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Caffeine modulates voluntary alcohol intake in mice depending on the access conditions: Involvement of adenosine receptors and the role of individual differences

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ABSTRACT

Caffeine is the most consumed psychoactive stimulant and the main active ingredient of energy drinks. Epidemiology studies have shown a positive correlation between the consumption of energy drinks and that of ethanol. The popular belief is that caffeine antagonizes the intoxicating effects of alcohol. Both drugs act on the adenosine system but have opposite effects. Caffeine is a methylxanthine that acts as a nonselective adenosine receptor antagonist, binding to A_1 and A_{2A} receptor subtypes. In contrast, ethanol increases extracellular adenosinergic tone. The purpose of this study was to examine the impact of a broad range of doses of caffeine and of selective adenosine A_1 and A_{2A} receptor antagonists on voluntary ethanol intake under different ethanol access conditions. C57BL/6 J male mice had access to ethanol (10% w/v) under different conditions: restricted (2 h in the dark), unrestricted (24 h access), or after 4 days of alcohol removal following several periods of unrestricted access. Mice reduced ethanol intake in the restricted access condition after receiving caffeine (20.0 mg/kg), or theophylline (20.0 mg/kg), another methylxanthine. Selective A_1 and A_{2A} adenosine receptor antagonists, or their combination, did not have any effect. However, under unrestricted access conditions caffeine and the adenosine A_{2A} receptor antagonist increased ethanol intake. After splitting animals into high, moderate and low ethanol consumers, caffeine (2.5–20.0 mg/kg) significantly increased ethanol consumption in moderate consumers with no effect on low or high consumers. In addition, after reintroducing ethanol access, caffeine (5.0 mg/kg) decreased ethanol consumption among moderate consumers. Thus, caffeine produced different effects on ethanol intake depending on the access condition and the baseline consumption of ethanol.

1. Introduction

Caffeine and alcohol are the two most consumed psychoactive substances in the world (Temple et al., 2017; Kalinowski and Humphreys, 2016). Interest in caffeine abuse has grown ever since the introduction to the market of so-called “energy drinks”. Although energy drinks contain several components with clear psychoactive effects, caffeine is the main active ingredient responsible for the behavioral and cognitive effects associated with these beverages (Giles et al., 2012). The concentration of caffeine in these drinks may range from modest to relatively high levels (50–500 mg caffeine per serving; Reissig et al., 2009; Arria and O'Brien, 2011).

A common pattern of alcohol consumption in young people is characterized by repeated bouts of heavy drinking followed by abstinence for hours and days. The combined intake of alcohol and “shots” of “energy drinks” is a relatively new phenomenon that is rising among young people. Combining caffeine with ethanol during binge drinking may stem from the popular belief that caffeine antagonizes intoxicating effects of alcohol, and improves social interactions (Weitzman et al., 2003; Reissig et al., 2009; Marczinski, 2011; Correa et al., 2014). Epidemiologic studies have shown that energy-drink users tend to show increased levels of alcohol consumption. The consumption of alcohol mixed with energy drinks in students is strongly associated with high-risk drinking behavior, including increased binge drinking, and more frequent episodes of weekly drunkenness (O'Brien et al., 2008; Patrick and Maggs, 2013).

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Pharmacological actions of caffeine are attributable to its activity as a non-selective A_1 and A_{2A} adenosine receptor antagonist (Fredholm et al., 1999; Ferré et al., 2018). On the other hand, ethanol has been suggested to increase the concentration of extracellular adenosine by facilitating adenosine release (Clark and Dar, 1989), and inhibiting adenosine re-uptake (Nagy et al., 1990; Butler and Prendergast, 2012). Secondarily, ethanol increases adenosine levels because acetate generated by ethanol metabolism promotes adenosine synthesis (Carmichael et al., 1991; Pardo et al., 2013a; López-Cruz et al., 2013). Several studies in rodents have provided information about the antagonistic interaction between acute caffeine on ethanol-modulated behaviors, such as intoxication, social interaction, or changes in preferences (López-Cruz et al., 2016; Okhuarobo et al., 2018; Correa et al., 2019; for a review see López-Cruz et al., 2013), all of which can be factors in ethanol consumption patterns. However, a limited number of studies have been performed to elucidate the impact of acute administration of caffeine ("shot"-like administration) on alcohol consumption in experimental animal models (Dietze and Kulkosky, 1991; Kunin et al., 2000; Rezvani et al., 2013; Okhuarobo et al., 2018). In these different studies the pathway of caffeine administration (IP, PO and SC), the availability of ethanol (24 h access, nocturnal or diurnal limited access), and the strain of rat (alcohol-preferring vs regular outbred rodents) are different depending on the study. Thus, a clear pattern of effects is difficult to discern.

It is even more difficult is to see a coherent pattern of results when assessing the impact of selective adenosine receptor antagonists on ethanol intake. Acute administration of an adenosine A_{2A} receptor antagonist (ANR 94) increased free ethanol intake in preferring rats (Micioni Di Bonaventura et al., 2012), and A_{2A} KO mice consumed more ethanol than their WT counterparts (Naassila et al., 2002). However, results in mice using the drinking in the dark (DID) model have found no effect of acute administration of the A_{2A} receptor antagonist MSX-3 (Fritz and Boehm, 2015). In alcohol-preferring rats self-administering ethanol in operant tasks, while ANR94 produced a mild increase in ethanol-reinforced responding (Micioni Di Bonaventura et al., 2012), the A_{2A} receptor antagonists SCH58261 (Adams et al., 2008), and DMPX reduced responding for ethanol in outbred rats (Thorsell et al., 2007). Even less information is available related to the role of A_1 receptor antagonists. Thus, in mice DPCPX dose-dependently decreased binge-like ethanol intake (Fritz and Boehm, 2015), as well as operant responding for alcohol in alcohol preferring rats (Adams et al., 2008), but had no effect in non-preferring rats (Arolfo et al., 2004).

Thus, the aim of the present study was to examine how acute administration of caffeine or of selective adenosine receptor antagonists affect free voluntary ethanol intake in a strain that shows high baseline ethanol consumption, C57BL/6 J mice. We assessed the acute pharmacological effects under different access conditions: in a restricted access condition which has been used to model voluntary intake of high amounts of ethanol in a short period of time in rodents (i.e., DID), or under free choice unlimited access conditions, a paradigm that allows self-regulation of alcohol intake and allows the appearance of individual differences. In addition, this unlimited access condition was also used to explore the effect of caffeine on alcohol reinstatement after it had been removed. In order to explore the effects of adenosine receptor antagonists on ethanol intake, a broad range of doses of the selective adenosine receptor antagonists MSX-3 (A_{2A} receptor antagonist) and CPT (A_1 receptor antagonist), separately or in combination, were acutely administered. Theophylline, another methylxanthine that is a metabolite of caffeine and has an analogous mechanism of action, was also assessed. Moreover, to account for possible non-specific effects of caffeine on palatability or calorie intake, sucrose consumption was also evaluated.

