Head running: Exercise, alcohol intake and frustrative nonreward

**Abstract**

Increased voluntary consumption of alcohol and other anxiolytics has been demonstrated in animals after experiencing frustrative reward devaluation (downshift) or omission. These results have been interpreted in terms of emotional self-medication. In the present study we analyzed whether voluntary physical activity reduces alcohol intake induced by reward downshift. Sixty-four male Wistar rats were divided into eight groups (n=8). Thirty-two (downshifted) animals received 32% sucrose during 10 preshift sessions (5 min), followed by 4% sucrose during 5 postshift sessions, whereas 32 (unshifted) controls were always exposed to 4% sucrose. Immediately after each consummatory session, animals were exposed to a 2-h two-bottle preference test involving 32% alcohol vs. water, or water vs. water. Half of the animals had also access to a wheel for voluntary running during the preference test. The results showed lower sucrose consumption in downshifted groups compared with unshifted controls (the frustrative reward downshift effect). Reward downshift significantly increased alcohol intake, this effect being absent in downshifted animals with access to the wheel. These findings suggest that physical exercise could be useful to prevent alcohol self-medication induced by frustrative nonreward.

*Key words*: alcohol consumption; emotional self-medication; frustration; physical activity, reward downshift

*Public significance statement*:

Human and non-human studies suggest that consumption-dependent reduction in negative affect promotes alcohol intake. This “self-medication behavior” has been observed in frustrating

situations involving reward loss. This study showed (in rats) that increased alcohol intake induced by a reward devaluation event was abolished by voluntary wheel running. Physical exercise could therefore be useful to prevent the maladaptive effects of frustration on drug use.

## Introduction

The consumption of psychoactive substances is a deeply rooted human practice since ancient times. Occasionally such practices can develop into maladaptive patterns characterized by a compulsive tendency to search and consume a substance, a loss of control for limited consumption, and the emergence of a negative emotional state when access to the drug is not possible (Koob, 2021).

Several different neurobehavioral approaches have been proposed to explain why people use drugs and eventually develop a substance-use disorder. Most of them focus on the (dopamine mesolimbic-dependent) acute pleasant/reinforcing properties of psychoactive substances (DiChiara & Bassareo, 2006; Koob, 2014; Uhl et al., 2019). The emotional self-medication hypothesis, however, suggests that the type of substance chosen for consumption depends on the extent to which that substance alleviates a range of negative affective states (Khantzian, 1985, 2013, Torres & Papini, 2016). According to this view, some clinical studies suggest that drug-use behavior is reinforced by a reduction in negative affect present in a variety of psychiatric and psychological conditions (Castaneda, 1994; DeMartini & Carey, 2011, Enman et al., 2014; Menary et al., 2011; Robinson et al., 2011), triggered by negative life events (Konopoka et al., 2013; McPhee et al., 2020), and associated with drug withdrawal (Koob & Volkow, 2016; Koob et al., 2020). Additional support for the emotional self-medication hypothesis derives from survey studies indicating that consumption-dependent reduction in negative affect is frequently cited as a factor promoting alcohol intake, among other drugs (e.g., Adams et al., 2012; Rodriguez et al., 2020). However, some studies have found weak associations between stress and drug-use (Preston et al., 2011), lack of relationships between high levels of emotional distress and reported substance use (Hall & Queener, 2007), moderate prevalence rates of self-

medication with alcohol and other drugs among individuals suffering from mood and anxiety disorders (Turner et al., 2018), no evidence of improvement in anxiety symptoms after drug consumption (Carrigan & Randall, 2003), and associations between substance use and symptoms exacerbation (Brady et al., 1990). These inconsistent results reveal the complexity of the relationship between aversive events/negative affect and drugs and alcohol intake, and the involvement of factors other than emotional regulation in drug intake.

Tests of the emotional self-medication hypothesis in nonhuman animals show that a number of physical and psychological aversive/stressing stimuli lead to increased voluntary alcohol drinking in rodents, although inconsistencies have also been reported (Becker et al., 2011; Sillaber & Henninger, 2004; Spanagel et al., 2014). Recent studies have extended these results to situations involving frustrative reward loss, that is, the sudden and unexpected reduction or omission of an expected reward (Amsel, 1992; Gray, 1987). In these studies, animals are exposed to two tasks in tandem: an *induction task* eventually involving reward loss, followed each day by a *preference test* providing a choice between an anxiolytic solution (e.g., alcohol) and water. In one study (Manzo et al., 2014), animals with extreme differences in emotional reactivity and anxiety (Roman high- and low-avoidance inbred rat strains; RHA-I and RLA-I; Fernández-Teruel et al., 2021) were exposed to appetitive (consummatory and instrumental) acquisition and extinction. Inmediately after each session, rats were exposed to an alcohol (2%) vs. water, two-bottle preference test. Anxious RLA-I rats showed greater preference and consumption of alcohol than less-anxious RHA-I rats after extinction (reward omission) sessions. Another study tested the effect of reward downshift (from 32% to 4% sucrose) in a consummatory task on the voluntary consumption of alcohol and the benzodiazepine anxiolytic chlordiazepoxide in Wistar rats. Again, animals increased anxiolytic

