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Psilocybin prevents reinstatement of alcohol seeking by disrupting the reconsolidation of alcohol-related memories

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Abstract

Background: For most psychiatric conditions, including alcohol use disorder (AUD), FDA approved pharmacological treatments are limited and their efficacy is restricted to only certain subgroups of patients. Scientific interest in the potential of psychedelic drugs has dramatically increased because of clinical preliminary evidence of efficacy in treating various psychiatric disorders. One of the most promising compounds belonging to this class of molecules is psilocybin. Here, to elucidate the therapeutic potential and treatment modalities of this drug, we investigated the effect of psilocybin on alcohol drinking and seeking in genetically selected Marchigian Sardinian alcohol preferring (msP) rats, a well validated animal model of AUD characterized by excessive drinking and seeking.

Methods: Using male and female msP rats we tested the effect of psilocybin on home cage voluntary alcohol consumption. We also tested the effect of the drug on the alcohol deprivation effect (ADE) model of relapse and on cue-induced reinstatement of alcohol seeking after a period of abstinence. Finally, we evaluated if psilocybin may disrupt the reconsolidation process of alcohol-related memory.

Results: Psilocybin did not reduce alcohol consumption, nor it prevented increased alcohol drinking after a period of forced abstinence and cue-induced reinstatement of alcohol-seeking. Noteworthy, in a memory retrieval-reconsolidation paradigm, psilocybin markedly attenuated resumption of alcohol seeking.

Conclusions: Altogether these data suggest that, despite psilocybin does not affect alcohol drinking and relapse, it may be highly effective if used to block the reconsolidation process of alcohol-related memories. This opens to the possibility of using this psychedelic drug in clinical settings in which AUD patients undergo procedures to recall the memory of alcohol and are then treated with psilocybin during the memory reconsolidation phase.

AUTHOR CONTRIBUTIONS

Conceptualization, RC; Formal Analysis, FB, RC; Investigation, FB, DC, RC; Resources, N.C, RC; Data Curation FB; Writing – Original Draft Preparation, FB, RC; Writing – Review & Editing, FB, RC; NC, Funding Acquisition, RC. 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.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Keywords

Psychedelics; Memory; Addiction; Alcoholism; Reward

Introduction

Alcohol use disorder (AUD) is one of the leading causes of preventable mortality, responsible for 3 million deaths per year (Witkiewitz, 2019) and represents a significant burden for affected patients and society due to its huge economic costs (Grant et al., 2017). Currently, the acetaldehyde dehydrogenase inhibitor disulfiram, acamprosate and the opioid receptor antagonist naltrexone are the only three FDA approved drugs for AUD therapy (Witkiewitz, 2019). However, the therapeutic efficacy of these pharmacological treatments is limited to only certain subgroups of patients (Litten et al., 2018).

An efficacious therapy should be able not only to reduce daily alcohol consumption but it should also prevent the risk of relapse. Environmental conditioning factors (i., e, cues predictive of alcohol availability) contributes considerably to relapse and maintenance of alcohol dependence and represents a major challenge in the treatment of alcoholism (Martin-Fardon and Weiss, 2013; Cooney et al., 1997; O'Brien et al., 1992; Monti et al., 1987).

Recent developments in the study of classic hallucinogens, combined with a re-appraisal of older literature, have led to a renewal of interest in possible therapeutic applications for these drugs, including the treatment of addiction. Psilocybin (3-[2-(dimethylamino) ethyl]-1H-indol-4-yl] dihydrogen phosphate), is one of these hallucinogens, that thanks to promising clinical preliminary results has gained a lot of attention. Psilocybin is a hallucinogenic substance contained in mushrooms of the genus psilocybe with high structural similarity with the neurotrasmitter serotonin (5-hydroxytryptamine, 5-HT), characterized by relative risk of toxicity and dependence if not appropriately employed (De Veen et al., 2017). After ingestion, the prodrug psilocybin is quickly degraded into its active metabolite psilocin, which is largely responsible for psilocybin psychotropic action (De Veen et al., 2017). Psilocybin binds to multiple 5-HT receptors activating them, but the highest affinity is for the 5-HT2A receptor; to a lesser extent it also activates the 5-HT1A receptor (McKenna et al., 1990). It has been shown to increase trait openness (MacLean et al., 2011), cognitive and behavioral flexibility (Gallimore, 2015) and to decrease depressive symptoms in terminally ill cancer patients (Grob et al., 2011). More important initial clinical findings suggest that psilocybin might be a valuable compound for the treatment of various form of addictions including alcoholism (Bogenschutz, 2013; Bogenschutz et al., 2015; De Veen et al., 2017; Johnson et al., 2014). An initial proof-of-concept study on the efficacy of psilocybin in alcohol dependence has showed a significant reduction in both percentage of drinking days and heavy drinking days with large effect sizes (Bogenschutz et al., 2015). Recently this study has been replicated in a larger cohort of AUD patients and the efficacy of psilocybin has been confirmed (Bogenschutz et al., 2022). These findings shed enthusiasm on the possibility of using this compound to treat AUD. Yet, the mechanism of action of psilocybin remains elusive and the optimization of its use is far to be determined. The possibility of testing this molecule in animal models of AUD would help to achieve these objectives.