2. Materials and methods

2.1. Subjects

Male C57BL/6JRccHsd mice (15–20 g) were purchased from Harlan Laboratories (Barcelona, Spain). Mice (N = 148) were 4 weeks old upon arrival to the laboratory. They were group housed until the ethanol drinking procedures started, and then they were individually housed for the rest of the intake experiments with standard lab chow ad libitum. After 7 days of acclimatization to the colony, animals started the drinking procedures. The colony was maintained at 22 ± 1 °C, with humidity control and 12-h light/dark cycles. All animals were under a protocol approved by the Institutional Animal Care and Use committee of Universitat Jaume I. All experimental procedures complied with directive 2010/63/EU of the European Parliament and of the Council, and with the "Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research", National Research Council 2003, USA. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

2.2. Pharmacological agents

Ethanol (Panreac Quimica S.A., Spain) was diluted to 10% (v/v), and sucrose (Sigma-Aldrich, S.A., Spain) was diluted to 5% (w/v), both in tap water. Non-selective adenosine receptor antagonists, caffeine and theophylline (Sigma-Aldrich, S.A., Spain), were dissolved in 0.9% w/v saline (final pH 7.4) and administered 30 min before testing. The adenosine A_1 selective receptor antagonist CPT (8-cyclopentyltheophylline) (Sigma-Aldrich, S.A., Spain) was dissolved in distilled water (final pH 8.0) and administered 20 min before testing. The adenosine A_{2A} selective receptor antagonist MSX-3 ((*E*)-phosphoric acid mono-[3-[8-[2-(3-methoxphenyl)vinyl]-7-methyl-2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl] ester disodium salt) was dissolved in saline (final pH 5.5) and administered 20 min before testing. MSX-3 was synthesized at the laboratory of Dr. Christa Müller (Universität Bonn, Germany). Saline solution (0.9% w/v) was used as vehicle control in all experiments. All drugs (with the exception of ethanol) were administered intraperitoneally (IP). Doses and lead times were selected based on potency and efficacy from previous studies done in mice in our laboratory (López-Cruz et al., 2014, 2016, 2018; Pardo et al., 2012, 2013b; Correa et al., 2018).

2.3. Apparatus and testing procedures

Baseline ethanol intake prior to pharmacological manipulations lasted 6 weeks. Ethanol (10% v/v) was available in 10.0 ml graduated cylinders with sipper tubes. During the last week of baseline, animals received one IP saline injections to avoid reaction to novelty during the test sessions. During the test phase, once a week, each subject received all doses of one of the drugs, including vehicle, in a randomly varied order.

Restricted access to one-bottle-in the dark cycle. We used a DID procedure modified from the original (Rhodes et al., 2005), an experimental setting that generates high levels of ethanol consumption. Beginning 3 h into the dark cycle, mice had their water bottles replaced by a 10 ml graduated cylinder containing alcohol (or sucrose depending on the experiment) for 2 h. During this period, the only fluid available was the test fluid. Animals were habituated to ethanol solutions during 4 days with free access (24 h) to two drinking bottles; one with tap water and the other one with ethanol (two days with 2% *v*/v and two days with 5% v/v). After this habituation period, and for the rest of the experiment, during the 2 h test session, animals had access to one tube containing ethanol (10% v/v), 5 days a week.

2.3.1. Unrestricted access condition with two bottles

Animals were habituated to ethanol solutions during 4 days with

free access (24 h) to two drinking bottles; one with tap water and the other one with ethanol (two days with 2% v/v and two days with 5% v/v). After these 4 habituation days and for the rest of the experiment animals had 24 h access to both solutions (i.e., water and 10% v/v ethanol). The position of the two bottles was alternated to prevent a placement effect. Ethanol intake data were collected after drug injection for the first 2 h of the dark cycle and also after 24 h. The same basic procedure was performed for the sucrose experiment although no habituation period was required.

2.3.2. Intermittent alcohol time-off after unlimited access condition

For this procedure, an independent group of animals was asigned to the unlimited access condition. The last 3 days before ethanol was removed, baseline intake was registered for the first 2 h of the dark cycle and also for 24 h. After the last day of baseline exposure, the first cycle of 4 days of no-access to alcohol started. The 5th day, caffeine was administered 30 min before mice had access again to ethanol for 4 consecutive days. These cycles were repeated two more times.

2.4. Data analyses

Experiments used a within-groups design, in which each animal received all drug doses in a randomly varied order. Normally distributed and homogenous data were evaluated by repeated measures analysis of variance (ANOVA). Further analyses were conducted by nonorthogonal planned comparisons using the overall error term to assess differences between each dose and the control condition (Altman et al., 1983; Keppel, 1991). All data were expressed as mean ± SEM, and significance was set at $p < 0.05$. STATISTICA 7 software was used.

3. Results

3.1. Experiment 1. Effect of caffeine on sucrose or ethanol intake under restricted access conditions

This experiment studied the impact of caffeine on the volume of voluntary ethanol or sucrose intake during 2 h in the dark cycle. A group of animals consumed sucrose (n = 11; Fig. 1C–D), and a different group consumed ethanol (n = 15; Fig. 1A–B). Repeated measures ANOVA revealed a significant effect of caffeine on ethanol intake as measured in total volume (in ml) ($F(4,56) = 9.34$; $p < 0.01$), or in g/kg ($F(4,56) = 8.03$; $p < 0.01$). Planned comparisons revealed a significant difference between vehicle and the highest dose of caffeine (20.0 mg/kg) ($p < 0.01$) on both variables. However, repeated measures ANOVA did not demonstrate a significant effect of caffeine on volume of sucrose intake (ml) ($F(4,40) = 1.73$; n.s.), nor on grams of sucrose consumed ($F(4,40) = 1.87$; n.s.).

