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Effect of caffeine on alcohol consumption and alcohol-induced conditioned place preference in rodents

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Abstract

Background: The aim of the study was to determine the effect of caffeine on alcohol consumption with or without deprivation and alcohol-induced conditioned place preference.

Methods: In the present study, we examined the effects of caffeine (2.5, 5 and 10 mg/kg) on alcohol consumption in Wistar rats with or without periods of deprivation in an unlimited-access, two-bottle, free choice drinking procedure after a stable baseline alcohol consumption was established. Conditioned place preference (CPP) was established by intraperitoneal injections of alcohol (2 g/kg) in a 12-day conditioning schedule in mice. The effect of caffeine (3 mg/kg) on CPP expression was determined by a final post-conditioning test following 12 conditioning sessions with alcohol. The effect of caffeine (3 mg/kg) on the reinstatement of alcohol-induced CPP was determined in a final post-conditioning test following 12 conditioning sessions with alcohol and the extinction of alcohol-induced CPP.

Results: Alcohol deprivation for 3 days did not result in alcohol deprivation effect (ADE). While caffeine (10 mg/kg) caused a significant ($p < 0.05$) reduction in alcohol consumption compared with the baseline following a period of alcohol deprivation, it did not cause a change in alcohol consumption compared with the baseline in the study without alcohol deprivation phase. Caffeine significantly ($p < 0.05$) reduced the expression of alcohol-induced CPP compared to saline and blocked the reinstatement of alcohol-induced CPP following the injection of a priming dose (0.4 g/kg) of alcohol.

Conclusions: Given that caffeine is an adenosine receptor antagonist, our findings suggest a role for adenosine receptors in the alcohol reward and alcohol-seeking behaviour.

Keywords: alcohol reward; caffeine; conditioned place preference; deprivation.

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Introduction

Alcohol addiction, commonly referred to as alcohol use disorder, is a chronic relapsing disorder caused by prolonged excessive alcohol use and is often accompanied by health and socio-economic burdens. It is the plague-like recurring end of a process that starts with the pleasures of the rewarding effects of alcohol. Thus, alcohol reward is important in the progression from controlled or occasional alcohol use to compulsive alcohol use (addiction) as well as return to addiction after a period of abstinence (relapse) [1, 2]. The desire to elicit the pleasures of alcohol rewarding effect (craving) often culminates in relapse, and a relapse to alcohol-seeking behaviour, on its own, often frustrates both pharmaco-therapeutic and psycho-therapeutic approaches to alcohol addiction treatment [1]. This underscores the need for more research efforts geared towards a better understanding of the processes involved in alcohol reward and relapse.

One of the commonly used models to study alcohol relapse in the laboratory is the alcohol deprivation effect, which is an increase in alcohol consumption after a period of forced abstinence (deprivation) [3]. The rewarding effects of alcohol can be measured in the different phases of the place conditioning paradigm (i.e. acquisition, expression and reinstatement) and each phase mimics a specific aspect of alcohol addiction, such as acquisition for initiation to drink, expression for craving and reinstatement for relapse. Thus, this model offers the advantage of studying the rewarding effects of alcohol and the aspects of alcohol addiction [4, 5].

Self-administered psychoactive substances, when administered together with alcohol, have resulted in interactions with important implications for alcohol rewarding

effects [6, 7]. Similarly, certain self administered psychoactive drugs can cause the reinstatement of alcohol use after a period of abstinence [7, 8]. Alcohol and caffeine are among the three most widely used self-administered psychoactive drugs for recreational purposes and both are often co-ingested. It is a common trend nowadays among alcohol drinkers to mix caffeinated energy drinks or beverages with alcohol [9].

Aside from this trend, empirical experimental evidence exists to justify a continued need to probe the effect of caffeine on alcohol use or alcohol-seeking behaviour [9, 10]. For instance, the activation of adenosine receptors [11] has been observed on account of an increase in extracellular levels of adenosine due to the inhibition of the adenosine re-uptake, following alcohol ingestion [12]. The modulation of the release of dopamine by adenosine receptors pathways, which is crucial in the rewarding effect of alcohol, has also been demonstrated. Another study reported that dopamine transmission is inhibited by the activation of adenosine A2 receptors and the administration of caffeine which is an antagonist to both the adenosine A1 and A2 receptors causing a surge in dopamine release [13]. Furthermore, treatment with the adenosine A2 receptor antagonist 3,7 –dimethyl-propargylxanthine, SCH58261, DMPX and adenosine A1 receptor antagonist DPCPX have been demonstrated to reduce alcohol-seeking behaviour, thereby suggesting a role for adenosine receptors in alcohol reward. Similarly, a high dose of caffeine has been demonstrated to cause a reduction in alcohol intake and preference, whereas a low dose increased alcohol intake and preference during free access to alcohol in rats [9]. Such collective evidence suggests a possible interactive pharmacology between alcohol rewarding effects and caffeine as well as the adenosine pathways in the brain.

Considering such evidence, the widespread consumption of alcohol and caffeine as well as the common practice of mixing alcohol and caffeinated beverages, it has become necessary to sustain the study of the interactive effects of caffeine on alcohol-induced reward and relapse to alcohol use, especially with models that have received little attention for this purpose. While the effect of caffeine on alcohol-induced reward modelled as oral alcohol self-administration or consumption without a period of deprivation in rodents has received some attention, the effect of caffeine on relapse modelled as increased in alcohol consumption following a period of deprivation (alcohol deprivation effect) has received little attention. Similarly, the effect of caffeine on the aspects of alcohol-induced conditioned reward using the condition place preference paradigm has received no attention.