To the best of our knowledge only two published studies explored the effect of psilocybin in laboratory animals. In one report it was shown that only subchronic treatment with psilocybin attenuates alcohol deprivation (ADE) induced excessive drinking (Meinhardt et al., 2020). In another study, it was shown that in rats with reduced cognitive flexibility and excessive alcohol seeking due to infralimbic cortex mGluR2 knockdown administration of psilocybin restores mGluR2 expression levels and attenuates the propensity to relapse (Meinhardt et al., 2021).

In the attempt to gain further insight on the therapeutic potential of psilocybin on AUD, here we investigated its effects on alcohol drinking and seeking in genetically selected Marchigian Sardinian alcohol-preferring (msP) rats, a well validated animal model of pathological drinking (Ciccocioppo et al., 2006; Borruto et al., 2021). Additionally, since rodent studies have found that psychedelics can evoke protracted change in the brain structure and induce molecular and cellular adaptations related to neuroplasticity (Cameron et al., 2018; Ly et al. 2018; De Vos et al., 2021; Jefsen et al., 2021), we south interesting to investigate the effect of psilocybin in a validated model of expression/reconsolidation of alcohol-related memory (Von der Goltz et al., 2009).

Materials and Methods

Animals

Male (N = 38) and female msP rats (N = 40) were used in the study. They were bred at the Department of Experimental Medicine of University of Camerino, Italy. For alcohol drinking experiments, they were individually housed on a reverse 12h light/dark cycle (lights off at 9 AM) in a temperature (20–22°C) and humidity (45–50%) controlled room. For the self-administration (SA) experiments, they were housed three per cage in a different room with a reverse 12h light/dark cycle (lights off at 8 AM). During the experiments, animals were offered free access to tap water and food pellets (4RF18, Mucedola, Settimo Milanese, Italy). All the animals were repeatedly handled for 5 minutes a day by same operators who performed the experiments to familiarize them to human contact. All efforts were made to minimize animal suffering and to reduce the number of animals used. Procedures were conducted in adherence with the European Community Council Directive for Care and Use of Laboratory Animals and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Prot 1D 58032).

Drugs

The alcohol drinking solution 10% (v/v) was prepared by diluting 95% alcohol (F.L.Carsetti, Camerino, Italy) with tap water. Psilocybin (3-[2-(dimethylamino) ethyl]-1H-indol-4-yl] dihydrogen phosphate, THC Pharm, Frankfurt am Main, Germany) was dissolved in sterile physiological saline and administered intraperitoneally (i.p.) at the volume of 1ml/kg. A single intraperitoneal (i.p.) dose of psilocybin (5 mg/kg) was used. This dose was chosen based on previous published reports in rodents, in which psilocybin has been administered at doses ranging between 1 mg/kg and 10 mg/kg (Meinhardt et al. 2020, Jefsen et al., 2019). To habituate animals to drug administration procedures, rats were i.p. injected three times with physiological saline prior to experiments.

Two-bottle choice paradigm

The two-bottle free-choice (2BC) paradigm (choice between water and 10% v/v alcohol) was used to measure alcohol drinking and preference (Koob et al., 2003; Tabakoff and Hoffman, 2000). Rats were given continuous access to 10% (v/v) alcohol and water under free access conditions. Fluids were delivered in two graduated drinking tubes with metallic drinking spouts. Consumption was measured by reading the volume that was consumed after 2, 8 and 24h after alcohol was offered to animals. The tubes were switched daily to avoid the development of side preference. Food intake was measured by weighing the containers. Alcohol, water and food intakes were calculated as absolute values at each time interval and are expressed as grams per kilogram (g/kg) to control for the influence of body weight differences (Becker and Lopez, 2004; Finn et al., 2007; Rimondini, Sommer, Heilig, 2003).

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 was equipped with two retractable levers, located in the front panel of the chamber, with a drinking reservoir placed in between, connected with a syringe pump. A house-light was located on the wall opposite to the levers. Behavioral sessions were controlled and recorded by a windows compatible PC equipped with Med-PC-5 software (Med Associates).