3.2. Experiment 2. Effect of adenosine receptor antagonists on ethanol intake under restricted access conditions

This study was conducted to determine if ethanol intake would be reduced after the administration of another methylxanthine, theophylline (0, 10.0 and 20.0 mg/kg; n = 15), or after the administration of the selective A_1 adenosine receptor antagonist CPT (0, 3.0, 6.0 and 9.0 mg/kg) (n = 7), the A_{2A} adenosine receptor antagonist MSX-3 (0, 3.0, 6.0 and 9.0 mg/kg; n = 8), or the combination of both CPT (0, 3.0, 6.0 or 9.0 mg/kg) plus the same dose of MSX-3 (0, 3.0, 6.0 or 9.0 mg/kg; n = 15). Repeated measures ANOVA showed that there was a significant effect of theophylline treatment on ethanol intake ($F(2,28) = 4.20$; $p < 0.05$) (Fig. 2A), and planned comparisons revealed that, as it was the case with caffeine, the highest dose of theophylline (20.0 mg/kg) showed a significant decline on ethanol intake ($p < 0.05$) compared to vehicle. However, repeated measures ANOVA for the A_1 receptor antagonist CPT ($F(3,18) = 0.18$; n.s. Fig. 2B), and for the A_{2A} receptor antagonist MSX-3 ($F(3,21) = 1.03$; n.s. Fig. 2C) alone, did not show a significant effect. The combination of both selective adenosine receptor antagonists (CPT + MSX3) showed no significant effect either ($F(3,11) = 0.27$; n.s. Fig. 2D).

3.3. Experiment 3. Effect of caffeine on ethanol, sucrose or water intake during the first two hours of the unrestricted access condition

The effect of caffeine on ethanol or water intake (ml) during the first 2 h of the dark cycle is shown in Fig. 3A and B (n = 15). Repeated measures ANOVA showed a significant effect of caffeine on ethanol intake ($F(4,56) = 2.82$; $p < 0.05$). Planned comparisons revealed a significant difference between vehicle and the three higher doses of caffeine (5.0 mg/kg, $p < 0.05$; 10 and 20.0 mg/kg, $p < 0.01$). However, there was not a significant effect of caffeine on water intake ($F(4,56) = 1.44$; n.s.).

The effect of caffeine on sucrose or water intake (ml) is shown in Fig. 3C and D (n = 13). Repeated measures ANOVA revealed a significant effect of caffeine on sucrose intake ($F(4,48) = 7.95$; $p < 0.01$). Planned comparisons revealed a significant difference between vehicle and the three higher doses of caffeine (5.0 and 10.0 mg/kg, $p < 0.05$; 20.0 mg/kg, $p < 0.01$). Repeated measures ANOVA did not demonstrate a significant effect of caffeine on water intake ($F(4,48) = 0.31$; n.s.).

3.4. Experiment 4. Effect of different adenosine receptor antagonists on ethanol intake under unrestricted access conditions: analyses of the first 2 h

Repeated measures ANOVA showed that there was a significant effect of caffeine on ethanol intake (g/kg) during the first 2 h of unrestricted access ($F(4,56) = 2.75$; $p < 0.05$) (Fig. 4A, n = 15). Planned comparisons revealed a significant difference between vehicle and 5.0 mg/kg ($p < 0.05$), 10.0 and 20.0 mg/kg of caffeine ($p < 0.01$). The results of theophylline (n = 10) analyzed with a repeated measures ANOVA showed no significant effect in the two first hours of ethanol access ($F(3,27) = 0.17$; n.s.) (Fig. 4B). The repeated measures ANOVA for the CPT results (n = 9) showed no significant effect ($F(3,24) = 0.43$; n.s.) of this drug (Fig. 4C). However, the repeated measures ANOVA for the selective A_{2A} adenosine receptor antagonist MSX-3 (n = 10) showed a significant effect ($F(3,27) = 3.60$; $p < 0.05$) (Fig. 4D). The higher dose of MSX-3 (9.0 mg/kg) was significantly different from vehicle ($p < 0.01$).

The repeated measures ANOVA for the 24 h data showed a significant effect of caffeine on ethanol intake (g/kg) under unlimited access conditions ($F(4,116) = 3.18$; $p < 0.01$) (Fig. 5A). Caffeine, at several doses, was significantly different from vehicle (2.5 and 10 mg/kg, $p < 0.05$; and 5 mg/kg, $p < 0.01$). Thus, when the entire period was analyzed, the impact of the lower dose of caffeine was bigger and the impact of the higher dose disappeared. The same patterns of results observed during the 2 first hours were observed when 24 h ethanol intake data were analyzed for the other adenosine receptor antagonists (supplemental material Fig. 2).

3.5. Experiment 5. Impact of caffeine on ethanol intake under unlimited access conditions: role of individual differences during 24 h access

The unrestricted access condition produced high variability on baseline ethanol intake among different mice. In order to see if caffeine modulated ethanol intake depending on the level of baseline consumption, a new group of animals (n = 30) was used and data were analyzed taking baseline levels of ethanol intake into account. Animals were divided in quartiles (see Fig. 6A) based on their baseline ethanol intake, and 3 groups were stablished: low consumers (Q1 mean = 5.66 ± 0.68 g/kg), moderate consumers (Q2 + 3 mean = 12.40 ± 1.50 g/kg), and high consumers (Q4 mean = 16.32 ± 0.41 g/kg). The one-way ANOVA revealed an overall significant difference between these groups on baseline ethanol intake