Therefore, the present study was conducted with the aim of investigating the effects of caffeine on alcohol consumption (following a period of deprivation in rats) and on the acquisition, expression and reinstatement of alcohol-induced conditioned place preference (CPP) in mice.

Materials and methods

Animals

Adult male Wistar rats and Swiss albino mice weighing between 150–250 g and 15–30 g, respectively, were used in the experiments. The rats were housed individually, while the mice were grouped and housed in cages with open-top wire racks in the Animal House Unit, where they had access to feed and water ad libitum. The Animal House Unit is under the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Nigeria. The animals were maintained on a 12:00/12:00 h light/dark cycle and allowed to acclimatize for a period of 7 days prior to the commencement of experiments. All the animals were handled in accordance with the international principles guiding the use and handling of experimental animals (National Institute of Health, USA, 2002).

Drugs

Caffeine was obtained from Lobal Chemie Laboratory Reagents and Fine Chemicals (India) and alcohol was obtained from Guangzhou Jhd Chemical Reagent Co., Ltd (China). Alcohol (10%v/v) was prepared by diluting 95% v/v in 0.9% normal saline. Caffeine was dissolved in 0.9% saline and injected between 8 am and 9 am for all experiments. When combined, caffeine and alcohol were administered in the same injection. All drugs were given through the intraperitoneal (IP) route.

Effect of caffeine on alcohol consumption following a period of alcohol deprivation

The Wistar rats housed in individual cages were exposed to a solution of 10% v/v alcohol for 3 consecutive days to initiate drinking, after which they were given free choice between water and 10% v/v alcohol contained in separate bottles for 24 h every day. The position of the bottles was alternated on a daily basis to control for side preferences. Following the exhibition of stable and reliable alcohol intake for several weeks, the rats were deprived of the alcohol solution for 3 days. During this period, the rats had access to two bottles containing water. On the next day following the period of deprivation, the rats were injected with caffeine (2.5, 5 or 10 mg/kg) or saline 15 min before being given free choice between water and alcohol and after 24 h [9] Alcohol consumption was measured by subtracting the volume remaining in the bottle after 24 h following each experiment from the volume of alcohol contained in the bottle (100 mL) at

the beginning of each experiment. A graduated measuring cylinder was used for the volume measurement. Spillage and evaporation were accounted for by placing two bottles, one containing alcohol (10% w/v) and the other water, in a separate cage that did not have any rats. The contents of these bottles were also measured just like the bottles in cages that had rats.

Effect of caffeine on alcohol consumption without a period of alcohol deprivation

The Wistar rats housed in individual cages were exposed to a solution of 10% v/v alcohol for 3 consecutive days to initiate drinking, after which they were given free choice between water and 10% v/v alcohol contained in separate bottles. The position of the bottles was alternated on a daily basis to control for side preferences. Following the exhibition of very low but stable and reliable alcohol intake for several weeks, the rats were injected with caffeine (2.5, 5 or 10 mg/kg) or saline 15 min before being given free choice between water and alcohol after 24 h [9]. Alcohol consumption was measured by subtracting the volume remaining in the bottle after 24 h following each experiment from the volume of alcohol contained in the bottle (100 mL) at the beginning of each experiment. A graduated measuring cylinder was used for the volume measurement. Spillage and evaporation were accounted for by placing two bottles, one containing alcohol (10% w/v) and the other water, in a separate cage that did not have any rats. The contents of these bottles were also measured just like the bottles in cages that had rats.

Conditioned place preference apparatus

Place preference conditioning was carried out as described in [14] with little modification. The CPP apparatus was made of ply board with the following dimensions: 45 (L) cm × 15 (W) cm × 15 (H) cm. The apparatus was divided into two compartments (20 cm × 15 cm × 15 cm) of equal size and a small central grey compartment (5 cm × 15 cm × 15 cm). One of the compartments was black with a smooth floor made of formica, while the other compartment was white with a rough floor made of aluminium wire mesh. This was done to achieve distinct visual and tactile cues in the two compartments. Removable doors made of plywood were used to close off the entrance to each of the compartments from the central compartment. The sides of the central compartment were made of glass for easy detection of crossing mice from the black to the white compartment and vice versa. The CPP procedure was divided into three phases: habituation, pre-conditioning and post-conditioning (Figure 1). All experiments were performed during the same time period each day (09.00–17.00 h) [5, 10].

Habituation/preconditioning

Habituation to the CPP apparatus was conducted for 3 days. On each day of habituation, the removable doors were removed from both compartments, after which each mouse was placed in the small central compartment and allowed to roam freely throughout the compartment for 15 min. The compartments were cleaned and de-odorised with hydrogen peroxide before the introduction of the next mouse. On the day following the completion of habituation, the pre-conditioning test was performed in the same manner as the habituation, except that the time spent in either the black or white compartment was recorded and this served as the pre-conditioning baseline. Each mouse was immediately returned to its cage following the completion of each test. Any mouse that displayed a strong unconditioned aversion (<33% of the session time spent in one side of the compartment during precondition) or preference (>66% of the session time spent in one side of the compartment during precondition) for any compartment was excluded from the study [5, 10].