consumption after reward downshift sessions, an effect that was not observed in unshifted groups (always receiving access to 4% sucrose), and in downshifted and unshifted groups exposed to water during the preference test (Manzo et al., 2015a; see also Donaire et al., 2022). Increased alcohol intake seemed to depend on its anxiolytic properties, as increased alcohol consumption observed after experiencing reward devaluation was accompanied by signs of anxiolysis in a test for anxiety administered immediately after the alcohol preference test (higher head-dipping frequency in the Hole-Board test) (Donaire et al., 2020). Interestingly, the augmented alcohol intake induced by reward loss was absent in animals receiving partial reinforcement training before experiencing the reward loss event (Manzo et al., 2015b), therefore suggesting that the impact of the frustrative induction task on drug intake can be prevented by treatments that increase resistance to frustration (Amsel, 1992). The present experiment aimed at extending this finding by identifying additional experimental manipulations to reduce or abolish the increased alcohol consumption induced by reward loss.

Physical activity has been extensively used as an adjunctive intervention for substance use disorders based on its physical and mental health benefits (Georgakouli et al. 2017; Jensen et al. 2019; Roessler, 2010; Weinstock et al., 2017), some of them dependent on its decreasing effects on negative affect (Abrantes et al., 2019; see however Cabé et al., 2021, for inconsistent results). Additional evidence from non-human animals' studies has shown reduced alcohol intake in subjects with previous or simultaneous access to alcohol and a wheel for voluntary running (Darlington et al. 2016; Ehringer et al., 2009; Engelhart et al., 1992; McMillan, 1976; McMillan et al., 1995), although negative results have also been found (Crews et al., 2004; Ozburn et al., 2008). Importantly here, 1 h of access to a running wheel three times per week reversed the increase in alcohol intake induced by social stress in mice, thus showing an effect of physical

activity on alcohol consumption induced by aversive stimuli (Reguilón et al., 2020). In the present study we investigated whether these results extend to aversive situations involving reward loss. To this aim, animals were exposed to a frustrative induction task (32%-to-4% vs. 4%-to-4% sucrose), followed daily by a free choice alcohol (32%) vs. water preference test. Half of the animals also had access to a wheel for voluntary running during the preference test. According to the evidence previously ~~revised~~ reviewed, we predicted: (a) suppressed sucrose consummatory behavior in downshifted (32-4) animals relative to unshifted (4-4) animals; (b) higher alcohol intake and preference in downshifted animals receiving alcohol in comparison with controls (unshifted rats with access to alcohol and downshifted rats with access to water); (c) reduced alcohol intake in downshifted animals with access to a wheel for running during the preference test compared with downshifted rats whose wheel was blocked.

## Methods

### Subjects

The subjects were 64 experimentally-naïve male Wistar rats (70 days; Envigo, Barcelona, Spain), weighing on average 318.55 g (± 33.83 g) at the beginning of the experiment. The number of animals per group (n = 8) was determined by a priori power analyses based on sucrose consumption data obtained in our laboratory. Rats were housed individually in polycarbonate cages (18 cm x 32 cm x 20.5 cm, L×W×H) with water and environmental enrichment continuously available, in a room with constant temperature (18-22ºC) and humidity (50-60%), with lights on between 08:00 and 20:00 h. Animals were food deprived and maintained within 82-85% of their ad lib weight. All the manipulations, measures and data in the study are reported. No animals were omitted from the study, and all animals completed the training sessions.

**Apparatus**

Reward downshift training involved eight original LI 836 boxes customized by Cibertec (Madrid, Spain), each measuring 29 cm× 24.5 cm × 35.5 cm (L×W×H). The back wall had a 3.2 cm x 3.9 hole through which a metallic sipper tube of a graduated cylinder was inserted. Boxes were place inside a standard lighted sound absorbing enclosure. Licking response was automatically registered with MED-PC-IV Program for Windows 7 in a computer located in the same room. The 32% (or 4%) sucrose solution was prepared w/w by mixing 32 g (or 4 g) of sucrose for every 68 g (or 96 g) of distilled water. These concentrations were selected on the basis of previous studies showing that this reward discrepancy is optimal to obtain a robust and consistent reward devaluation effect (e.g., Donaire et al., 2022; Flaherty, 1996; Papini & Pellegrini, 2006).

The preference test was conducted in an adjacent experimental room with eight polycarbonate boxes measuring 21 cm x 45 cm x 24 cm (L x H x W), each equipped with a sliding door to give access to a 9 cm high x 34 cm diameter wheel running. Wheels were located in the right side of the boxes. Recording of the running behavior (number of laps) was conducted with MED-PC Program for Windows 7 in a computer located in the same room. Fluid consumption was measured by weighing the bottles (250 ml polypropylene bottles with metallic nipple) before and after each preference session (“Smart Weigh" Precision Scale, TS500). Alcohol (Ethanol 96% Extra Pure Ph Eur, Merck) was diluted in tap water on a v/v basis. Each bottle contained 40 ml of alcohol solution, prepared by mixing 166.66 (32%) ml of alcohol in 500 ml of tap water. This alcohol concentration was selected based on the increase in consumption after reward downshift observed in previous studies (Donaire et al., 2022) and

because it led to consumption levels in line with research on the pharmacological effects of alcohol intake in rodents (e.g., Carnicella et al., 2011).