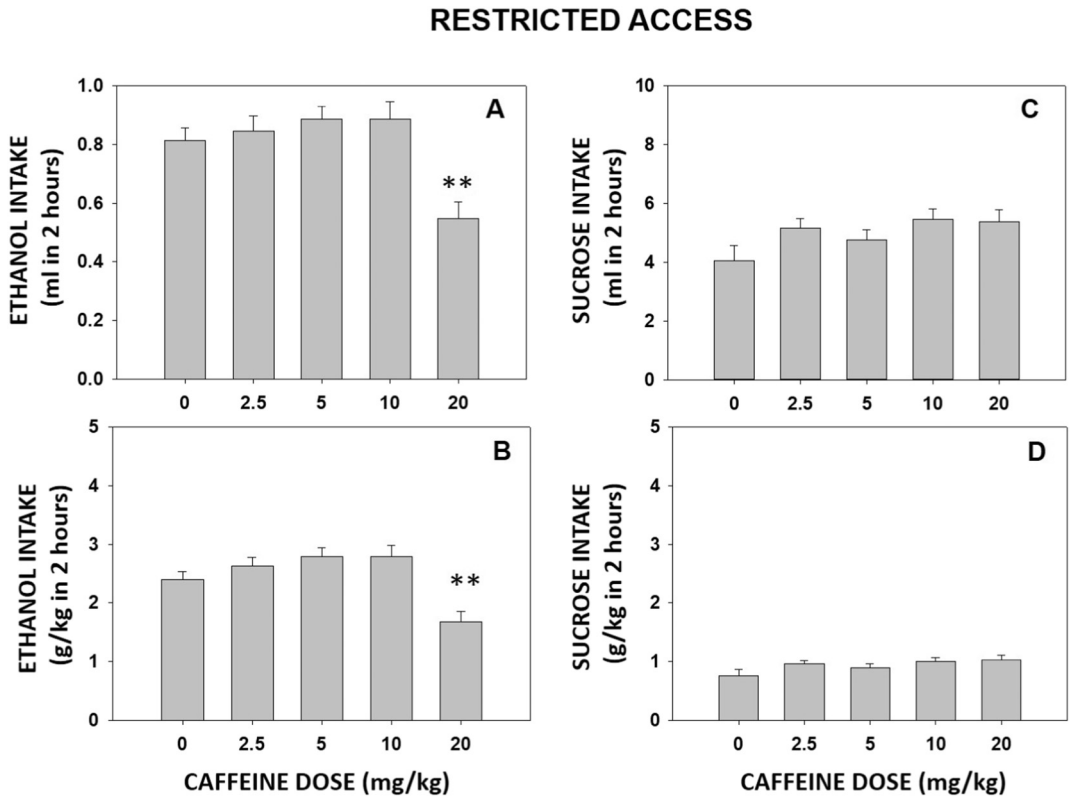

Fig. 1. Effect of caffeine on ethanol (A–B) or on sucrose intake (C–D) in a 2 h restricted access paradigm. Mean ± S.E.M. milliliters (A, C) or grams (B, D) consumed per kilogram of body weight. **p < 0.01 significantly different from vehicle.

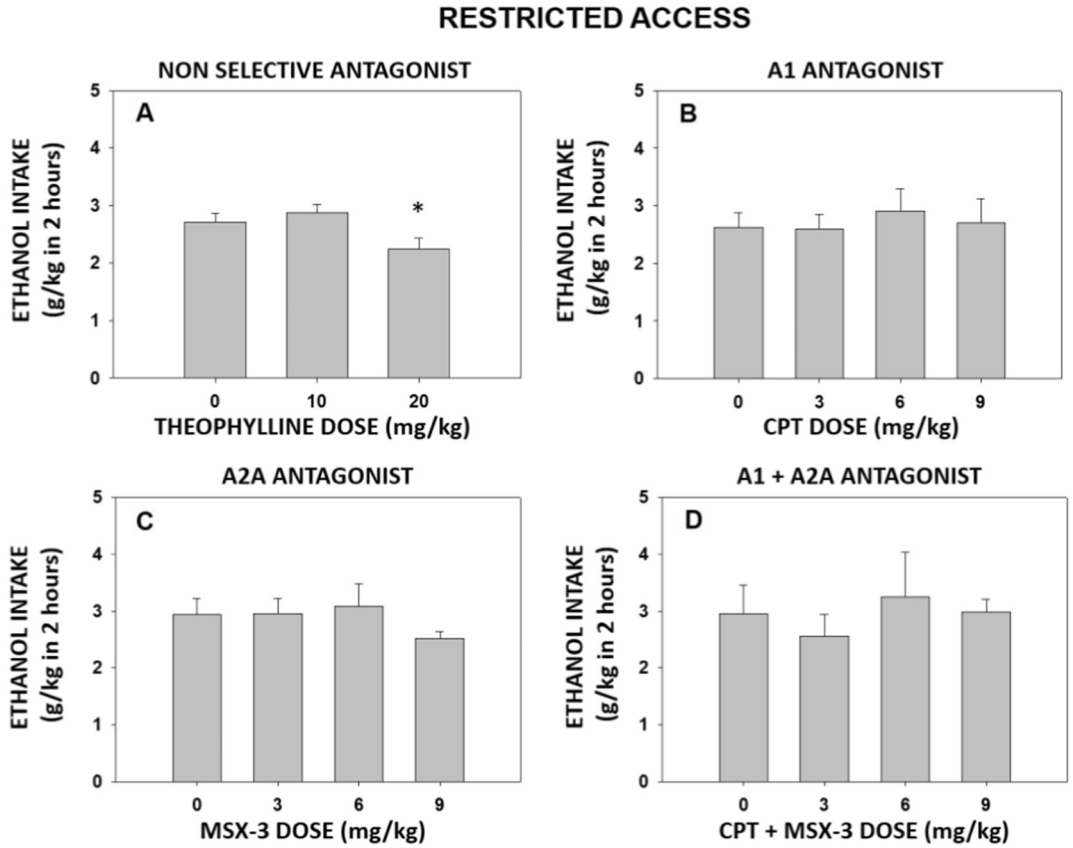

Fig. 2. Effect of theophylline (A), CPT (B), MSX-3 (C) and CPT + MSX-3 (D) on ethanol intake in a 2 h restricted access paradigm. Mean ± S.E.M. g/kg of ethanol consumed. *p < 0.05 significantly different from vehicle.

