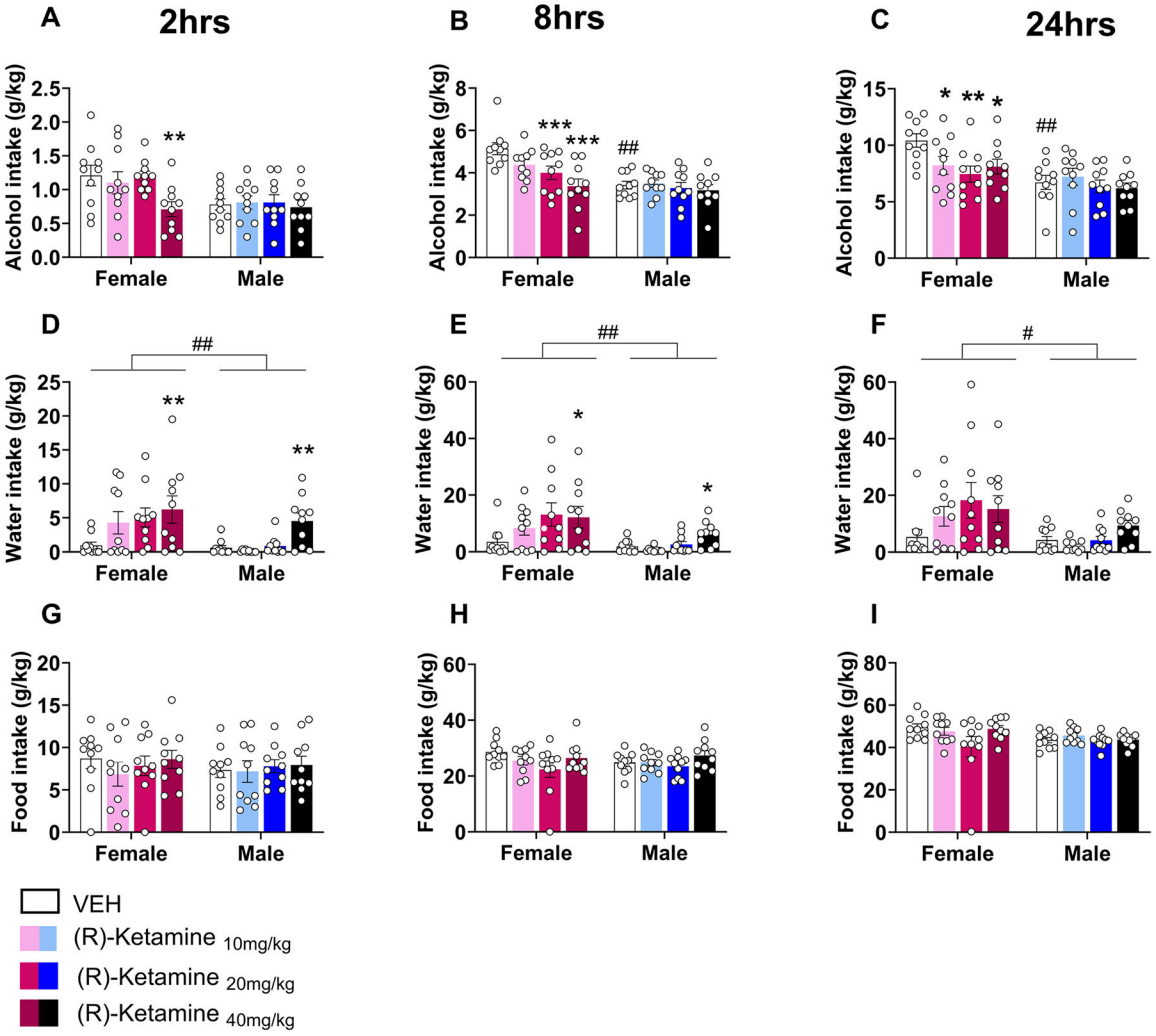

Fig. 1.