**Procedure**

Subjects were matched by weight, $F < 1$, and randomly assigned to Groups 32/Alcohol, 32/Alcohol + Wheel, 32/Water, 32/Water + Wheel, 4/Alcohol, 4/Alcohol + Wheel, 4/Water, and 4/Water + Wheel, respectively ($n = 8$). For the induction task, a 5-min habituation session in the consummatory box without fluids preceded sucrose consummatory training. On days 1–10 (preshift phase) animals had free access to 32% (or 4%) sucrose. On Days 11-15 (postshift phase), all animals received 4% sucrose. Each session lasted 5 min starting from the first contact with the sipper tube. Rats were transported in squads of eight animals, one from each experimental condition. The dependent variable was lick frequency (number of licks during the 5-min session).

Immediately after each sucrose consummatory session, rats were tested in a 2-h, 2-bottle alcohol -32%- (or water) vs. water preference test. Animals were first habituated for four days to the two-bottle procedure with both bottles containing tap water (see Manzo et al., 2015a). All bottles were weighed before and after the preference test to assess the amount of fluid consumed. The location of the bottles was changed daily to minimize position preferences. Half of the animals had access to the wheel for voluntary running during the preference test, whereas in the other half the wheel was available but locked. The dependent variables were the amount of alcohol (g) consumed transformed by the weight of the animal in the same day (g/kg), and the number of wheel turns for each session. A preference ratio for alcohol was also calculated by dividing the consumption on each target bottle (alcohol or water, ml/kg) by the total

consumption for each preference test session. A preference ratio above 0.5 reflects preference for alcohol over water, and below 0.5 reflects preference for water over alcohol; 0.5 implies no preference for either fluid. To calculate a preference ratio in groups given access to water in both bottles, a bottle was arbitrarily designated the target bottle for each animal.

**Statistical analysis**

Analyses of variance were calculated for each dependent variable with an alpha value set at the 0.05 level. Partial eta square ($\eta^2$) was used to assess effect size. Preshift data were analyzed by calculating the mean consumption for sessions 8-10 (preshift terminal performance, T). Sucrose intake (ml/kg), alcohol intake (g/kg), and alcohol preference were subjected to a Contrast (32% vs. 4%) by Drug (alcohol vs. water) by Wheel (with vs. without wheel) by Session (T, and 11 to 15) analysis of variance, with Session as a repeated-measure factor. Wheel running data were subjected to a Contrast (32% vs. 4%) by Drug (alcohol vs. water) by Session (T, 11 to 15) analysis of variance. Planned Bonferroni comparisons were also calculated to compare means of interest to the research (Castañeda et al., 1993), so that we could answer questions such as whether or not a 32-to 4% sucrose devaluation induced consummatory suppression and increased alcohol consumption and preference, and whether wheel running prevented the increased alcohol consumption observed in animals exposed to sucrose devaluation. To further test whether the influence of wheel running on alcohol intake could be interpreted in terms of response competition, Pearson correlation coefficients were calculated between alcohol ~~(or water)~~ consumption (g/kg) and wheel turns on every postshift session ($p < 0.05$). All statistical tests were conducted with the IBM SPSS Statistics 27.0 package.

## Results

Figure 1 shows the results of the induction task involving a 32-to-4% sucrose downshift during preshift (T) and postshift (11-15) sessions.