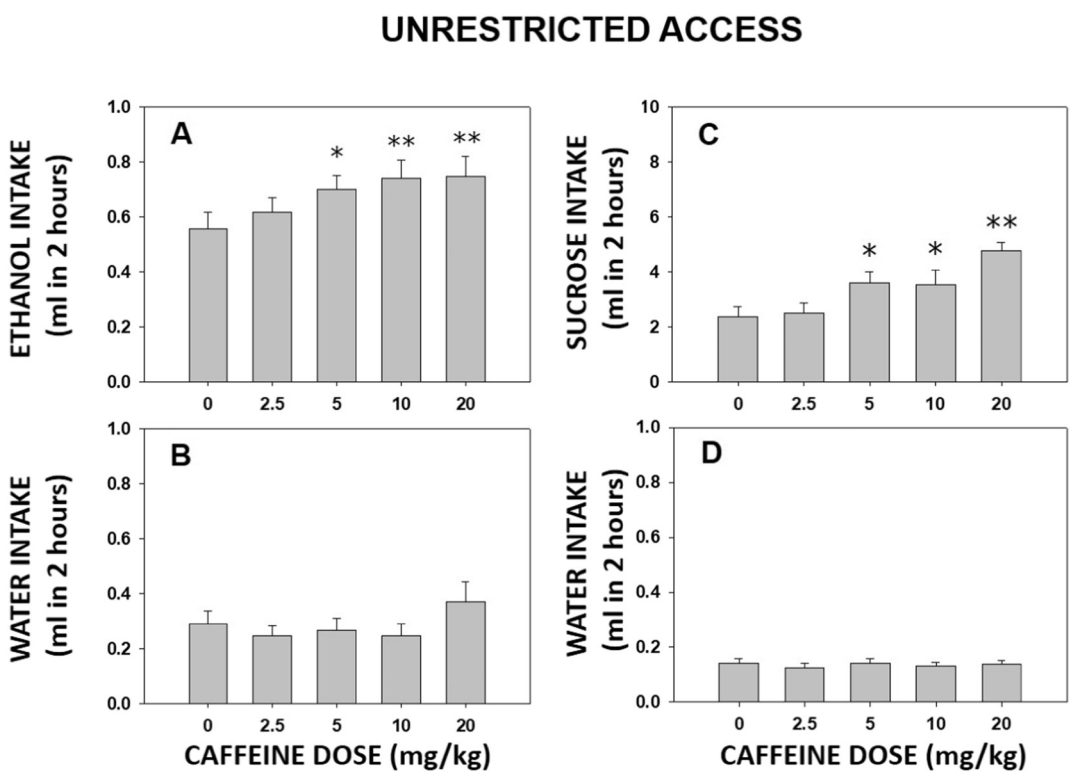

Fig. 3. Effect of caffeine on concurrent ethanol or water intake (A, B), or on concurrent sucrose or water intake (C, D), during the first 2 h of the dark cycle under non-restricted-24 h access conditions. Mean ± S.E.M. ml of fluid consumed. *p < 0.05, **p < 0.01 significantly different from vehicle.

during 24 h ($F(2,27) = 96.504$; $p < 0.05$).

After administering caffeine to the 3 baseline groups (Fig. 6B–D), the repeated measures ANOVA for the low consumers did not show a significant effect on ethanol intake ($F(4,28) = 0.60$; n.s), and the same was true for the high consumers group ($F(4,28) = 1.29$; n.s.). However, repeated measures ANOVA showed that there was a significant effect of caffeine on the moderate group ($F(4,52) = 3.00$; $p < 0.05$), and planned comparisons revealed that all doses increased significantly ethanol intake (2.5 and 20 mg/kg $p < 0.05$; 5.0 and 10.0 mg/kg, $p < 0.01$).

3.6. Experiment 6. Effect of caffeine on ethanol reinstatement

In order to see if caffeine had any impact on ethanol intake during repeated episodes of time-off and reintroduction (see Fig. 7 for a schematic of the procedure), saline or caffeine (5.0 mg/kg) were administered to two different groups of mice (n = 20 total) on the first day of every reintroduction cycle, and ethanol consumption was evaluated during 24 h. Animals with moderate levels of ethanol consumption were selected. Average baseline (BL) intake (before the first injection started) was similar for both groups; saline or caffeine.

The two-way ANOVA (time × treatment) for ethanol intake during the first 2 h revealed a significant effect of time ($F(12,216) = 2.76$; $p < 0.01$), but no significant effect of caffeine treatment ($F(1,18) = 0.47$; n.s.), and no interaction ($F(4,72) = 1.216$; n.s.) (Fig. 8A). However, the two-way factorial ANOVA (time vs drug) for ethanol intake during 24 h after the injection showed a significant effect of time ($F(12,216) = 2.43$; $p < 0.01$), no significant effect of treatment ($F(1,18) = 0.02$; n.s.), but a significant interaction ($F(12,216) = 2.48$; $p < 0.01$). Planned comparisons between the corresponding BL group and the reintroduction day showed a significant change in ethanol intake in the group that received caffeine. The group treated with caffeine showed significant differences from its own baseline on the first and second day of the second ethanol reinstatement cycle ($p < 0.01$, $p < 0.05$ respectively), and on every day of the third ethanol reinstatement cycle ($p < 0.01$ for the first two days; and $p < 0.05$ for the second first days). Within the saline group there was a significant difference between baseline levels of ethanol intake and the first day of the second ethanol reinstatement cycle ($p < 0.05$) (Fig. 8B).

4. Discussion

In the present study, we investigated the effect of caffeine on voluntary ethanol intake under different parameters of access: restricted, unlimited, or unlimited after intermittent time-off, in the alcohol preferring C57BL/6 J strain of mice. The restricted access procedure facilitates the amount of drinking, taking advantage of the innate tendency of rodents to consume higher levels of food and drink solutions during the dark phase of the circadian cycle (Rhodes et al., 2005; Rodríguez-Ortega et al., 2018). Consistent with previous research in outbred rats (Dietze and Kulkosky, 1991), the highest dose of caffeine administered in the present study (20.0 mg/kg) decreased voluntary alcohol consumption although not sucrose.

While the DID paradigm explores high levels of ethanol consumption over a short period of time, other procedures such as the unlimited 24 h access can give information about how animals regulate ethanol intake when other solutions are present, and can give information about individual differences in this regulation. Thus, the effect of caffeine was also studied when animals had no time restriction. Caffeine dose dependently (5.0–20.0 mg/kg) increased ethanol intake during the first two hours of unlimited access, and this effect was also seen with sucrose intake. Thus, it seems that caffeine can have different, and even opposite, effects on ethanol or sucrose intake depending on the access conditions; when animals drink high volumes of ethanol (around 0.8 ml) or sucrose (around 4.0 ml) because it is the only time and the only solution available (i.e. in the DID procedure), caffeine did not change or tended to reduce consumption. Moreover, the present results reveal a strong difference in the reinforcing value of both solutions: the volume of ethanol consumed is around 1/4th of that of sucrose in both type of access conditions. Thus, it is possible that the subtle, but