Effect of acute (R)-ketamine (0, 10, 20, 40 mg/kg) on alcohol, water, and food intake in a two-bottle choice paradigm in male and female msP rats at 2, 8 and 24 h. **A**) At the 2-hour time point, administration of (R)-ketamine at the highest dose (40 mg/kg) significantly reduced alcohol intake exclusively in female rats, with no effect in males. **B**) At the 8-hour time point, (R)-ketamine at doses of 20 and 40 mg/kg significantly decreased alcohol consumption in female rats only. Vehicle-treated male rats exhibited lower alcohol intake compared to the female control group. **C**) At the 24-hour time point, (R)-ketamine significantly reduced alcohol intake in female rats at all tested doses (10, 20, and 40 mg/kg) relative to the vehicle-treated group. Consistent with earlier observations, vehicle-treated males showed lower alcohol intake than female controls. **D**, **E**, **F**) Water intake was generally higher in female rats compared to males. (R)-ketamine at 40 mg/kg increased water consumption in both sexes at the 2- and 8-hour time points. **G**, **H**, **I**) Food intake did not differ between sexes and was not affected by the treatment at 2, 8 and 24 h. Data are presented as mean ± SEM. *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ indicate significant differences between (R)-ketamine and vehicle-treated (VEH) group.; $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ show a significant difference between male vs. females

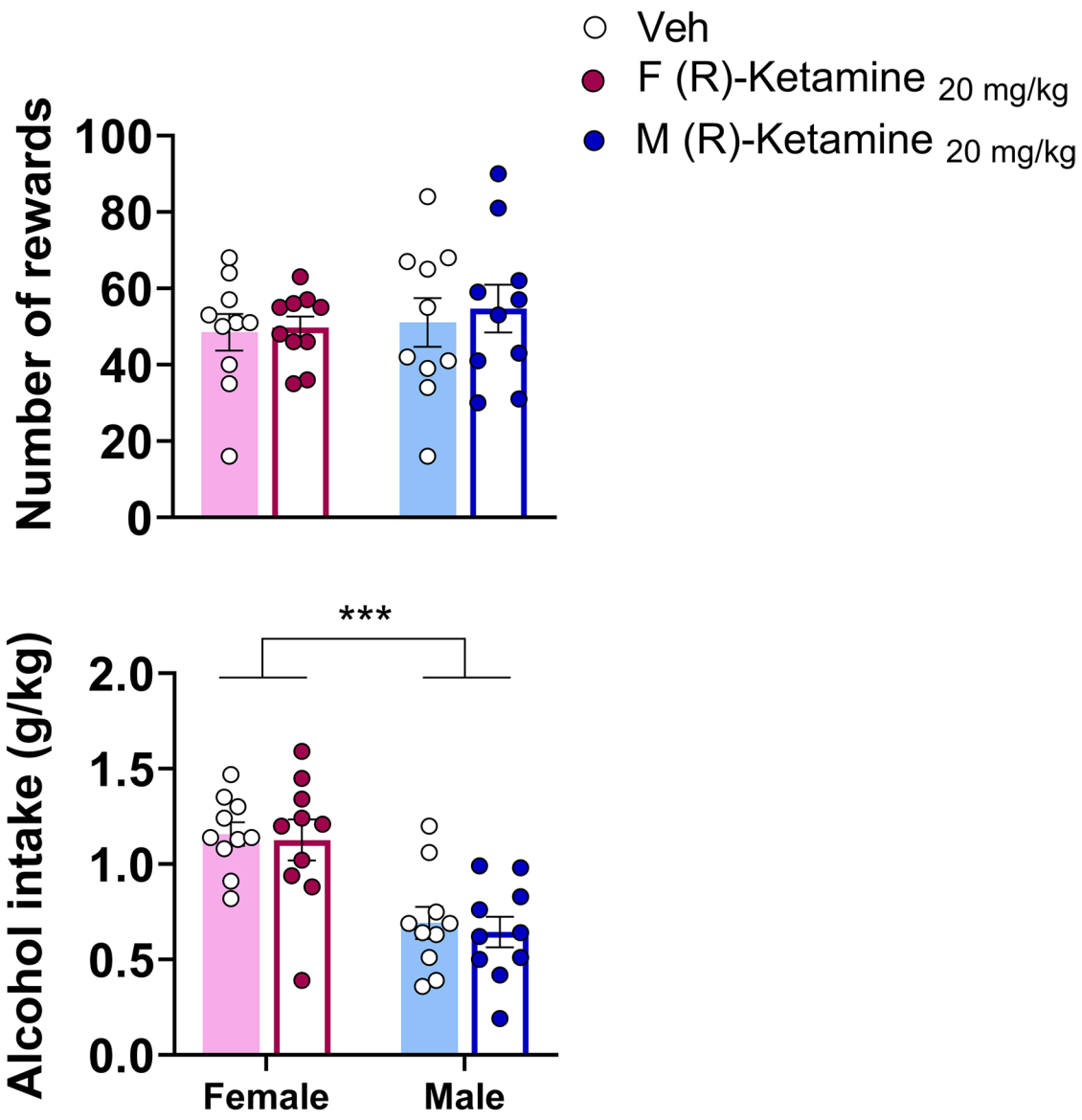

Fig. 2.