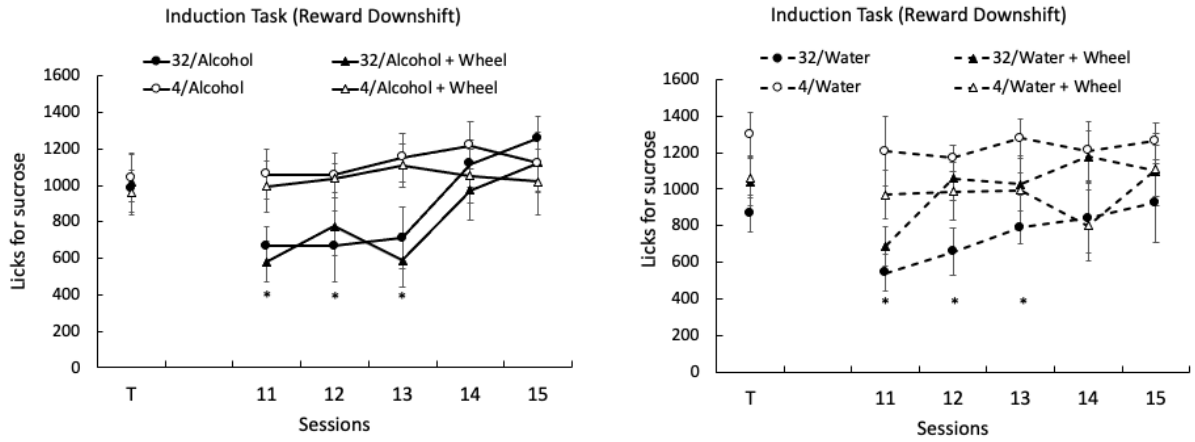


**Figure 1.** Mean number of licks for sucrose (±SEM) during the preshift phase (T, average of the last 3 sessions, 8-10) and during the postshift phase (sessions 11-15) of the induction task. *: unshifted (4) groups *vs*. downshifted (32) groups, $p<0.05$.

A Contrast by Drug by Wheel by Session analysis revealed a statistically significant effect of Contrast, $F(1, 56) = 8.396$, $p = 0.005$, $\eta^2p = 0.130$; Session, $F(5, 280) = 6.784$, $p = 0.0001$, $\eta^2p = 0.108$; and a Contrast by Session interaction, $F (5, 280) = 5.125$, $p = 0.0001$, $\eta^2p = 0.084$. Bonferroni tests revealed statistically significant differences between downshifted (32) and unshifted (4) groups on postshift sessions 11, $F(1, 56) = 22.345$, $p = 0.0001$, $\eta^2p = 0.285$; 12, $F(1, 56) = 8.193$, $p < 0.006$, $\eta^2p = 0.128$; and 13, $F(1, 56) = 14.233$, $p = 0.0001$, $\eta^2p = 0.203$. Therefore, regardless the Drug (alcohol, water) or the Wheel (with, without) condition, animals exposed to sucrose devaluation from 32% to 4% showed lower fluid intake of the devalued 4% sucrose solution compared with animals receiving 4% sucrose throughout training.

Table 1 shows the average of alcohol consumption (g/kg) across sessions (T, sessions 11 to 15) in groups receiving alcohol. In order to analyze whether wheel running prevented increased alcohol intake triggered by sucrose downshift, we focused on the terminal preshift vs. average posthift performance of unshifted (4) and downshifted (32) groups with access to alcohol ~~(32/Alcohol + Wheel and 32/Alcohol)~~. As no effects of wheel were obtained in unshifted controls (4/Alcohol + Wheel vs. 4/Alcohol, $F(1,14) = 0.363$, $p = 0.557$, $\eta^2p = 0.025$), Figure 2 presents the individual and averaged results of groups 32/Alcohol + Wheel and 32/Alcohol.

**Table 1**

Mean (± SEM) alcohol consumption (g/kg) in devalued downshifted and unshifted groups receiving alcohol with and without simultaneous access to a wheel for voluntary running.

| | *Preshift Phase* | | *Postshift Phase* | | | |
|---|---|---|---|---|---|---|
| *Group* | T | 11 | 12 | 13 | 14 | 15 |
| 32/Alcohol + Wheel | 3.86 (± 0.61) | 6.21 (± 0.75) | 9.33((± 3.57) | 6.23 (±1.02) | 8.84 (±3.33) | 4.62 (±0.54) |
| 32/Alcohol | 2.63 (± 0.26) | 2.38 (± 0.33) | 2.84 (± 0.33) | 2.96 (± 0.41) | 2.96 (± 0.50) | 2.42 (± 0.45) |
| 4/Alcohol + Wheel | 3.42 (± 0.31) | 4.61 (± 0.57) | 6.74 (± 0.46) | 3.69 (± 0.46) | 4.09 (± 0.43) | 7.25 (± 3.77) |
| 4/Alcohol | 3.18 (± 0.34) | 3.00 (± 0.34) | 7.44 (± 4,38) | 6.17 (± 2.84) | 3.07 (± 0.51) | 2.84 (± 0.47) |

*Note*: T: average of the last 3 sessions (8-10). Postshift sessions 11 to 15.