Conditioning

Daily conditioning sessions that lasted 30 min commenced the next day after the preconditioning session and lasted for 12 consecutive days. The mice received saline (10 mL/kg) or alcohol (2 g/kg) via IP injections on alternate days and in alternate compartments (black and white). Each mouse received alcohol in the compartment it least preferred during the pre-conditioning session and received saline in the most preferred compartment. Alcohol administration was done on days 1, 3, 5, 7, 9 and 11 and saline on days 2, 4, 6, 8, 10 and 12 of the conditioning phase. The mice in the saline control group received saline in both compartments on alternate days, such that saline was received in the black compartment on even day and in the white compartment on odd days for 12 days. During the conditioning days, each mouse was restricted to the appropriate compartment for 30 min following saline or alcohol injection. The mice were then returned to their home cage after this time [5, 10].

Post-conditioning

The post-conditioning test was performed 24 h following the last conditioning session on day 13. The removable doors sealing the two compartments from the central compartment were removed. Each mouse was placed in the central grey compartment and allowed to explore freely throughout the apparatus for 15 min, as described for the preconditioning test, and the time spent in each compartment was recorded. The CPP (conditioning scores) in seconds were calculated by subtracting the time spent by each mouse in the alcohol-paired compartment during the post-conditioning sessions from the time spent in the same compartment during the pre-conditioning sessions. A positive value from subtraction was taken as CPP, whereas a negative value was taken as conditioned place aversion (CPA) [5, 10].

The effect of caffeine on the acquisition of alcohol-induced conditioned place preference

To achieve this, separate groups of mice were conditioned with a combination of alcohol (2 g/kg) and caffeine (3 mg/kg) via IP injection or alcohol (2mg/kg) via IP injection, as described earlier [10].

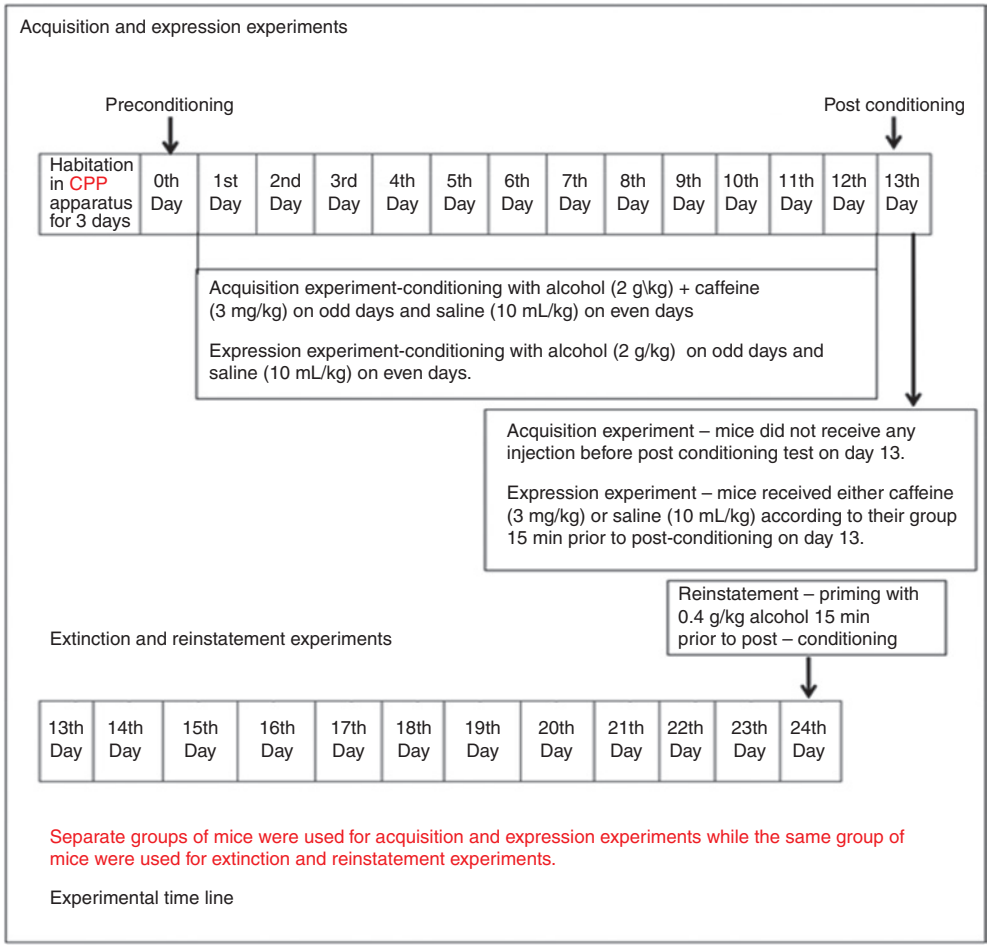

Figure 1: The experimental time line and manipulations.

The effect of caffeine on the expression of alcohol-induced conditioned place preference

To achieve this, separate groups of mice (n = 5–7) received IP injections of caffeine (3 mg/kg) or saline (10 mL/kg) 24 h following the last conditioning session (day 13), and 15 min prior to the post-conditioning test [10].

The effect of caffeine on the alcohol priming re-instatement of alcohol-induced conditioned place preference

After 24 h following the post-conditioning test, the mice were made to observe daily CPP tests, in which each mouse was allowed to explore freely throughout the apparatus for 15 min, as described earlier until the preference for the alcohol-paired compartment became similar to that obtained during the pre-conditioning session. At this point, alcohol-induced CPP was said to have been extinguished or abolished. The day following the last extinction session, separate groups of mice received either saline or caffeine (3 mg/kg, IP) 15 min before a priming injection of a low dose of alcohol (0.4 g/kg, IP). About 15 min following the priming injection of alcohol, the mice were allowed free access to roam both compartments for another 15 min, and the time spent in each compartment was recorded [10].

