Published in final edited form as:

Behav Neurosci. 2009 December ; 123(6): 1271–1278. doi:10.1037/a0017610.

Effects of ethanol and caffeine on behavior in C57BL/6 mice in the plus-maze discriminative avoidance task

Danielle Gulick and Thomas J. Gould*

Department of Psychology, Neuroscience Program, Center for Substance Abuse Research Temple University, Philadelphia, PA 19122

Abstract

Introduction—Caffeine is frequently consumed concurrent to or immediately following ethanol consumption. Identifying how caffeine and ethanol interact to modulate behavior is essential to understanding the co-use of these drugs. The plus-maze discriminative avoidance task (PMDAT) allows within-subject measurement of learning, anxiety, and locomotion.

Methods—For training, each mouse was placed in the center of the plus-maze for five minutes and each time that the mouse entered the aversive enclosed arm, a light and white noise were turned on. At testing, each mouse was returned to the center of the maze for three minutes. No cues were turned on during testing.

Results—Ethanol (1.0-1.4 g/kg) dose-dependently decreased anxiety and learning, and increased locomotion. Caffeine (5.0-40.0 mg/kg) dose-dependently increased anxiety and decreased locomotion and learning. Caffeine failed to reverse ethanol-induced learning deficits. However, 1.4 g/kg ethanol blocked the anxiogenic effect of caffeine.

Discussion—Although caffeine and ethanol interact to modulate behavior in the PMDAT, caffeine does not reverse ethanol-induced learning deficits. Ethanol-induced anxiolysis may contribute to alcohol consumption, while ethanol's blockade of caffeine-induced anxiogenesis may contribute to co-use.

Keywords

Addiction; Alcohol; Anxiety; Learning and Memory; Locomotion

Introduction

Alcohol is one of the most frequently-consumed psychoactive drugs in our society, despite well-known cognitive deficits associated with both acute and chronic alcohol consumption (Ryback, 1971; Wilkinson & Poulos, 1987). In addition, caffeine – a stimulant (Cohen, Welzl, & Battig, 1991) and psychoactive drug (Fredholm, Battig, Holmen, Nehlig, & Zvartau, 1999) – has become a common ingredient in alcoholic beverages (Marczinski & Fillmore, 2006). Anecdotal evidence suggests that the frequent co-consumption of ethanol and caffeine is due to the ability of caffeine to reverse ethanol-induced cognitive and motor deficits. Support

* Corresponding Author Department of Psychology Center for Substance Abuse Research Weiss Hall Temple University Philadelphia, PA 19122 Tel: (215) 204-7495 Fax: (215) 204-5539 tgould@temple.edu .

Publisher's Disclaimer: The following manuscript is the final accepted manuscript. It has not been subjected to the final copyediting, fact-checking, and proofreading required for formal publication. It is not the definitive, publisher-authenticated version. The American Psychological Association and its Council of Editors disclaim any responsibility or liabilities for errors or omissions of this manuscript version, any version derived from this manuscript by NIH, or other third parties. The published version is available at www.apa.org/pubs/journals/bne

for this hypothesis comes from studies demonstrating that caffeine antagonizes ethanol-induced changes in psychomotor performance (Drake, Roehrs, Turner, Scofield, & Roth, 2003; Fillmore, 2003; Franks, Hagedorn, Hensley, Hensley, & Starmer, 1975; Liguori & Robinson, 2001; Marczynski & Fillmore, 2003). These studies demonstrate that caffeine modulates the effects of ethanol on measures of attention and sedation; however, it is unclear whether caffeine is able to reverse ethanol-induced deficits in learning.

The current research examined whether caffeine interacts to modulate ethanol-induced changes in anxiety, locomotion, and learning in the plus-maze discriminative avoidance task (PMDAT). Developed by Silva and colleagues (1997), the PMDAT uses an elevated plus-maze consisting of two opposing, open arms and two opposing, enclosed arms. During training, animals are free to explore all four arms but are conditioned to avoid one enclosed arm (the aversive arm) by the presentation of both light and white noise stimuli when they enter that arm. Time spent in the open arms, compared to time in all arms, is used as a measure of anxiety. Time in the aversive enclosed arm compared to time in the non-aversive enclosed arm is used as a measure of learning. Total entries into all arms are used as a measure of locomotion. On testing day, animals are again free to explore all four arms, with no presentation of the conditioning stimuli, and the same measures observed at training are recorded. Thus, the PMDAT allows measurement of learning, anxiety, and locomotion at both training and testing within each subject.

Although the interactive effects of ethanol and caffeine on behavior in the PMDAT have yet to be examined, the effects of each drug alone have been studied in this task. In mice, ethanol impairs learning, decreases anxiety, and produces dose-dependent, biphasic effects on locomotion in the PMDAT (Kameda *et al.*, 2007) and nicotine, a stimulant like caffeine, reverses ethanol-induced learning deficits in the PMDAT (Gulick & Gould, 2009b). Caffeine impairs learning and increases anxiety when administered alone but reverses benzodiazepine-induced learning deficits when the drugs are co-administered; the effect of caffeine on total arm entries in the PMDAT has not been reported (Silva & Frussa-Filho, 2000). Both ethanol and benzodiazepines act as agonists at γ -aminobutyric acid (GABA) receptors (Hevers & Luddens, 1998). Based on the evidence that ethanol-induced learning deficits can be reversed by the stimulant nicotine, and that caffeine reverses benzodiazepine-induced learning deficits, caffeine may reverse ethanol-induced learning deficits in this task.