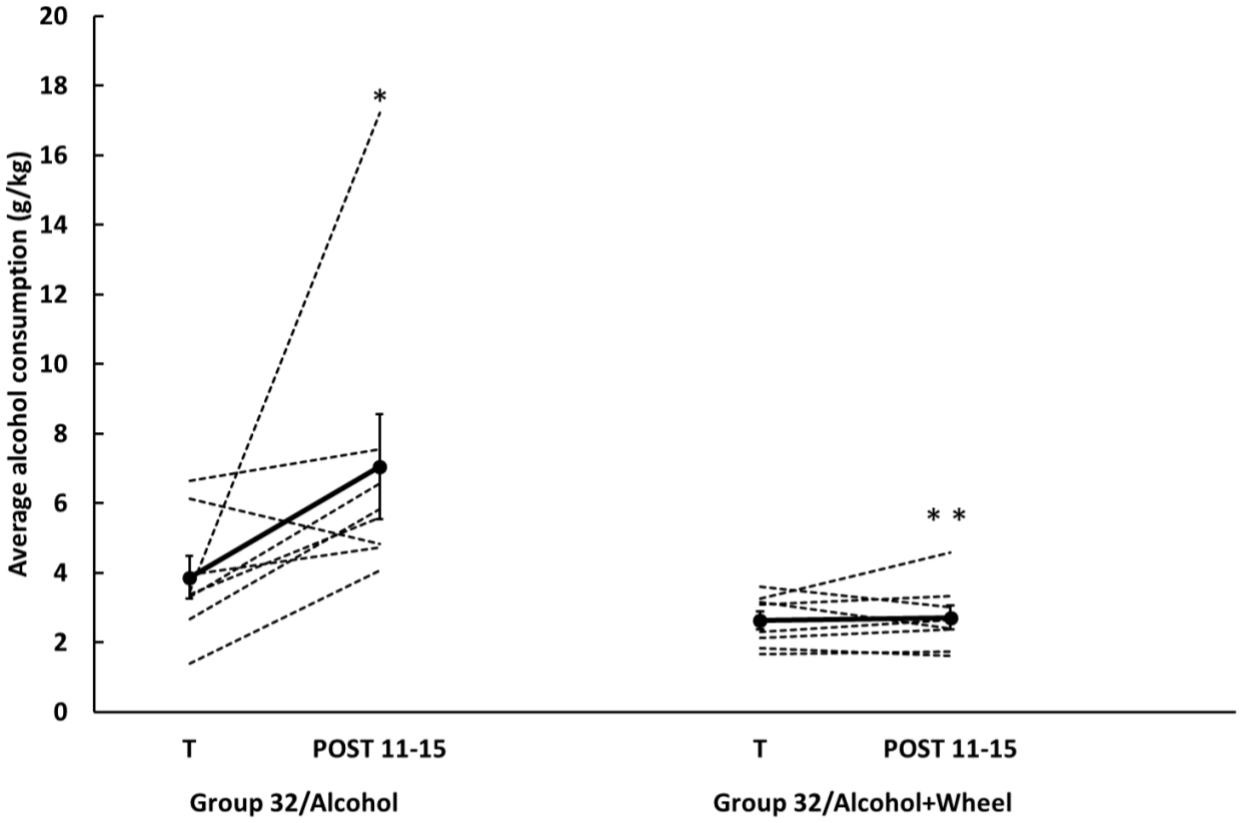


**Figure 2.** Individual data (dashed lines) and mean ±SEM (undotted lines) of alcohol consumption (g/kg) in the preshift phase (T, average of the last 3 sessions, 8-10) and the postshift phase (average of the sessions 11 to 15) of groups 32/Alcohol and 32/Alcohol + Wheel. *: T *vs*. POST 11-15 in Group 32/Alcohol, $p<0.05$. **: Group 32/Alcohol *vs.* Group 32/Alcohol + Wheel in POST 11-15, $p < 0.05$.

A Wheel by Phase (T, Post) analysis involving groups 32/Alcohol and 32/Alcohol + Wheel yielded a statistically significant effect of Wheel, $F(1, 14) = 10.299$, $p = 0.006$, $\eta^2p = 0.424$. The Wheel by Phase interaction was marginally significant, $F(1,14) = 3.654$, $p = 0.077$, $\eta^2p = 0.207$. Importantly, only group 32/Alcohol showed increased alcohol consumption in postshift phase in comparison with preshift phase, $F(1, 14) = 7.715$, $p = 0.015$, $\eta^2p = 0.355$. In

addition, group 32/Alcohol showed higher alcohol consumption compared with 32/Alcohol + Wheel only in posthift phase F(1, 14) = 7.900, p = 0.014, $\eta^2$p = 0.361.

The impact of reward devaluation and wheel access on alcohol consumption were also analyzed in terms of alcohol preference differences across alcohol groups and sessions (Figure 3).