Statistical analysis

Statistical analysis was done using graph pad prism 4. The data were analyzed using unpaired t-test, one and two way analysis of variance (ANOVA), followed by Turkey's post hoc test. Data represent mean ± SEM. Statistical significance was considered at $p < 0.05$.

Results

The effect of caffeine on alcohol consumption following a period of deprivation

ANOVA revealed an overall effect of caffeine treatment (IP) on alcohol consumption ($F(3, 31) = 5.582$, $p < 0.0035$). There was no significant difference in alcohol consumption post-deprivation following injection with 4 mL/kg saline, 2.5 and 5 mg/kg caffeine compared with their respective baseline consumption. However, 10 mg/kg caffeine injection significantly ($p < 0.05$) reduced alcohol consumption post-deprivation compared with the baseline consumption. In

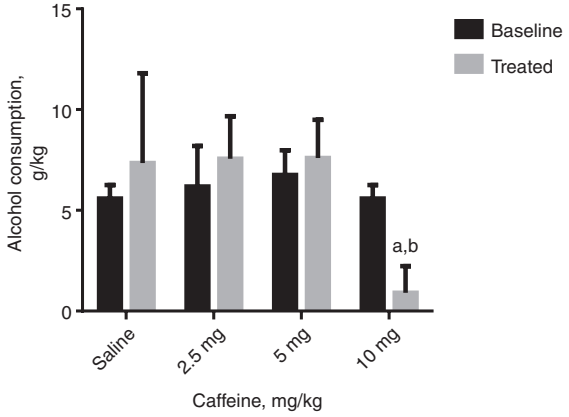

Figure 2: The effect of the intraperitoneal injection of caffeine on alcohol consumption following deprivation.
The values represents mean ± SEM. [a]$p < 0.01$ vs. saline, 2.5 mg and 5 mg caffeine injections, [b]$p < 0.05$ vs. baseline alcohol consumption of 10 mg/kg caffeine-treated rats.

addition, rats injected with 10 mg/kg of caffeine showed a significant ($p < 0.01$) reduction in alcohol consumption post-deprivation compared with those injected with saline and the other rats injected with 2.5 and 5 mg/kg of caffeine (Figure 2).

The effect of caffeine on alcohol consumption without a period of deprivation

ANOVA revealed an overall effect of caffeine treatment (IP) on alcohol consumption ($F(3, 28) = 4.807$, $p < 0.0080$). There was no change in alcohol consumption following saline injection compared with the baseline consumption. On the one hand, caffeine (2.5 mg/kg) caused a change in alcohol consumption that was not statistically significant compared with the baseline. On the other hand, while the rats treated with caffeine (5 mg/kg) showed a significant ($p < 0.05$) increase in alcohol consumption compared with their baseline consumption and the consumption of saline-treated rats, caffeine-treated (10 mg/kg) rats did not show a change in alcohol consumption compared with their baseline consumption and that of saline-treated rats, but showed a significant decrease in alcohol consumption compared with the alcohol consumption of caffeine-treated (5 mg/kg) rats (Figure 3).

The effect of caffeine on the acquisition of alcohol-induced conditioned place preference

The one-way ANOVA revealed an overall effect of caffeine treatment (IP) on the acquisition of alcohol-induced CPP ($F(2, 11) = 23.27$, $p < 0.0001$). The conditioning sessions with alcohol alone and in combination with caffeine caused a significant ($p < 0.001$) increase in time spent in the alcohol-paired compartment (acquisition of conditioned place preference) compared with saline during the post-conditioning session. The saline-treated mice spent less time in the alcohol-paired compartment during the post-conditioning session. This resulted in a negative value after subtraction from the preconditioning scores, indicating a conditioned place aversion for the compartment. In comparison, the acquisition of CPP as indicated by the time spent in the alcohol-paired compartment, caused by a combination of

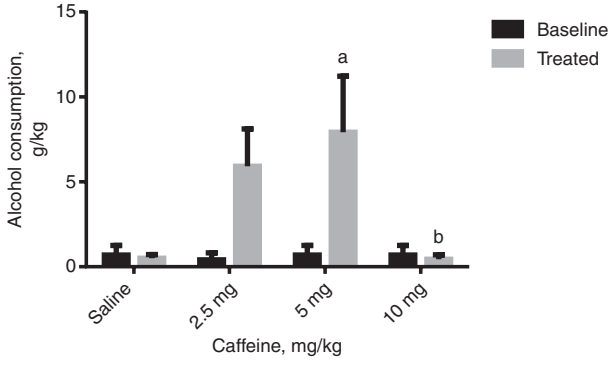

Figure 3: The effect of the intraperitoneal injection of caffeine on alcohol consumption without deprivation.
The values represent mean ± SEM (n = 5). [a]$p < 0.05$ vs. baseline alcohol consumption of 5 mg/kg caffeine-treated rats and alcohol consumption of saline-treated rats, [b]$p < 0.05$ vs. alcohol consumption of 5 mg/kg caffeine-treated rats.