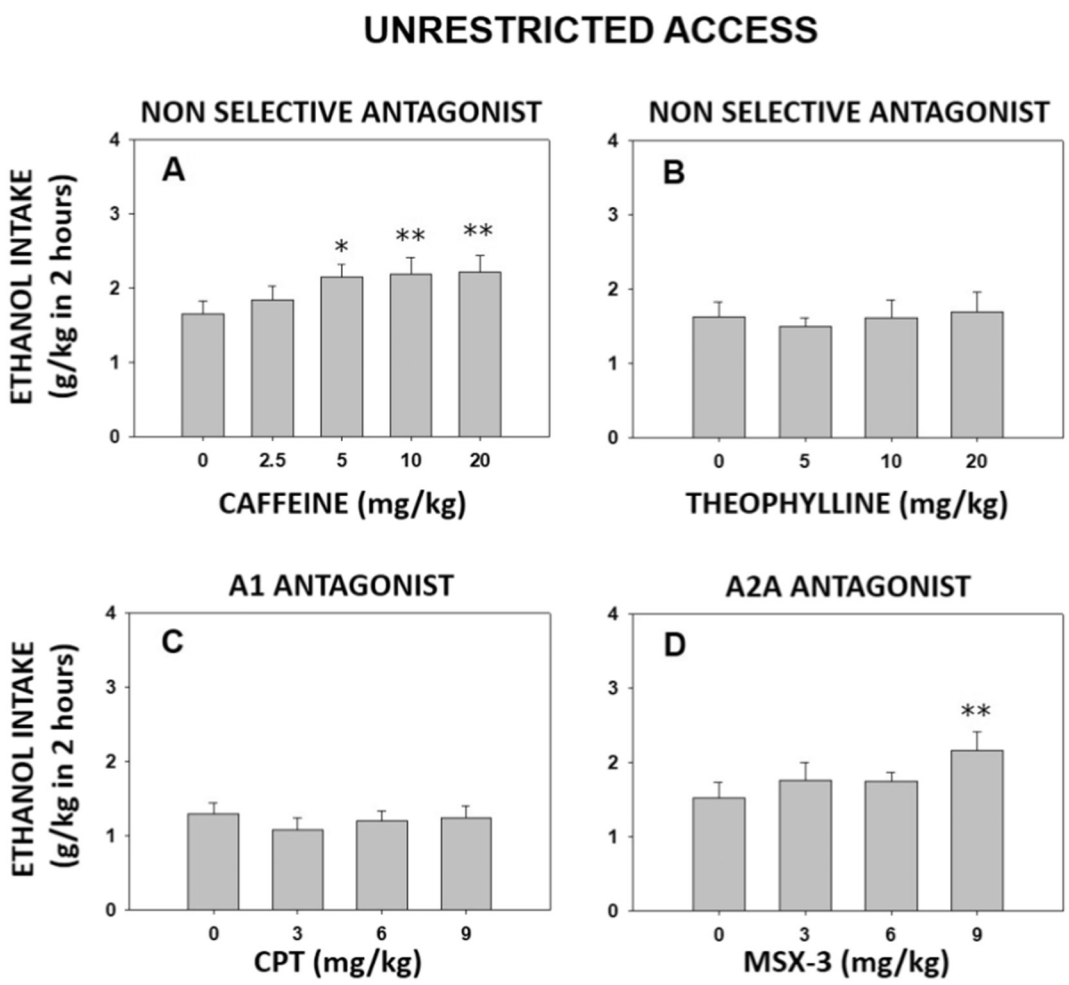

Fig. 4. Effect of caffeine (A), theophylline (B), CPT (C) and MSX-3 (D) on ethanol intake during the first 2 h of the dark cycle in a 24 h access condition. Mean ± S.E.M. g/kg of ethanol consumed. *p < 0.05, **p < 0.01 significantly different from vehicle.

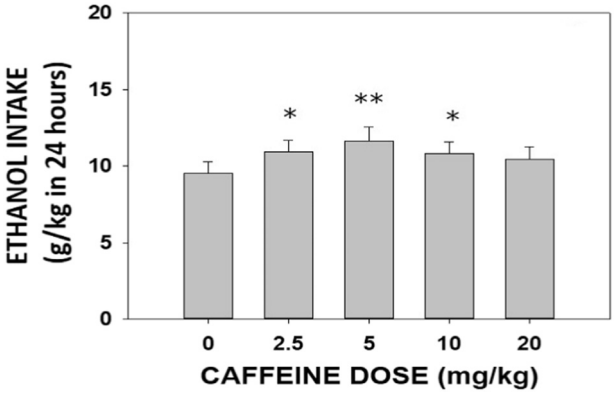

Fig. 5. Effect of caffeine on voluntary ethanol intake during 24 h under unrestricted access conditions. Mean ± S.E.M. g/kg of ethanol consumed. *p < 0.05, **p < 0.01 significantly different from vehicle.

significant, effect of the high dose of caffeine on ethanol intake under restricted access, is not effective enough when sucrose consumption is so high. In support of this possibility, although 20.0 mg/kg of caffeine did not affect sucrose intake in the DID procedure, in a pilot study with 40.0 mg/kg, caffeine did reduce both ethanol as well as sucrose intake. This non-specific reduction of intake was seen also in rats eating standard food or saline solutions after high doses of caffeine (40.0–50.0 mg/kg) (Dietze and Kulkosky, 1991; Nadal et al., 1995). Thus, it seems that high doses of caffeine can have anorectic properties, in a similar way that other psychostimulants do (Cousins et al., 1994). However, when ethanol (or in other groups sucrose) is constantly present and mice have water as an alternative drinking solution, they drink less in two hours (around 0.5 ml of ethanol in one experiment, and 2 ml of sucrose in the other) and thus, caffeine can more easily increase the amount of ethanol that animals consume. These data on sucrose are consistent with previous results from our laboratory using a different strain of mice and a different type of sucrose delivery (pellets); animals given one of the doses used in the present study (20 mg/kg) ate more sucrose pellets (Correa et al., 2018). Taking all these results into consideration, and acknowledging the strong difference in the reinforcing value of both type of reinforcers (10% ethanol vs 5% sucrose solutions), it still seems that caffeine, at high enough doses, changes patterns of consumption that are dependent on the access condition (restriction, effort, novelty, etc) (Correa et al., 2018). Although in previous studies with CD1 mice, we have not detected a change in ethanol content in blood after caffeine administration (López-Cruz et al., 2014), in the present results we cannot rule out the possibility that the effects of caffeine might be related to the changes in ethanol metabolism or clearance of caffeine.