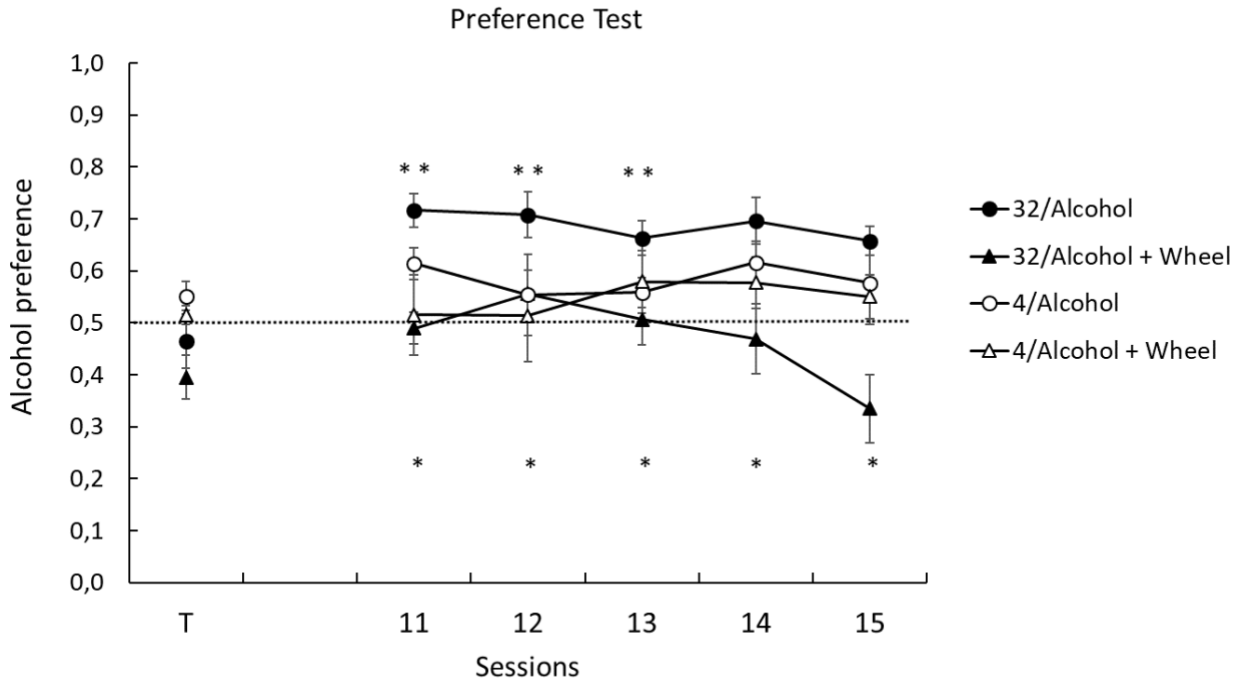


***Figure 3.*** Mean (±SEM) alcohol preference across preshift (T) and postshift sessions (11-15) and in groups receiving alcohol. *: group 32/Alcohol *vs.* group 32/Alcohol + Wheel in postshift sessions, p<0.05. **: postshift sessions *vs.* T in group 32/Alcohol, p < 0.05.

A Contrast by Wheel by Session analysis showed statistically significant main effects of Session, F(5, 140) = 3.280, p = 0.008, $\eta^2$p = 0.105; and Wheel, F(1, 28) = 16.168, p = 0.0001, $\eta^2$p = 0.366. There were also significant Session by Contrast, F(5, 140) = 2.370, p = 0.042, $\eta^2$p =

0.078; and Contrast by Wheel, F(1, 28) = 7.558, p = 0.010, $\eta^2$p = 0.213, interactions. To simplify the statistical analysis of these results, we focused on analyzing whether alcohol preference in downshifted animals was modulated by wheel running. There were statistically significant differences between groups 32/Alcohol and 32/Alcohol + Wheel only in postshift sessions, Fs(1, 14) > 5.630, ps < 0.034, $\eta^2$ps > 0.286. Moreover, alcohol preference was lower in preshift (T) phase in comparison with postshift sessions 11, 12 and 13 in group 32/Alcohol (ps < 0.045), but not in group 32/Alcohol + Wheel (ps > 0.230).

Figure 4 shows the number of wheel turns registered in downshifted and unshifted groups exposed to alcohol or water in the preference test.

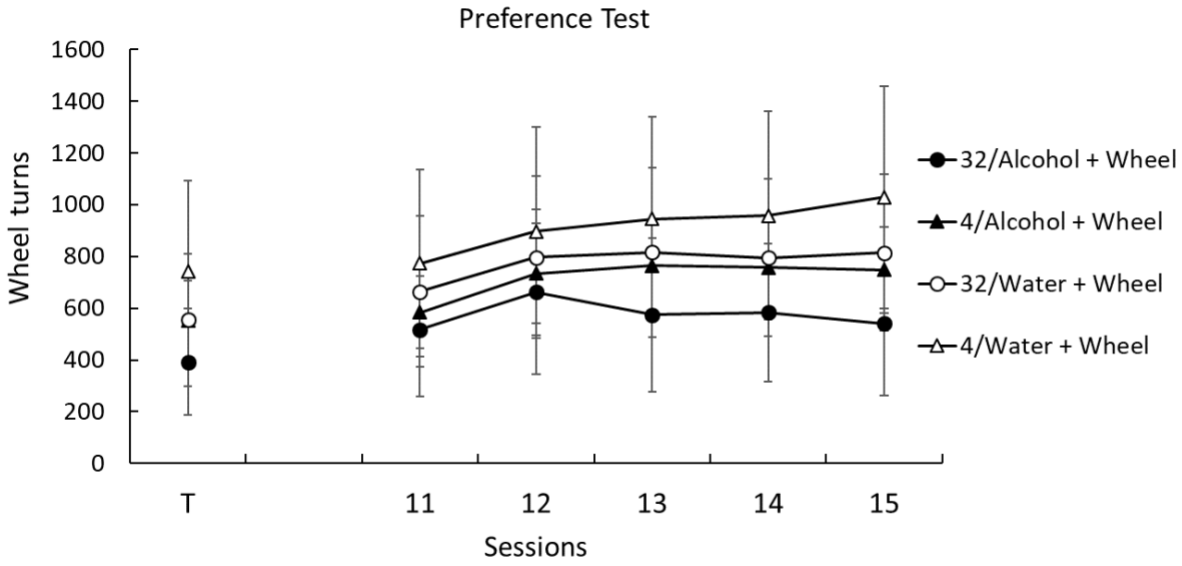


**Figure 4.** Mean wheel turns (±SEM) of the groups with access to a running-wheel during the preshift (T) and postshift phases (11-15).