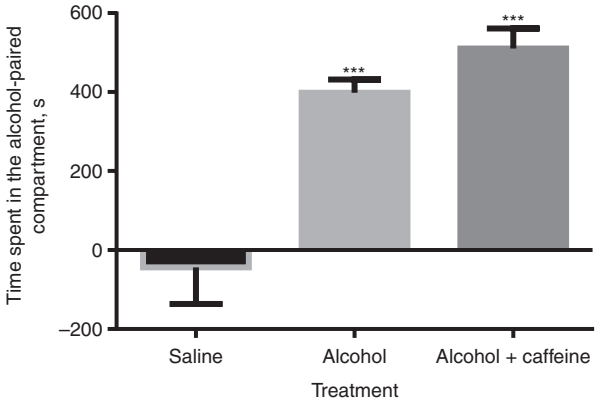

Figure 4: The effect of caffeine (3 mg/kg) on the acquisition of alcohol induced conditioned place preference.
The values represent mean ± SEM (n = 5–7). ***$p < 0.001$ vs. saline. Alcohol vs. alcohol + caffeine ($p = 0.09$).

alcohol and caffeine, was not significantly different from that caused by alcohol alone (Figure 4).

The effect of caffeine on the expression of alcohol-induced conditioned place preference

The results of the Student's t-test demonstrated that ($t = 2.938$, $df = 9$ $p < 0.0165$) the mice injected with caffeine (3 mg/kg) before the post-conditioning test exhibited a significant ($p < 0.05$) reduction in the expression of CPP, as indicated by a reduction in the time spent in the alcohol-paired compartment (CPP) compared with the saline-injected mice following 12 conditioning sessions with alcohol (Figure 5).

The effect of caffeine on the low-dose priming reinstatement of alcohol-induced conditioned place preference.

The two-way ANOVA revealed an overall effect of caffeine and saline on the reinstatement of alcohol-induced CPP ($F(1, 20) = 8.929$, $p < 0.0073$). Caffeine blocked the reinstatement of extinct alcohol-induced CPP following the priming injection of low-dose alcohol (0.4 mg/kg), as indicated by the near zero time spent in the alcohol-paired compartment. However, saline caused a significant ($p < 0.05$) reinstatement of the extinct alcohol-induced CPP following priming injection of low-dose alcohol (0.4 mg/kg), as indicated by the increase in time spent in the alcohol-paired compartment post-reinstatement compared with the time spent in the same compartment pre-reinstatement (Figure 6).

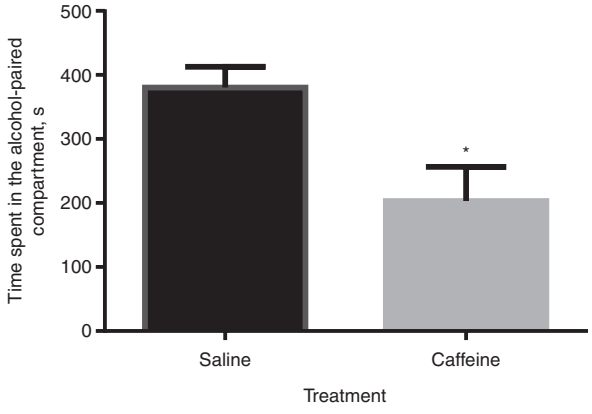

Figure 5: The effect of caffeine (3 mg/kg) on the expression of alcohol-induced conditioned place preference.
The values represent mean ± SEM (n = 5). *p < 0.05 vs. saline.

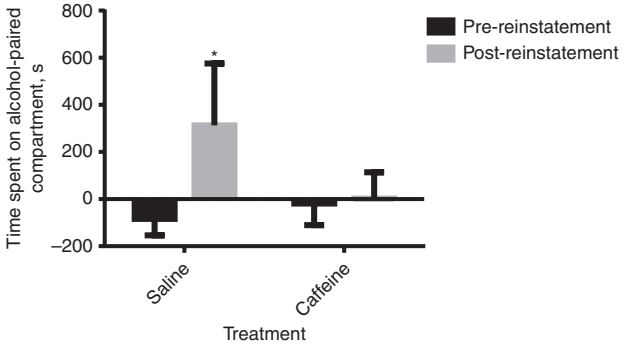

Figure 6: The effect of caffeine (3 mg/kg) on the alcohol priming-induced reinstatement of alcohol-induced conditioned place preference.
The values represent mean ± SEM (n = 5). *p < 0.05 vs. time spent in the alcohol-paired compartment pre-reinstatement.

Discussion

A robust but temporary increase in alcohol consumption over baseline consumption, following a period of forced abstinence or deprivation, is an important criterion for determining the occurrence of the alcohol deprivation effect (ADE) [3, 15]. Given such information, the observation that, following treatment with saline, the Wistar rats exhibited a consumption pattern post-deprivation, which was not significantly different from their consumption pattern pre-deprivation (baseline), does not exactly satisfy the criterion for alcohol deprivation effect. This is contrary to other reports which demonstrated ADE [3, 15, 16]. In these reports, the rats were exposed to multiple deprivation phases and increasing alcohol concentrations. Hence, the absence of ADE in the control animals may be due to the use of a single deprivation phase and alcohol concentration in our study. Consequently, it is difficult to discuss the effects of caffeine in our study in the context of ADE given that the control mice did not exhibit ADE. However, it is evident from our findings that the lower and intermediate doses of caffeine used in our study did not affect alcohol consumption after a period of deprivation. Our curiosity to determine whether lower doses of caffeine would increase alcohol consumption following a period of deprivation (ADE) was partly triggered by an earlier report, which suggested that caffeine at a low dose of 5 mg/kg had a priming effect resulting in increased alcohol consumption in an unlimited access, two = bottle free choice paradigm in both alcohol naive

and experienced rats [9, 17]. Thus, we hypothesized that the priming effect of lower doses of caffeine may trigger ADE, given that the priming stimulus is a potent trigger of ADE [18, 19]. However, contrary to our hypothesis, the lower doses of caffeine used in our study did not cause priming and, as such, no relapse-like drinking (ADE) was observed.