The studies involving additional adenosine receptor antagonists indicate that theophylline (another methylxanthine, with A_1 and A_{2A} adenosine receptor activity) also decreased voluntary ethanol intake at the highest dose used (20.0 mg/kg) in the DID procedure, but did not produce any effect on sucrose consumption in this procedure (supplemental material Fig. 1), which is consistent with the caffeine results. However, this methylxantine had no impact under unrestricted access

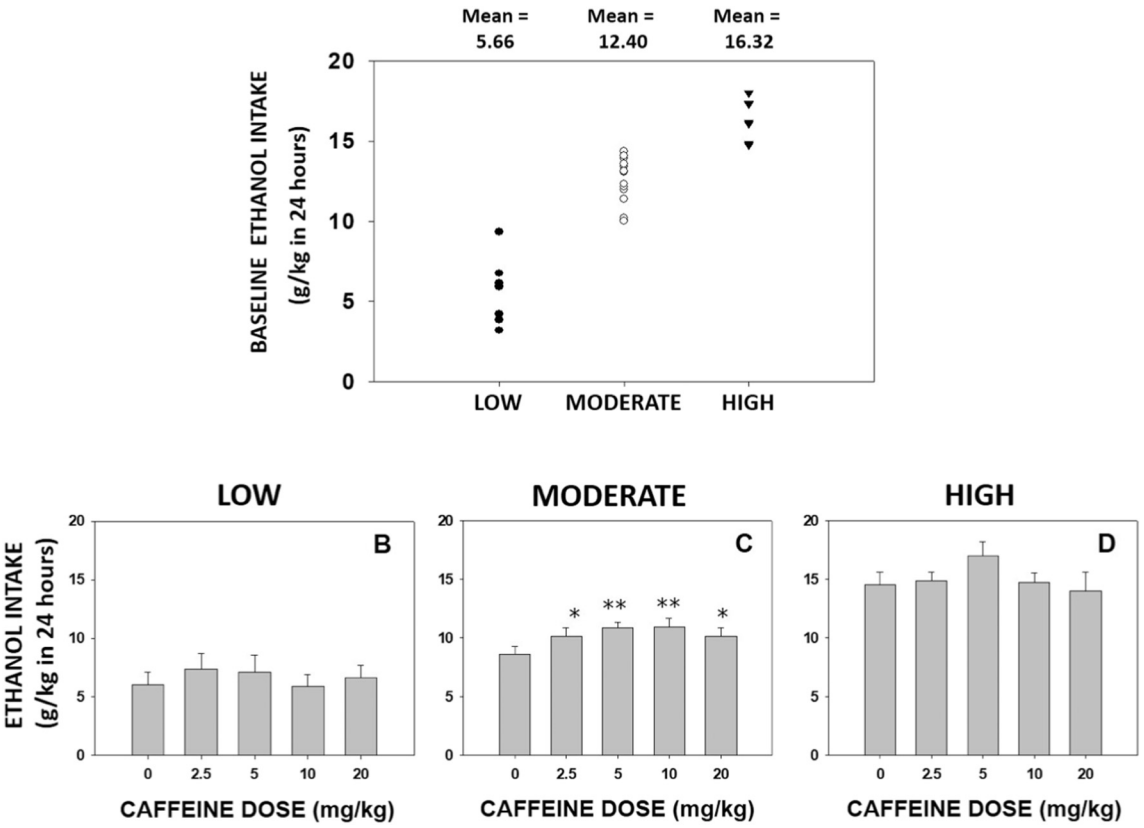

Fig. 6. Individual average scores of 3 days of 24 h-baseline ethanol intake (g/kg) (A). Mice were divided in three groups (low, moderate and high ethanol consumers) using quartiles. Effect of caffeine on voluntary ethanol intake among low (B), moderate (C), and high (D) subgroups of ethanol drinkers. Mean ± S.E.M. g/kg of ethanol consumed. *$p < 0.05$, **$p < 0.01$ significantly different from vehicle.

conditions probably showing less effectiveness than caffeine. Previous research in our laboratory has shown that theophylline produces behavioral changes in mice at the same dose range that caffeine, although it seems to be less efficacious than caffeine (López-Cruz et al., 2014). Thus, although both methylxantines produce anxiogenic and ataxic effects at high doses (higher than the present doses), a reduction of ethanol intake but not sucrose intake, does not clearly support these side effects as responsible for ethanol intake reduction. On the other hand, in rats, subchronic administration of theophylline increased ethanol consumption that was available concurrently to water under

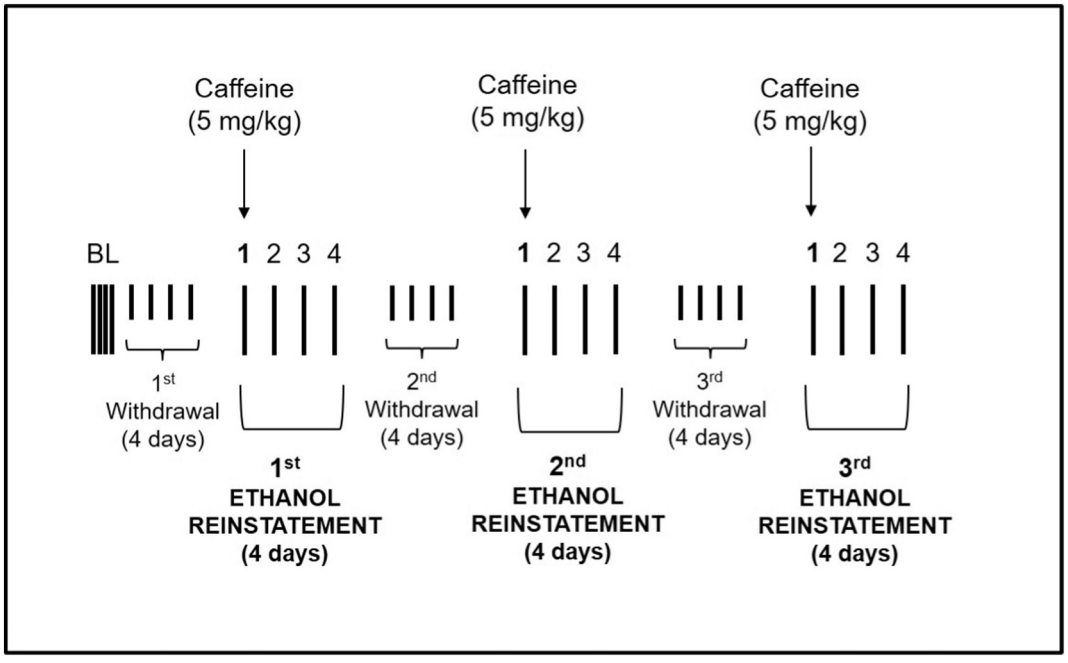

Fig. 7. Schematic diagram of the ethanol reinstatement procedure.