Self-administration training

Animals were trained to self-administer 10% (v/v) alcohol for five days a week, in 30 min daily sessions under a fixed-ratio 1 (FR1) schedule of reinforcement. Before the start of operant training, in addition to water rats were given intermittent access to 10% (v/v) alcohol in their home cage for one week to familiarize them to this solution. On the first day of operant training, rats were given 15 hours access to a single lever (right lever) that produced 0.1 ml deliveries of water on a fixed-ratio 1 (FR1) schedule of reinforcement with ad libitum food available on the floor of the operant chamber. Afterwards, animals were trained to respond for 10% (v/v) alcohol, in 30 minutes daily sessions under a FR1 schedule of reinforcement. Operant sessions started with lever insertion and ended with levers retraction. Responses at the right (active) lever were reinforced with 0.1 ml of alcohol solution delivered in the drinking reservoir. Reinforcement delivery was followed by 5sec time-out (TO). Discriminative stimuli predictive of ethanol availability (S) consisted in an orange flavor extract deposited on the bedding of the operant chamber before every session. Additionally, each active lever press resulting in ethanol delivery was accompanied by illumination of a house light stimulus (CS). During the TO active lever responses were recorded but not reinforced. Throughout the sessions, responses at the left (inactive) lever were recorded but had no scheduled consequences. All training sessions were performed during the dark phase of their light/dark cycle and were carried out for 5 days a week for 4 weeks.

EXPERIMENT 1: *Effect of psilocybin on a two-bottle choice drinking paradigm in msP rats.*—Adult male (N=12) and female (N=14) msP rats were given continuous

access to 10% alcohol and water under free access conditions to achieve a stable baseline of drinking and high preference for alcohol. Once baseline drinking was reached, rats were divided into two groups with similar alcohol intake during the last 3 days of training. Each group consisted of male and female animals equally distributed. In rodents, right after administration, psilocybin evokes a motor repertoire (i.e., head twitches, locomotor impairment, inhibition of exploration) that lead to unspecific actions on alcohol drinking and seeking. Hence, to ensure that such motor effects would not have interfered with our investigation we gave psilocybin (5 mg/kg) or its vehicle i.p. 12h before the beginning of the alcohol drinking monitoring phase (at the start of the dark phase of the light/dark cycle). Alcohol, water and food intakes (g/kg) were recorded after 2, 8 and 24h.

EXPERIMENT 2: *Effect of psilocybin on alcohol deprivation-induced increase of drinking in msP rats.*—Male (N=10) and female (N=10) msP rats were given continuous access to 10% alcohol and water under free access conditions until they achieved a stable baseline of drinking and high preference for alcohol. Once baseline drinking was reached, they were subjected to an alcohol deprivation phase of three weeks. At the end of this period, animals were divided into two groups to receive an i.p. injection of psilocybin (5 mg/kg) or vehicle given i.p. Male and female rats were equally distributed in the two groups. Treatment occurred 12 h before reintroducing alcohol to ensure that any behavioral effects would be due not to the potential hallucinogenic effects of psilocybin. Alcohol, water and food intake was measured at 2, 8 and 24h and the occurrence of an Alcohol Deprivation Effect (ADE) was determined.

EXPERIMENT 3: *Effect of psilocybin on cue-induced reinstatement of alcohol-seeking.*—Male (N=8) and female (N=8) msP rats were trained to self-administer 10% (v/v) alcohol solution as described above. All training sessions were performed during the dark phase of their light/dark cycle until achieving a stable baseline of operant responding. Subsequently, all animals were subjected to a period of forced abstinence of 21 days during which they were left undisturbed in their home cages. On the 21st day of abstinence, animals were divided into two groups balanced for sex and alcohol self-administration baseline. Rats were injected i.p. with psilocybin (5mg/kg) or vehicle 12h before the reinstatement test. For reinstatement rats were returned in the operant chambers but lever pressing was no longer reinforced by alcohol delivery. The total number of responses at the active and inactive levers were recorded for 30 min.

EXPERIMENT 4: *Effect of psilocybin on reconsolidation of alcohol-related memory.*—Male (N=8) and female (N=8) msP rats were trained to self-administer 10% (v/v) alcohol until a stable self-administration baseline was achieved as previously described. For the next three weeks rats were left undisturbed in their home cages until drug test begun. For the memory retrieval-reconsolidation test male and female rats were equally distributed into 2 groups to receive i.p. psilocybin (5 m/kg) or vehicle. For memory retrieval rats were returned in 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., 2007). Immediately after this

5-min memory reactivation session, rats received psilocybin or its vehicle and were returned in their homecages until the next day. 24h 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.

2.7 Statistical analysis

In the 2BC experiment the effects of psilocybin on alcohol, water, and food intake was evaluated by a two-way analysis of variance (ANOVA), with “treatment” as between-subject factor and “time” as a within-subject factor. The effect of psilocybin on ADE was analyzed by a two-way ANOVA with “treatment” as between-subject factor and “ADE” as within-subject factor. An unpaired Student’s t-test was used to analyze the effect of psilocybin in preventing cue- induced reinstatement of alcohol-seeking. Active and inactive lever responses were analyzed separately. Data from memory reactivation were analyzed by two-way ANOVA with “treatment” as between-subject factor and “time” as within-subject factor. Active and inactive lever responses were analyzed separately. Dunnet’s test was used for post-hoc analysis when appropriate. For analysis data from male and female rats were pooled and statistical significance was set at $p<0.05$.