The significant reduction in alcohol consumption pattern post-deprivation following treatment with high dose caffeine (10 mg/kg) is in consonance with an earlier report by Rezvani et al. [9]. Considering the knowledge that caffeine is an antagonist at the adenosine receptors A1 and A2 [20, 21], this finding suggests that the antagonism of the adenosinergic receptors may have important implications for alcohol use following a period of deprivation.

In our study, we observed that some rats exhibited very low (<0.7 g/kg) baseline alcohol consumption after the drinking initiation process. Given that a dose of 5 mg/kg caffeine has been suggested to have a priming effect in rats with high baseline consumption [9, 17], we decided to test whether this dose as well as a lower and higher dose of caffeine would cause priming in these animals to increase alcohol consumption. Under the circumstances of our study, a dose of 5 mg/kg caffeine caused an increase in alcohol consumption over the baseline consumption while the lower (2.5 mg/kg) and higher (10 mg/kg) doses did not cause a change in consumption. However, our observation that the lower and higher doses of caffeine did not cause a significant change in alcohol consumption is somewhat different from an earlier report, which demonstrated that the doses of 3 mg/kg and 10 mg/kg of 3,7-Dimethylpropargylxanthine, an adenosine A2a receptor antagonist, reduced lever pressing for ethanol in Wistar rats [22]. While this report utilized the operant ethanol self-administration paradigm and adenosine A2 antagonist, our study utilized a different paradigm and caffeine, which is an antagonist at both the adenosine A1 and A2 receptors. This may account for the difference in results observed between the two studies. While this observation is contrary to our findings in the deprivation study, it is in accordance with earlier reports on the effect of caffeine on alcohol consumption in the Wistar and other strains of rats, although the rats in these reports had relatively higher baseline alcohol consumption compared with the rats in the current study [9, 17]. Our evidence indicates that caffeine (5 mg/kg) may have primed the rats to the rewarding or reinforcing effects of alcohol, thus leading to increased consumption.

Furthermore, we observed that caffeine at an intermediate dose of 5 mg/kg increased alcohol consumption, while lower and higher doses of 2.5 mg/kg and 10 mg/kg, respectively, did not affect alcohol consumption. These findings suggest that caffeine has a bi-modal effect on alcohol consumption. This observation is not in consonance with an earlier report, which demonstrated that the adenosine A1 receptor antagonist at low, intermediate and high doses caused a decrease in binge-like alcohol intake in mice. The same report also demonstrated that the adenosine A2 receptor antagonist did not affect alcohol intake in mice, suggesting that the A1 receptors may have a more relevant role in alcohol consumption [23]. The difference in results between this study and ours may be due to the differences in experimental design and animal species used in both studies.

A different experimental manipulation of adding caffeinated energy drinks containing high amounts of caffeine to alcoholic solution has demonstrated an increase in alcohol consumption, which increased with an increase in alcohol concentration in Wistar rats [24]. On the one hand, evidence shows that adolescent caffeine exposure does not alter alcohol consumption. On the other hand, adolescent exposure to a mixture of caffeine and alcohol rather than caffeine or alcohol alone results in unique changes in locomotion, delta FosB expression, cocaine CPP and saccharin preference in mice [25, 26]. It would be worthwhile to look at the effects of co-consumption on other aspects of alcohol addiction, such as craving and relapse, apart from the alcohol reward modelled as consumption.

The acquisition of alcohol-induced CPP, which follows repeated conditioning with alcohol, mimics the development of alcohol addiction [10, 27]. This condition is characterized by the transition from controlled to compulsive alcohol use due to repeated alcohol use. Therefore, the lack of significant difference between the magnitude of CPP caused by alcohol and a combination of alcohol and caffeine in our study suggests that no interaction occurred between caffeine and alcohol to potentiate the magnitude of CPP produced by the combination. Moreover, given that the presence of a rewarding stimulus and learned association between the rewarding stimulus and environmental cues are two important factors that are necessary for the acquisition of CPP [7, 28], our findings suggest that caffeine neither altered the rewarding stimulus produced by alcohol nor the learned association between the rewarding effect of alcohol and environmental cues. It is unlikely that our finding is due to the dose of caffeine used, because this dose has been demonstrated to be border line as regards the induction of significant place preference and stimulation of locomotion, thus presumably making it easy to detect any interactive effect in these behaviors [10]. Little wonder, therefore, that our findings

corroborate an earlier report, which demonstrated that caffeine is a weaker reinforcer than alcohol with a potential for producing a 'ceiling' reinforcing effect, as well as an absence of difference between the magnitude of CPP induced by alcohol and a combination of caffeine and alcohol in adult male C57BL/6J mice [10].

While a role for the adenosinergic system has been suggested in the acquisition of alcohol drinking in oral self-administration studies, as evidenced by the facilitation of alcohol intake in Wistar rats by caffeine [17], our evidence suggest that the adenosinergic system may have no role in the acquisition of alcohol-induced CPP. The differences in experimental procedures, animal species and dose of caffeine between our study and the self-administration studies may explain these observed disparities in results. Above all, it should be appreciated that CPP and self-administration studies measure different aspects of reward, and as such, differences in the development of addiction process and expression of aspects of addiction can be expected from both paradigms.