A Contrast by Drug by Session analysis revealed only a statistically significant Session effect, F (5, 140) = 10.420, p = 0.001, $\eta^2$p = 0.271, thus showing an increase in wheel running across sessions regardless the Contrast (32 vs. 4) or the Drug (alcohol vs. water) condition.

Finally, statistically significant Pearson correlations between alcohol intake (g/kg) and wheel turns were not obtained on postshift sessions, indicating ~~relations between -15, r(32) = 0.561, p = 0.001, indicating that animals showing higher alcohol intake also showed more wheel running. On all other postshift sessions there was simply~~ no relation between measures of drinking and running.

## Discussion

In the present study, animals were exposed to a 32-to-4% sucrose downshift manipulation followed by access to alcohol vs. water for voluntary drinking with/without simultaneous access to a wheel for voluntary running. We aimed at analyzing whether physical exercise provided by a movable wheel would reduce the augmented alcohol consumption repeatedly observed after experiencing a reward loss event (Donaire et al., 2018, 2020; Manzo et al., 2014; Manzo et al., 2015a). Compared with unshifted (4) controls, downshifted (32) animals showed lower sucrose consumption during the postshift (downshift) phase. Importantly, the augmented alcohol intake and preference (postshift > preshift phase) registered in rats exposed to reward downshift was absent in animals with simultaneous access to a wheel for running, thus suggesting an attenuating effect of physical exercise on augmented alcohol intake induced by reward loss.

The 32-to-4 sucrose manipulation in the present experiment negatively affected consummatory response regardless the subsequent drug (alcohol vs. water) or wheel (with, without) condition. There is extensive behavioral, hormonal, pharmacological, psychogenetic and neurobiological evidence indicating that animals exposed to unexpected reward loss exhibit

a behavioral impairment that relies on the emergence of a negative emotional response (referred to as frustration, disappointment, anxiety, or psychological pain; see Amsel, 1992; Flaherty, 1996; Gray, 1987; Papini et al., 2015). According to this view, forced administration of anxiolytics (alcohol, benzodiazepines, barbiturates) before the reward downshift episode significantly attenuates consummatory suppression (see Flaherty, 1996, for review). The finding that experiencing reward downshift in turn increases subsequent voluntary anxiolytics consumption also supports an interpretation of the reward downshift effect in terms of negative emotion (Manzo et al., 2015a; Donaire et al., 2018, 2020, 2022; present results). Similar results have been obtained with other reward loss and drug administration paradigms (Ginsburg & Lamb, 2018; Podlesnik et al., 2006; Vasquez et al., 2021), thus showing the usefulness of animal models of reward loss to analyze the impact of negative emotions on drug use and abuse.

The increase in alcohol consumption observed in animals exposed to reward downshift may alternatively be explained in terms of resurgence. This phenomenon refers to the recurrence or recovery of a previously reinforced response when the reinforcement for a more recently reinforced response is discontinued (Bouton & Trask, 2016; Podlesnik et al., 2006; Shahan & Sweeney, 2011). There is evidence in humans and non-human animals that drug-seeking relapse can be precipitated by loss of alternative non-drug reinforcement (Ginsburg & Lamb, 2018; Podlesnik et al., 2006; Quick et al., 2011). According to this view, 32/Alcohol animals increased alcohol intake as a way to replace the reduction in reinforcement experienced from drinking a downshifted sucrose solution. An interpretation of the present results in terms of resurgence, however, has some limitations. First, both the induction task and the preference test were consummatory (rather than operant) and were presented consecutively in the same day, rather than successively (in two differentiated training phases) as used in the resurgence paradigm.

Second, alcohol consumption and preference increased from a baseline level, not from an absent (previously extinguished) behavior. Finally, "resurgence" of alcohol use would require evidence that alcohol was a source of reinforcement (based on its pleasant effects), so that the "loss" of sucrose increased behaviors (drinking) aimed at obtaining the alternative reinforcer (32% alcohol). However, in the present experiment animals did not show preference for alcohol vs. water in the preshift phase (see T in Figure 3), a result that is consistent with previous studies involving high doses of ethanol (Pautassi, 2019). The increase in preference levels observed in group 32/Alcohol after experiencing reward downshift suggests that such preference for alcohol was dependent on the reduction of negative affect induced by sucrose devaluation.