Results

EXPERIMENT 1: *Effect of psilocybin on a two-bottle choice drinking paradigm in msP rats.*

Two-way ANOVA analysis on alcohol drinking revealed an overall effect of time [$F_{(2,24)} = 212.7$; $p < 0.0001$], but not a significant effect of treatment [$F_{(1,24)} = 1.3$; $p > 0.05$] or time x treatment interaction [$F_{(2,48)} = 1.4$; $p > 0.05$] (Figure 1A). Analysis of water consumption revealed a significant effect of time [$F_{(2,24)} = 22.5$; $p < 0.0001$] and treatment [$F_{(1,24)} = 4.3$; $p < 0.05$], but not time x treatment interaction [$F_{(2,48)} = 1.8$; $p > 0.05$] (Figure 1B). Analysis of food consumption showed an effect of time [$F_{(2,24)} = 622.6$; $p < 0.0001$], but not treatment [$F_{(1,24)} = 0.05$; $p > 0.05$] or time x treatment interaction [$F_{(2,48)} = 0.2$; $p > 0.05$] (Figure 1C).

EXPERIMENT 2: *Effect of psilocybin on alcohol deprivation-induced increase of drinking in msP rats.*

The mean value of alcohol intake (g/kg) baseline of the last 3 days drinking were: vehicle group 7.8 ± 0.8; psilocybin group: 7.2 ± 0.9. Two-way ANOVA on alcohol intake revealed an overall effect of ADE [$F_{(1,18)} = 7$; $p < 0.05$], but no significant effect of treatment [$F_{(1,18)} = 1.1$; $p > 0.05$] or ADE x treatment interaction [$F_{(1,18)} = 0.5$; $p > 0.05$] (Figure 2A). As shown by student’s t-test water (Figure 2B) and food (Figure 2C) intakes were also not affected by the treatment ($t(18) = 1.3$; $p > 0.05$ and $t(18) = 0.3$; $p > 0.05$, respectively).

EXPERIMENT 3: *Effect of psilocybin on a cue-induced reinstatement of alcohol-seeking.*

The mean± SEM values of operant responding of the last 3 self-administration training days were 51.5 ± 4 and 51 ± 3.8 for the vehicle and the psilocybin groups, respectively. When after 21 days of forced abstinence rats were returned to the chambers to evaluate

reinstatement of alcohol seeking the mean± SEM values of active lever presses for the control and the psilocybin groups were 65.2 ± 6.8 and 65.2 ± 6.5, respectively. Unpaired student's t-test revealed no significant ($t(14) = 0.04$; $p > 0.05$) difference between the two groups (Figure 3a). Inactive lever responding was also unaffected ($t(14) = 0.4$; $p > 0.05$) by treatment (Figure b).

EXPERIMENT 4: *Effect of psilocybin on reconsolidation of alcohol-related memory.*

The mean ± SEM values of active lever presses relative to the last 3 self-administration training days were 58 ± 7 and 56.6 ± 5 for the vehicle and the psilocybin group, respectively. In the 5-min retrieval phase active lever responses were 20.6 ± 7.4 for the vehicle group and 23.1 ± 7 for the psilocybin group. When 24 h after psilocybin treatment the reinstatement test was evaluated (Figure 4B, a), two-way ANOVA revealed a significant effect of treatment [$F_{(1,14)} = 9.6$; $p < 0.01$] and time [$F_{(2,14)} = 9.7$; $p < 0.01$]. No significant treatment x time was detected [$F_{(2,28)} = 1.9$; $p > 0.05$]. A two-way ANOVA applied to inactive lever responding (Figure 4B, c) showed no overall effect of time [$F_{(2,14)} = 0.7$; $p > 0.05$], treatment [$F_{(1,14)} = 0.5$; $p > 0.05$] or time x treatment interaction [$F_{(2.28)} = 0.1$; $p > 0.05$].

Discussion

Results showed that a single injection of psilocybin did not reduce two-bottle choice voluntary alcohol intake in msP rats. Similarly, psilocybin did not prevent either the increase of drinking after a period of forced abstinence or cue-induced reinstatement of alcohol-seeking. Future studies will have to address the effect of psilocybin on stress induced reinstatement of alcohol seeking as it was not investigated here. Noteworthy, in a memory recall-reconsolidation task we observed that psilocybin, given shortly after the self-administration memory retrieval procedure, attenuated drug seeking when rats were re-exposed to the self-administration environment. Inactive lever responding, water and food intake were not modified by psilocybin, suggesting treatment specificity.