The CPP paradigm is widely used in measuring the rewarding properties of drugs, and established anti-craving drugs are able to reduce the expression of drug-induced CPP [5, 29]. Hence, the reduction of alcohol-induced CPP by caffeine observed in our study suggests the anti-craving effect of caffeine at the doses tested. Given that craving is motivated by the need to elicit pleasures of reward [30], this finding also suggests a reduction of the rewarding effect of alcohol by caffeine at the doses tested. Similar to our evidence is the evidence from another report involving self-administration studies, which demonstrated a reduction in alcohol consumption by high doses (10 and 20 mg) of caffeine. While these doses of caffeine are high and the dose used in our study can be best described as borderline, both reports suggest one thing: that caffeine at certain doses may reduce the rewarding effect of alcohol. In the same report, a lower dose of 5 mg/kg caffeine caused an increase in alcohol consumption in alcohol preferring rat strain, suggesting that caffeine may have a bi-modal effect on alcohol consumption and, by extension, reward. Moreover, the increase in consumption caused by a lower dose was suggested to be due to priming or cue-like effect of the lower dose, which sensitized the rats to the rewarding effect of alcohol and led to their increase consumption [9].

Caffeine enhances the release of dopamine by removing the inhibitory effect of adenosine on the dopamine release. Thus, it is possible that caffeine replaces alcohol's effect on dopamine and reward, as both of them cause dopamine release. Thus, it is possible that caffeine satisfies the need for dopamine (and reward), such that the animals do not need to stay in the alcohol-paired compartment or drink alcohol in the self-administration study mentioned earlier to obtain the pleasures of reward. This may especially be the case as the animals were injected with caffeine minutes before they were allowed access to the alcohol-paired environment. Thus, the animals who experienced dopamine release following caffeine administration may no longer find it necessary to spend more time in the alcohol-paired compartment to experience dopamine surge and the subsequent reward. Hence, the decrease in the expression of CPP observed in our study may be due to this possibility.

A major factor that may lead to difficulties in interpreting the results of the CPP experiments is the testing of drugs that impair memory without having any effect on the reward pathway. This is because such drugs may reduce the amount of time spent in the alcohol-paired compartment, thus reducing the expression of CPP and, in the process, give misleading false positive results [5]. Caffeine is a well-established drug that is devoid of any memory impairment but rather has a positive effect on memory/cognition [31, 32]. Thus, the reduction in expression of CPP by caffeine seen in our study could not have been due to drug-induced memory impairment or cognitive deficit. Contrary to our report, another report that looked at the effect of caffeine exposure on ethanol-induced conditioned taste aversion has shown that caffeine exposure does not influence alcohol-induced conditioned behavior in mice. This disparity in results may well be due to differences in the experimental approach and manipulations in both reports [33].

To the best of our knowledge, this is the first report on the effect of caffeine on craving as an aspect of alcohol addiction modelled as CPP. This report also suggests the involvement of the adenosinergic receptors in craving as an alcohol-seeking behavior. The CPP is finding increasing use in screening compounds for anti-relapse properties, and well known anti-relapse compounds prevent reinstatement of drug-induced CPP in this paradigm [5, 34]. Consequently, the prevention of the reinstatement of alcohol-induced CPP by caffeine indicates that caffeine, at the doses tested, may have anti-relapse properties. This is somewhat expected because relapse-like drinking is fueled by the need to obtain the rewarding effects of alcohol [35], and given that the same dose of caffeine is shown to reduce CPP (reward) expression (craving) in our study, we conclude that the same dose blocked the reinstatement of extinguished alcohol-induced CPP.

Put together, the evidence presented in this study demonstrates that caffeine has a bi-modal effect on alcohol consumption with or without deprivation; moreover, alcohol reduced the expression and blocked the

reinstatement of alcohol-induced CPP. Given that alcohol oral self-administration and CPP in the general model alcohol reward, and the expression and reinstatement of CPP model craving and relapse, respectively, our findings suggest a possible role for the adenosinergic receptor system in alcohol reward and alcohol seeking-behavior, such as craving and relapse. Our findings also indicate that the adenosinergic system holds promise for the development of new pharmaco-therapeutic options for the treatment of alcohol addiction.