Methods

Subjects

Male C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME) were tested at 8–12 weeks of age (20–30 g). Mice were housed in groups of 4 mice per cage and had *ad libitum* access to food and water. A 12-hr light–dark cycle (lights on at 7:00 am) was maintained, with all testing done between 9:00 am and 5:00 pm. Procedures were approved by the Temple University Institutional Animal Care and Use Committee.

Drugs

Caffeine was procured from Sigma (St. Louis, MO). Ethanol was procured from Fisher Scientific (Pittsburgh, PA). Ethanol and caffeine were prepared in physiological saline and administered via intraperitoneal injection (i.p.). Caffeine (5–40 mg/kg) was administered 30 minutes before training and ethanol (1.0 or 1.4 g/kg) was administered 15 minutes before training (dosing based on Kameda *et al.*, 2007; Silva & Frussa-Filho, 2000). Caffeine doses were also based on the finding that 20 mg/kg of caffeine in rodents corresponds to approximately 4 cups of coffee (Fredholm *et al.*, 1999), but caffeine is metabolized up to 6 times faster in mice than in humans (for review, Nehlig & Debry, 1994). Injection volume for

caffeine was 0.01 ml/g body weight, and for ethanol it was 20% vol/vol in saline. Controls received physiological saline.

Apparatus

The modified elevated plus-maze consisted of a wood base and grey Plexiglas floors and walls with no top. The entire maze was 36" off the ground, with two opposing enclosed (walled) arms ($12 \times 3 \times 6$ in, $L \times W \times H$, 5 lx) and two opposing open arms (12×3 in, $L \times W$, 10 lx). A 75-watt lamp (600 lx) was placed directly over the aversive arm, and a speaker connected to a noise generator (85 dB) was placed directly below the same arm.

Procedure

For the training session, each mouse was placed in the center of the apparatus and, for a period of five minutes, the time spent in each arm or in the center area was recorded by a researcher who was blind to the drug treatment. Total entries into the arms were also recorded. Each time that the mouse entered the aversive arm, the 75-watt light and the 85-dB white noise were turned on by the researcher. Both cues were turned off when the mouse exited the aversive arm.

For the testing session 24 hours later, each mouse was returned to the center of the apparatus and, for a period of three minutes, was again free to explore the maze. Time in each arm and arm entries were again recorded. No cues were turned on during the testing session. The maze was cleaned with 70% ethanol before each training and testing session (methods based on Kameda *et al.*, 2007; Silva & Frussa-Filho, 2000). Prior research has demonstrated that conditioned avoidance of the aversive arm requires the tone and light stimuli to be presented during training trials (Gulick & Gould, 2009b).

Scoring

Entry into an arm was counted when all four paws crossed into that arm. Time in the center arm was not counted toward any measure. Total number of entries per minute into all arms was used as an index of locomotion. Percent time in the aversive enclosed arm vs. percent time in the non-aversive enclosed arm (time in arm/time in all arms) was used as an index of learning (increased time in the aversive enclosed arm and decreased time in the non-aversive enclosed arm = decreased learning), and percent time in the open arms (time in open arms/time in all arms) was used as an index of anxiety (increased time in the open arms = decreased anxiety).

Statistical Analyses

Differences between groups were analyzed by one-way or two-way analysis of variance (ANOVA). Levene's test for homogeneity of variance was used to determine appropriate post-hoc tests, and either Tukey's (homogeneous variability) or Duncan's (non-homogeneous variability) post-hoc tests were run at the level of $p < 0.05$. Statistics were calculated with SPSS (Version 14; SPSS, Chicago, IL).

Effects of Ethanol and Caffeine in the PMDAT

In our previous study with nicotine and ethanol, we examined the dose-dependent effects of ethanol on plus-maze discriminative avoidance (Gulick & Gould, 2009b). In the current study, we first constructed a dose-response curve for the effects of caffeine on plus-maze discriminative avoidance. Caffeine (5-40 mg/kg) or saline was administered i.p. 30 minutes before training in the PMDAT (doses and timing based on Silva and Frussa-Filho, 2000). To determine whether the effects of caffeine on learning are due to state-dependent effects, we then compared the effects of caffeine (40 mg/kg) or saline before training or before training and testing on behavior. Finally, to determine whether caffeine interacts with ethanol to

modulate anxiety, locomotion, or learning, we co-administered doses of both drugs that have been shown to alter behavior in the PMDAT. In the first interaction study, we used a dose of ethanol (1.0 g/kg) that has been shown to decrease learning in the PMDAT. Caffeine (20 or 40 mg/kg) or saline was administered i.p. 30 minutes before training and 1.0 g/kg ethanol (Gulick & Gould, 2009b) or saline was administered i.p. 15 minutes before training in the plus-maze. Caffeine failed to reverse the learning deficit associated with the low, non-anxiolytic dose of ethanol. Thus, in order to assess whether caffeine may reverse the learning deficit associated with an anxiolytic dose of ethanol, we also conducted an interaction study using the highest dose of caffeine (40 mg/kg) and a dose of ethanol (1.4 g/kg) previously demonstrated to be anxiolytic in the plus-maze (Gulick & Gould, 2009c; Kameda *et al.*, 2007).