The caloric contribution of the 32% alcohol solution could also underlie the augmented alcohol consumption observed in food-restricted animals exposed to a reduction in sucrose solution (from 32% to 4%). Nevertheless, a similar increase in fluid consumption was observed in downshifted animals with subsequent access to a solution containing chlordiazepoxide (Manzo et al., 2015a), an anxiolytic substance lacking caloric value. Alternatively, anxiolysis derived from alcohol intake could rest on the ability of a potent response (such as drinking) to interfere with the negative emotional state induced by reward downshift, rather than on its pharmacological properties per se (Papini & Dudley, 1997). However, downshifted groups with access to water showed no evidence of change in fluid intake after experiencing reward downshift, suggesting that just performing the licking response was not sufficient to reduce negative affect (see comparable results but on the lack of positive consequences for just licking in Ruiz et al., 2016). The lack of impact of the sucrose downshift manipulation on wheel running (a response also known to have reinforcing properties; e.g., Belke & Pierce, 2016) also makes this interpretation unlikely.

The present results are in accordance with studies showing increased voluntary alcohol consumption and preference in non-human animals exposed to a variety of aversive stimuli, including uncontrollable foot shocks, physical restraint, forced swimming, social isolation, social defeat and odor predator, among others (e.g., Anderson et al., 2016; Anisman and Waller, 1974; Lynch et al., 1999; Manjoch et al., 2016; Nash & Maickel, 1985; Newman et al., 2018; Thompson et al., 2020; Wolffgramm 1990). Reported data are also concordant with human studies showing increased alcohol use and abuse in patients with psychiatric pathologies, healthy subjects exposed to a variety of negative events, and alcohol-dependent subjects experiencing withdrawal (e.g., Anderson et al., 2016; Becker et al., 2011; Briand & Blendy, 2010; Gil-Rivas & McWhorter, 2013; Koob, 2014). Overall, these results have been interpreted in terms of emotional self-medication, suggesting that the anxiolytic effects of alcohol reduce negative affect and provide a source of reinforcement for drug intake behavior (Blume et al., 2000; Hall & Queener, 2007; Khantzian, 2013).

The most important result obtained in the present study refers to the abolishing effect of voluntary wheel running on augmented alcohol intake and preference induced by reward downshift: animals with simultaneous access to alcohol and a wheel for running did not show increased alcohol intake after experiencing reward downshift, a result that cannot be explained on the basis of response (fluid intake vs. running) competition (see the absence of negative correlations between alcohol intake and wheel turns in the Results section). The reduction in sucrose concentration during the postshift phase was not accompanied by changes in alcohol consumption or preference provided rats could ran in a wheel, which increased slightly across sessions. The absence of changes in alcohol intake from preshift to postshift phases reveals that its potential reinforcing effect was not substituted by the alternative running reinforcer.

There is extensive evidence showing the usefulness of physical activity as an effective treatment for drug (including alcohol) use disorders (Cabé et al., 2021; Georgakouli et al. 2017; Jensen et al. 2019; Roessler, 2010; Weinstock et al., 2017). In line with these clinical results, simultaneous access to a wheel for exercising significantly reduces alcohol consumption and preference and modifies alcohol drinking patterns in rodents (Darlington et al., 2016; Ehringer et al., 2009; Hammer et al., 2010; McMillan et al., 1995; Ozburn et al., 2008), albeit null and opposite results have also been reported (Crews et al., 2004; Werme et al., 2002). However, only a few studies have analyzed the extent to which physical activity influences alcohol consumption induced by aversive/stressful stimuli. In one such study (Reguilón et al., 2020), mice received 4 sessions of repeated social defeat and 1 h of access to a running wheel three times per week. Once this phase concluded, animals were trained in an operant alcohol (6%) self-administration procedure. Social defeat increased motivation to obtain alcohol and alcohol intake, an effect that was reversed by previous voluntary wheel running.

Although the mechanisms underlying the impact of physical exercise on drug use and abuse remains unclear (Lynch et al., 2013), the fact that exercise activates the dopaminergic brain reward system suggest that physical activity could serve as an effective hedonic substitute to drugs, promoting the normal functioning of the brain reward and anti-reward systems (Abrantes & Blevins, 2019; Darlington et al., 2016; Ozburn, 2008). According to this view, intense exercise has been shown to decrease alcohol craving in recovering alcoholics (Ussher et al., 2004). Similarly, previous access to voluntary exercise reduces anxiety-like behavior in rats, whereas withdrawal from exercise access enhances alcohol intake (Lynch et al., 2019).

In the present study, reward downshift increased alcohol intake without affecting wheel running, whereas wheel running abolished the effect of reward downshift on alcohol

consumption. These results suggest that although wheel running was not an effective alternative reinforcer to alcohol intake, its ameliorating effects on negative affect (see Abrantes et al., 2019) could contribute to reduce alcohol intake after experiencing reward loss. In accordance to this, animals exposed to a frustrative reward omission task showed lower hormonal and behavioral signs of anxiety when they had previous exercise training in comparison with controls (Taylor et al., 2019). Whether or not the present data can be interpreted in terms of hedonic substitution will have to be addressed in future studies to determine the usefulness of physical exercise to prevent the maladaptive effects of frustration on drug use.

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