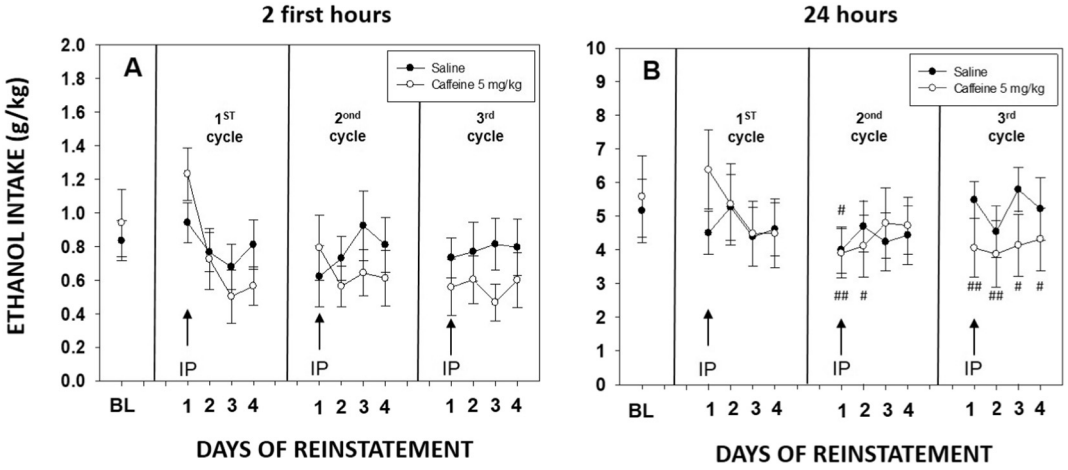

Fig. 8. Effect of caffeine on voluntary ethanol intake in the first 2 h of the dark cycle (A), or after 24 h (B) during repeated cycles of 4 days of ethanol removal and reintroduction. Mean ± S.E.M. g/kg of ethanol consumed. #p < 0.05, ##p < 0.01 significantly different from baseline (BL) in each treatment group.

unrestricted conditions (Gatch and Selvig, 2002). Additionally, because pharmacological actions of caffeine and methylxantines are attributable to its activity as a non-selective A_1 and A_{2A} adenosine receptor antagonist (Fredholm et al., 1999; Ferré et al., 2018), we studied the effect of selective adenosine receptor antagonists on voluntary ethanol intake under restricted and unrestricted access conditions. In the DID experiment, administration of CPT, an A_1 adenosine receptor antagonist, or MSX-3, an A_{2A} adenosine receptor antagonist, did not exhibit any effect on ethanol intake, neither separately nor in combination. The lack of effect of MSX-3 in the DID paradigm is consistent with a previous study (Fritz and Boehm, 2015). However, in that study the A_1 receptor antagonist DPCPX was found to dose-dependently decrease binge-like ethanol intake (Fritz and Boehm, 2015) in a similar manner to caffeine and theophylline in the present results. Contrary to what was expected, a range of combined doses of an A_1 plus an A_{2A} receptor antagonist (trying to mimic the mechanism of action for methylxantines), did not change ethanol consumption in the present study. Similarly, in an operant behavior study, the A_1 receptor antagonists DPCPX given in combination with the adenosine A_{2A} receptor antagonist SCH 58261 had no effect in alcohol-preferring rats trained to self-administer alcohol in operant boxes (Adams et al., 2008).

In the unrestricted access condition, only the A_{2A} receptor antagonist increased ethanol intake similarly to caffeine. A_{2A} receptor antagonists have been clearly implicated in modulating ethanol intake. Thus, data from free (non-operant) access to ethanol indicate that A_{2A} knockout mice tested in a 48 h access two-bottle choice task displayed higher ethanol intake than WT mice (Naassila et al., 2002). Similarly, acute administration of the A_{2A} receptor antagonist ANR94 increased ethanol intake in alcohol-preferring rats (Micioni Di Bonaventura et al., 2012). The C57BL/6 J strain of mice used in the present experiments can be considered an ethanol preferring strain. However, the adenosine A_1 receptor antagonist CPT did not produce any effect. Thus, all these results seem to indicate that the increase intake observed after caffeine administration under unrestricted access conditions could be mediated by actions on A_{2A} receptors.

Additionally, because 24 h free access allows the expression of individual differences we decided to analyze the data from that test taking into account differences in basal ethanol intake. Although caffeine had a biphasic effect on ethanol intake when taking into account all the animals, when the analyses used the two extreme quartiles as the statistical criteria to divide animals into high, middle and low ethanol consumers, we did not find any impact of caffeine among the low or high consumers. There was no effect of caffeine on 24 h water intake in any of the groups. Nevertheless, animals that consume moderate levels of ethanol were significantly affected by caffeine. Whether the moderate-consuming mice increase ethanol intake because caffeine increases they motivation for seeking alcohol or decreases they perception of their limit tolerance to alcohol, is still unresolved question. Moreover, it is possible that low consuming animals do not increase ethanol consumption because of some side effect such as anxiogenic actions of caffeine, and probably caffeine could not increase intake among the high consumers due to a ceiling effect. Further studies should address all those possibilities, and should increase the number of animals used in the two extreme groups.

A predominant feature in human alcohol abuse is the reported desire or “craving” to consume ethanol, along with frequent episodes of drinking after periods of abstinence. These and other factors may be responsible for the relapse to uncontrolled ethanol drinking (Heyser et al., 1997). When relapse occurs after a period of abstinence, ethanol drinking temporarily increases, a phenomenon known as the “alcohol deprivation effect” (Sinclair, 1979; Heyser et al., 1997). In the present study, we examined the impact of an acute caffeine injection after several cycles of forced time-off on ethanol reinstatement in an unrestricted access paradigm. Our results show that, after the second time-off cycle, caffeine (5.0 mg/kg) reduced ethanol consumption compared to BL, even during days when caffeine was not administered. Thus, the impact of caffeine emerged after repeated cycles. Our results do not show the alcohol deprivation effect after the first 4 days of ethanol removal, probably due to the fact that our animals did not show signs of dependence during withdrawal because their levels of intake were moderate. Thus, consistent with previous data in rats (Rezvani et al., 2013; Okhuarobo et al., 2018), our data in mice show that caffeine administered after ethanol removal can prevent ethanol reinstatement, at least among moderate consumers.

In summary, the present results using acute administration of caffeine, show that when ethanol consumption is very high (in the DID paradigm and among the high consumers in the free access paradigm) caffeine does not increase ethanol consumption, and even tends to reduce it. In addition, among low consumers, caffeine does not increase ethanol intake either. Thus, only among moderate consumers that have free access to ethanol and have other sources of fluid (water), caffeine does increase ethanol consumption, and that effect seems to be mediated by adenosine A_{2A} receptors. However, these present studies address only a way in which caffeine can affect ethanol consumption. Since caffeine is consumed normally in a repeated manner, further studies should address the impact of how chronic administration of caffeine (from moderate to high levels) can affect ethanol intake.

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Declaration of competing interest

Authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pbb.2019.172789.

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