Sofar only a few studies have explored the effect of psilocybin on alcohol drinking in rodents. Meinhardt et al. applied three different treatment schedules to determine its effects on ADE. Results revealed that sub-chronic treatment with 1 mg/kg psilocybin produced a short-lasting anti-relapse effect. Conversely, 4 weeks microdosing (0.1 mg/kg) or acute administration of higher doses (2.5 mg/kg) showed no effects (Meinhardt et al., 2020). More recently the same research group found a link between psilocybin treatment, expression mGluR2 and reduction of alcohol drinking. In this study, in fact, Meinhardt and co-workers observed that administration of psilocybin (1 and 2,5mg/kg) restored mGluR2 expression and prevented alcohol relapse in rats in which infralimbic cortex mGluR2 knockdown generated a phenotype with reduced cognitive flexibility and excessive alcohol seeking (Meinhardt et al., 2021). Altogether these data indicate that, in rodents, psilocybin attenuates the motivation for alcohol only under specific circumstances and to a small extend. This is in contrast with recent clinical data suggesting a remarkable drug effect in AUD patients (Bogenschutz et al., 2014; Bogenschutz et al., 2022). Clinical studies using hallucinogens are largely based on integrated therapeutic approaches in which pharmacological and psychosocial-assisted therapies are delivered together (Bogenschutz, 2013; Johnson et al.,

2008). Hence, it is possible that the modest efficacy of psilocybin on alcohol drinking in rats depends on the fact that this drug exerts its therapeutic actions especially in conjunction with psychotherapy and that the therapeutic effects are related to the subjective psychedelic experience, aspects of psychedelics-assisted therapy that cannot be captured by animal models.

Several studies have demonstrated that long-term memory formation and development of drug addiction share common molecular mechanisms and neuronal circuitries (Hyman et al., 2006; Kelley, 2004). Consistently, 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 (McGaugh, 2000; Dudai, 2004). Previous studies demonstrated that following retrieval, cocaine, opioids, alcohol-related memories undergo a reconsolidation process and that administration of protein synthesis inhibitor anysomicin or NMDA receptor antagonists interfering with this process and reduced subsequent drug seeking and relapse (Xue et al., 2012; Von der Goltz, 2009; Bernardi et al., 2007; Robinson and Franklin, 2007; Hellemans et al., 2006; Lee et al, 2006; Lee et al., 2005; Miller and Marshall, 2005). Here we demonstrated that psilocybin given right after the alcohol memory retrieval task attenuated reinstatement of drug seeking when such alcohol memory was subsequently recalled. This finding has important clinical implications since it opens to the possibility of using psilocybin to prevent drug craving and relapse. For example, one possibility is to use it in human laboratory settings in which drug-related memories are artificially recalled and soon after the patient is treated with psilocybin. This practice could be easily integrated into already used psychotherapeutic approaches. Recently, the N-methyl D-aspartate (NMDA) antagonist ketamine, which use is currently experiencing a renaissance due to its rapid and novel anti-depressive and anti-addictive actions (Worrell and Gould, 2021; Ivan Ezquerra-Romano et al., 2018; Lapidus et al., 2014), has been reported to disrupt maladaptive reward memories in hazardous drinkers when administered immediately after memory retrieval producing beneficial effects in AUD patients (Das et al., 2019). This clinical evidence, together with our finding, highlights the promise of using reconsolidation interference as a valid and alternative approach to treat AUD with hallucinogens based therapies.

The neurobiological mechanism underlying the effect of psilocybin on memory reconsolidation is at present unknown. Laboratory animals experiments on its effects on cognition are very sparse, are based on different paradigms and do not bring uniform results, which makes difficult to formulate hypotheses. In the Morris water maze test in rodents psilocybin and its active metabolite psilocin disrupt memory retrieval (Rambousek et al., 2014). Considering that psilocybin is a potent 5_HT2A agonist and that interference with this receptor in the medial Prefrontal Cortex produces profound changes in the retrieval and consolidation of recognition memory (Morici et al., 2022; Morici et al., 2015), it is tempting to hypothesize that altered 5-HT2A transmission is responsible the effect the drug observed here. However, given the scarcity of studies with specific serotonin receptor ligands on alcohol-related memory reconsolidation tasks it is hard to provide solid ground

to the hypothesis that psilocybin acts through 5-HT2A mediate mechanisms. In humans the effect of psilocybin on memory has been explored in few studies leading to mixed results. For instance, it has been shown that it reduces attentional tracking ability but had no effect on spatial working memory (Carter et al., 2005). In other studies, it has been reported that autobiographical memories can be more easily accessed following psilocybin while working memory can be impaired at high drug doses (Healy, 2021). At molecular level it was found that a single dose of psilocybin led to an increase in dendritic spine formation and spine size in fronto-cortical neurons and enhances excitatory neurotransmission (Shao et al., 2021). These changes are long lasting and are consistent with the possibility that acting on brain plasticity psilocybin may affect memory processing. Electrophysiological and brain imaging studies demonstrated a widespread desynchronization of brain activity, increased entropic state and global reduction of brain connectivity after psylocibin which may negatively affect memory acquisition/consolidation mechanisms (Muthukumaraswamy et al., 2013;Vejmola et al., 2021; Golden et al., 2022; McCulloch et al., 2022). However, recent studies in depressed patients tested for the enduring effects of psilocybin therapy showed global increase in brain network integration and enhanced cognitive flexibility up to 3–4 weeks from treatment (Doss et al., 2021; Daws et al., 2022). Altogether these findings, despite not being conclusive, provide strong support to the possibility that psilocybin can interfere with memory processing.