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References

1. Howard CB. Alcohol dependence, withdrawal, and relapse. Alcohol Res Health 2008;31:348–61.
2. Wickizer TM. State-level estimates of the economic costs of alcohol and drug abuse. J Health Care Finance 2013;39:71–84.
3. Heyser CJ, Moc K, Koob GF. Effects of naltrexone alone and in combination with acamprosate on the alcohol deprivation effect in rats. Neuropsychopharmacology 2003;28:1463.
4. Liu Y, Le Foll B, Liu Y, Wang X, Lu L. Conditioned place preference induced by licit drugs: establishment, extinction, and reinstatement. ScientificWorldJournal 2008;8:1228–45.
5. Khan Y, Pandy V. Methanolic extract of Morinda citrifolia L. (Noni) unripe fruit attenuates ethanol-induced conditioned place preferences in mice. Front Pharmacol 2016;7:1–8.
6. Patkina NA, Zvartau EE. Caffeine place conditioning in rats: comparison with cocaine and ethanol. Eur Neuropsychopharmacol 1998;8:287–91.
7. Vidal-Infer A, Roger-Sánchez C, Daza-Losada M, Aguilar MA, Miñarro J, Rodríguez-Arias M. Role of the dopaminergic system in the acquisition, expression and reinstatement of MDMA-induced conditioned place preference in adolescent mice. PLoS One 2012;7:1–12.
8. Self DW, Nestler EJ. Relapse to drug-seeking: neural and molecular mechanisms. Drug Alcohol Depend 1998;51:49–60.
9. Rezvani AH, Sexton HG, Johnson J, Wells C, Gordon K, Levin ED. Effects of caffeine on alcohol consumption and nicotine self-administration in rats. Alcohol Clin Exp Res 2013;37:1609–17.
10. Hilbert ML, May CE, Griffin WC. Conditioned reinforcement and locomotor activating effects of caffeine and ethanol combinations in mice. Pharmacol Biochem Behav 2013;110:168–73.
11. Yao L, Arolfo MP, Dohrman DP, Jiang Z, Fan P, Fuchs S, et al. βγ Dimers mediate synergy of dopamine D2 and adenosine A2 receptor-stimulated PKA signaling and regulate ethanol consumption. Cell 2002;109:733–43.
12. Nagy LE, Diamond I, Casso DJ, Franklin C, Gordon AS. Ethanol increases extracellular adenosine by inhibiting adenosine uptake via the nucleoside transporter. J Biol Chem 1990;265:1946–51.
13. Shook BC, Jackson PF. Adenosine A2A receptor antagonists and Parkinson's disease. ACS Chem. Neurosci 2011;2:555–67.
14. Cunningham CL, Gremel CM, Groblewski PA. Drug-induced conditioned place preference and aversion in mice. Nat Protoc 2006;1:1662–70.
15. Vengeliene V, Bilbao A, Spanagel R. The alcohol deprivation effect model for studying relapse behavior: a comparison between rats and mice. Alcohol 2014;48:313–20.
16. Koros E, Piasecki J, Kostowski W, Bienkowski P. Development of alcohol deprivation effect in rats: lack of correlation with saccharin drinking and locomotor activity. Alcohol and Alcohol 1999;34:542–50.
17. Kunin D, Gaskin S, Rogan F, Smith BR, Amit Z. Caffeine promotes ethanol drinking in rats: examination using a limited-access free choice paradigm. Alcohol 2000;21:271–7.
18. Le AD, Poulos CX, Harding S, Watchus J, Juzytsch W, Shaham Y. Effects of naltrexone and fluoxetine on alcohol self-administration and reinstatement of alcohol seeking induced by priming injections of alcohol and exposure to stress. Neuropsychopharmacology 1999;21:435–44.
19. Spanagel R, Hölter SM. Long-term alcohol self-administration with repeated alcohol deprivation phases: an animal model of alcoholism? Alcohol and Alcohol 1999;34:231–43.
20. Basheer R, Strecker RE, Thakkar MM, McCarley RW. Adenosine and sleep–wake regulation. Prog Neurobiol 2004;73:379–96.
21. Fisone G, Borgkvist A, Usiello A. Caffeine as a psychomotor stimulant: mechanism of action. Cell Mol Life Sci 2004;61:857–72.
22. Thorsell A, Johnson J, Heilig M. Effect of the adenosine A2a receptor antagonist 3, 7-dimethyl-propargylxanthine on anxiety-like and depression-like behavior and alcohol consumption in Wistar rats. Alcohol Clin Exp Res 2007;31:1302–7.
23. Fritz BM, Boehm II SL. Adenosinergic regulation of binge-like ethanol drinking and associated locomotor effects in male C57BL/6J mice. Pharmacol Biochem Behav 2015;135:83–9.
24. Roldán M, Echeverry-Alzate V, Bühler KM, Sánchez-Diez IJ, Calleja-Conde J, Olmos P, et al. Red Bull® energy drink increases consumption of higher concentrations of alcohol. Addict Biol 2017; doi: 10.1111/adb. 12560.
25. Robins MT, Lu J, Van Rijn RM. Unique behavioral and neurochemical effects induced by repeated adolescent consumption of caffeine-mixed alcohol in C57BL/6 Mice. PLoS One 2016;11:e0158189.
26. Robins MT, DeFriel JN, van Rijn RM. Adolescent intake of caffeinated energy drinks does not affect adult alcohol consumption in C57BL/6 and BALB/c mice. Alcohol 2016;54:1–9.
27. Biała G, Kotlińska J. Blockade of the acquisition of ethanol-induced conditioned place preference by N-methyl-D-aspartate receptor antagonists. Alcohol and Alcohol 1999;34:175–82.
28. Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? Psychopharmacology 2000;153:31–43.

29. Kuzmin A, Sandin J, Terenius L, Ögren SO. Acquisition, expression, and reinstatement of ethanol-induced conditioned place preference in mice: effects of opioid receptor-like 1 receptor agonists and naloxone. J Pharmacol Exp Ther 2003;304:310–8.
30. Blum K, Liu Y, Shriner R, S Gold M. Reward circuitry dopaminergic activation regulates food and drug craving behavior. Curr Pharm Des 2011;17:1158–67.
31. Angelucci ME, Cesario C, Hiroi RH, Rosalen PL, Cunha CD. Effects of caffeine on learning and memory in rats tested in the Morris water maze. Braz J Med Biol Res 2002;35:1201–8.
32. Jarvis MJ. Does caffeine intake enhance absolute levels of cognitive performance? Psychopharmacology 1993;110:45–52.
33. May CE, Haun HL, Griffin WC. Sensitization and tolerance following repeated exposure to caffeine and alcohol in mice. Alcohol Clin Exp Res 2015;39:1443–52.
34. Mcgeehan AJ, Olive MF. The anti-relapse compound acamprosate inhibits the development of a conditioned place preference to ethanol and cocaine but not morphine. Br J Pharmacol 2003;138:9–12.
35. Quintanilla ME, Tampier L, Karahanian E, Rivera-Meza M, Herrera-Marschitz M, Israel Y. Reward and relapse: complete gene-induced dissociation in an animal model of alcohol dependence. Alcohol Clin Exp Res 2012:36:517–22.