Effect of (R)-ketamine on alcohol self-administration. (**A**) Mean total number of alcohol rewards (**B**) Alcohol intake expressed in g/kg. Data are expressed as mean ± SEM. *** $p <$ 0.001 significant difference between females vs. males

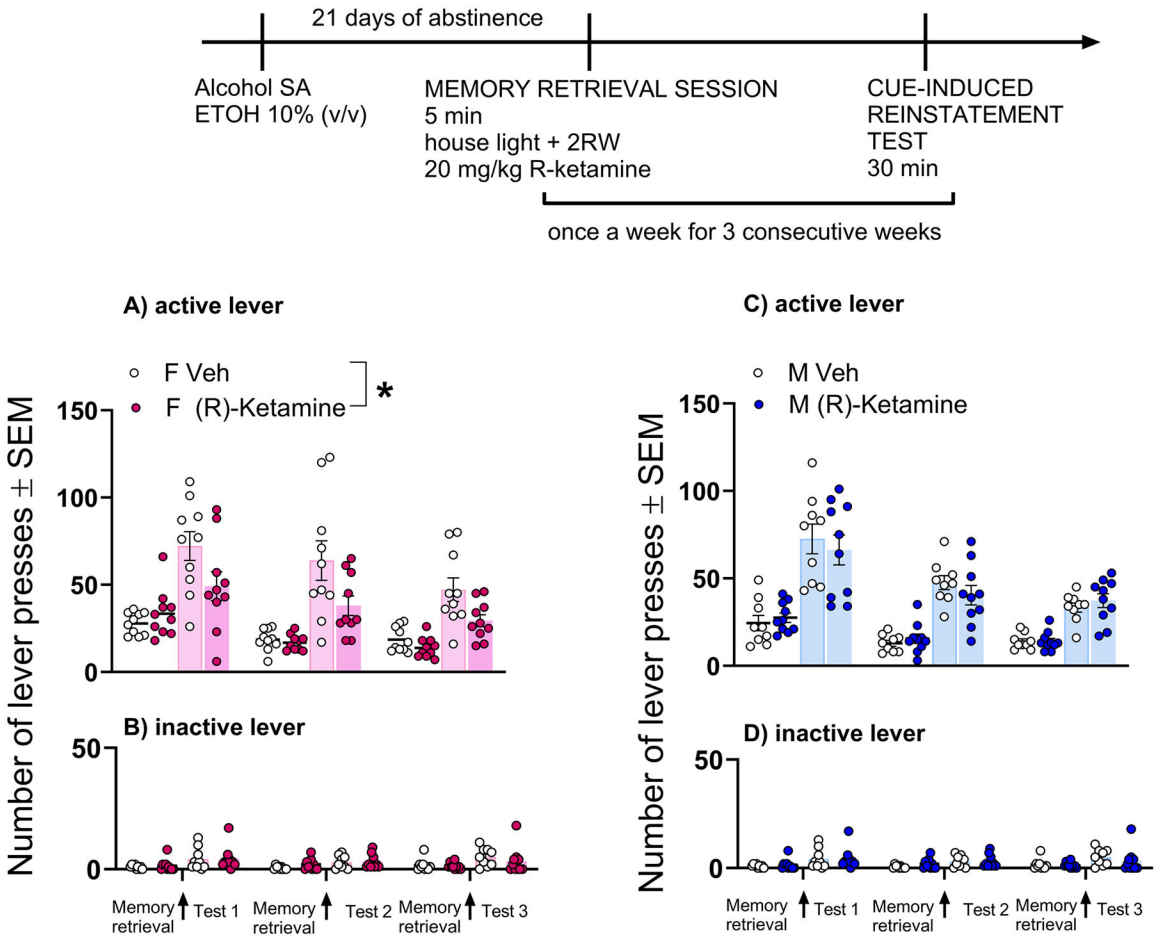

Fig. 3.

Effect of (R)-ketamine on the reconsolidation of alcohol-related memories. (**A**) In females, (R)-Ketamine significantly reduced responding at the previously alcohol paired lever. (**B**) Responding at the inactive lever was not affected by the treatment; (**C**) In males (R)-Ketamine did not modify responding at the previously alcohol active lever: (**D**) Responding at the inactive lever was also not affected by the treatment. Data are expressed as mean ± SEM. *$p < 0.05$ significant difference between (R)-ketamine and vehicle-treated (VEH) group