In conclusion, our results confirm the modest effect of psilocybin in reducing alcohol drinking in rodents. However, we found that if the drug is given shortly after an alcohol self-administration memory retrieval procedure, it robustly attenuates subsequent drug seeking. This finding has important clinical implications since disruption of alcohol-related memories may represent a valid therapeutic approach for relapse prevention (Barak and Goltseker, 2021; Das et al., 2019). By inference, it is possible to hypothesize that this therapeutic approach can be extended to other psychiatric disorders in which presentation of environmental cues exacerbates the disease. One example is post-traumatic stress disorders (PTSD), a pathological condition that develops after exposure to a traumatic event subsequently triggering intrusive fearful memories and anxiety. A wealth of studies has demonstrated high comorbidity between AUD and PTSD (Petrakis and Simpson, 2017). Hence, the observation that psilocybin can disrupt memories reconsolidation processes opens to the possibility of using this drug to treat patients with comorbid AUD and PTSD.

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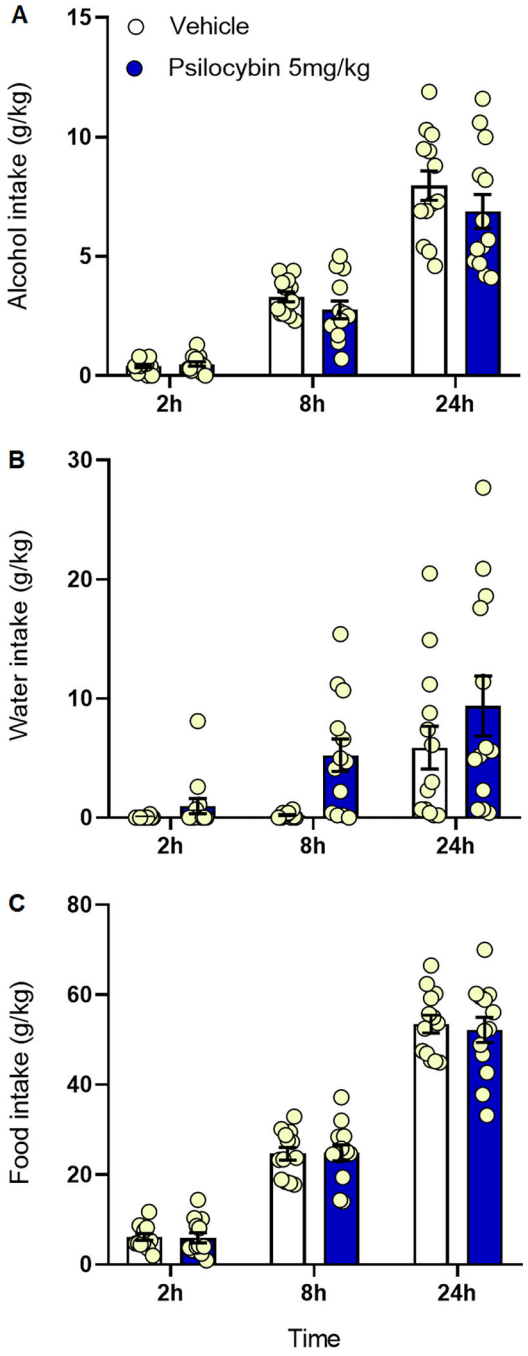

Figure 1. Effect of psilocybin on alcohol consumption in a two-bottle choice drinking.

(**A**). Psilocybin given 12 h before test did not affect: **A**) alcohol; **B**) water; **C**) or food intake. Data are expressed as mean ± SEM.

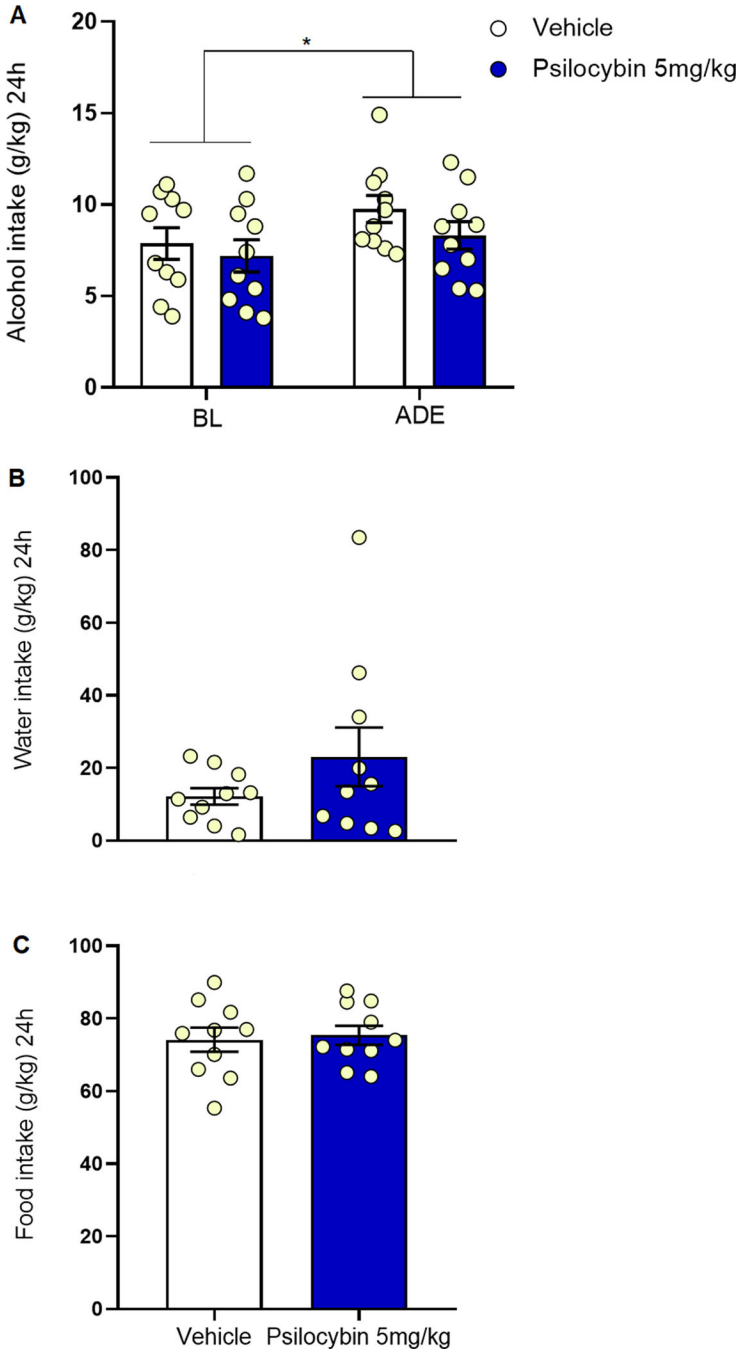

Figure 2. Effect of psilocybin on alcohol deprivation effect.

Baseline (BL) represent the mean value of the last three days of alcohol drinking prior to deprivation. **A**) Once alcohol was returned after the deprivation period a significant ADE effect was detected that was not modified by treatment. **B**) Water and **C**) food intakes were also not affected by psilocybin. Data are expressed as mean ± SEM. * $p < 0.05$ significant difference from baseline drinking.

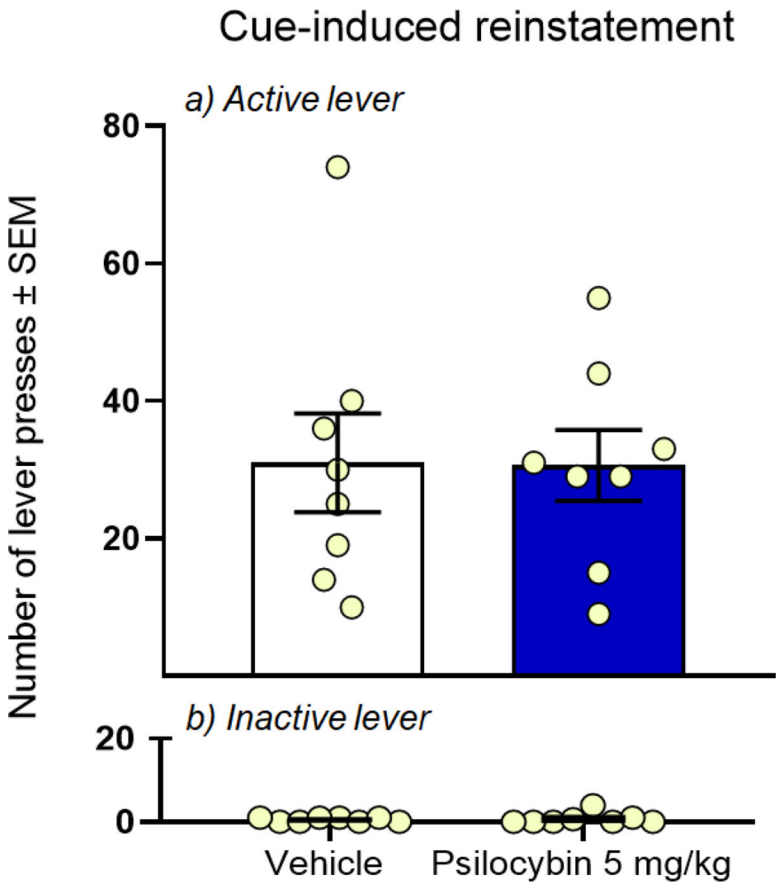

Figure 3. Effect of psilocybin on cue-induced reinstatement of alcohol-seeking.

MsP rats were treated with psilocybin or vehicle 12h before the reinstatement session. Blue and pink circles indicate male and female rats, respectively. Data are expressed as mean ± SEM of: **a)** total number of responses at the active and **b)** inactive levers.

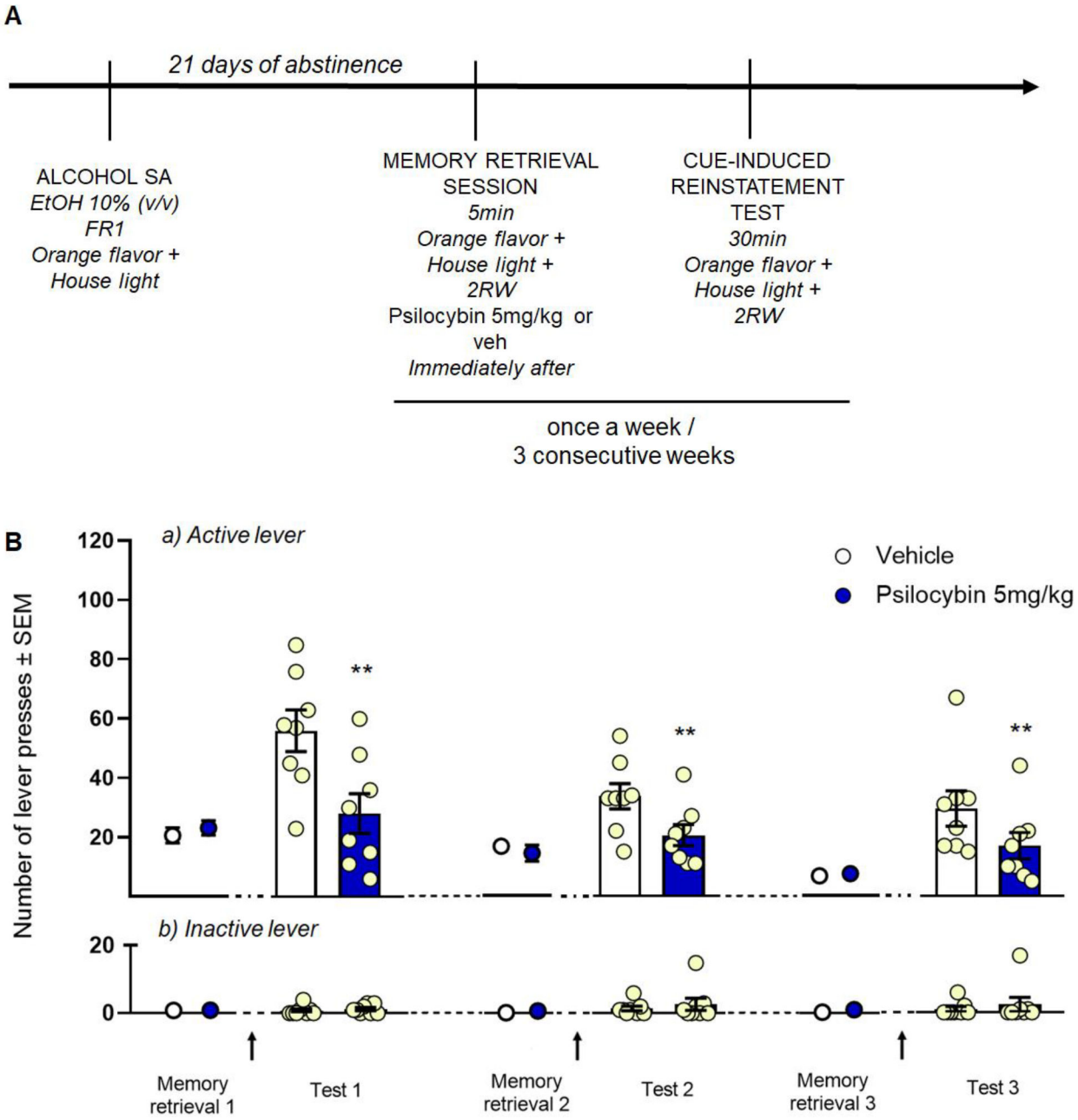

Figure 4. Effect of psilocybin on reconsolidation of alcohol-related memory.

A) Timeline presentation of conducted experiments. **B**) Arrows indicate administration of either psilocybin or vehicle. In the three tests carried out psilocybin significantly reduced alcohol-related lever presses but not inactive lever responding. Blue and pink circles indicate male and female rats, respectively. Data are expressed as mean ± SEM of **a)** total responses at the active and **b)** total responses at inactive lever. * *p < 0.01 significant difference from baseline drinking.