Results

Effects of Caffeine alone

Percent time in the aversive arm—Caffeine administered at training had no effect on learning-related behavior at training but altered learning assessed at testing. At training, two-way ANOVA revealed significant effects of arm, $F(1,35) = 88.56$, $p < 0.001$ but no effect of drug and no significant interaction. Tukey's post-hoc tests revealed that all groups spent more time in the non-aversive arm than the aversive arm ($p < 0.05$). At testing, two-way ANOVA revealed no significant effect of drug treatment, but a significant effect of arm, $F(1,35) = 48.27$, $p < 0.001$, as well as a significant interaction, $F(5,31) = 25.53$, $p < 0.001$. Tukey's post-hoc tests revealed that all groups except the group administered 40 mg/kg caffeine spent significantly more time in the non-aversive arm than the aversive arm ($p < 0.05$) while the group administered 40 mg/kg caffeine spent a similar amount of time in each arm. In addition, the group administered 40 mg/kg caffeine spent more time in the aversive arm and less time in the non-aversive arm than saline controls ($p < 0.05$) (Figure 1 A-B). Thus, a high dose of caffeine administered at training produced a learning deficit at the test session.

To determine if the learning deficit induced by the highest dose of caffeine (40 mg/kg) reflected a state-dependent process, we administered caffeine (40 mg/kg) before training only or before training and testing. At training, two-way ANOVA revealed no significant effect of drug treatment, but a significant effect of arm, $F(1,17) = 90.12$, $p < 0.001$, as well as a significant interaction, $F(2,16) = 6.89$, $p < 0.01$ (Figure 2 A). Duncan's post-hoc tests revealed that all groups spent significantly more time in the non-aversive arm than the aversive arm ($p < 0.05$). In addition, the group administered 40 mg/kg caffeine at training only spent less time in the aversive arm than saline controls ($p < 0.05$). There was no significant effect of arm or drug on percent time in the aversive arm at testing, but there was a significant interaction, $F(2,16) = 24.54$, $p < 0.001$ (Figure 2 B). Tukey's post-hoc tests demonstrated that both groups administered caffeine spent significantly more time in the aversive arm and less time in the non-aversive arm than saline controls at testing ($p < 0.05$). However, there were no differences between the groups administered caffeine at training and at training and testing. Thus, the learning deficit associated with the 40 mg/kg dose of caffeine is not due to state-dependent effects.

Percent time in the open arms—During training, there was a significant effect of caffeine dose on percent time in the open arms, $F(4,36) = 2.65$, $p < 0.05$. Duncan's post-hoc tests revealed that the groups administered 10-40 mg/kg caffeine spent a significantly lower percentage of time in the open arms compared to saline controls ($p < 0.05$). There was no significant effect of caffeine dose on percent time in the open arms during testing (Figure 1 C-D). As expected, higher doses of caffeine increased anxiety on training day; however, there were no changes in anxiety at testing when mice received caffeine at training only.

Total entries into all arms—Two-way ANOVA revealed no effect of drug and no drug by session interactive effect on entries per minute at training and testing (Figure 1 E-F). Thus, the effects of caffeine on anxiety and learning were not associated with changes in locomotion. However, there was a significant effect of session on entries per minute ($F(1,76) = 9.02$, $p < 0.001$), due to a decrease in entries between training and testing across groups, suggesting habituation to the plus-maze.

Interactions of 1.0 g/kg Ethanol and Caffeine in the PMDAT

Percent time in the aversive arm—Since nicotine, a stimulant, has been shown to reverse ethanol-induced learning deficit in the PMDAT, we examined whether caffeine, another stimulant frequently consumed with ethanol, could produce the same reversal. Caffeine (20 and 40 mg/kg) and ethanol (1.0 g/kg) were administered before training. For training, two-way ANOVA revealed a significant effect of drug, $F(5,30) = 4.73$, $p < 0.01$, and of arm, $F(1,34) = 81.4$, $p < 0.001$, as well as a drug by arm interaction, $F(5,30) = 6.95$, $p < 0.001$. Duncan's post-hoc tests revealed that all groups spent more time in the non-aversive arm than in the aversive arm ($p < 0.05$), and the groups administered caffeine alone spent more time in the non-aversive arm than saline controls ($p < 0.05$). For testing, two-way ANOVA revealed no effect of group but a significant effect of arm, $F(5,30) = 92.53$, $p < 0.001$ and a significant interaction, $F(5,30) = 18.32$, $p < 0.001$. Tukey's post-hoc tests revealed that the groups administered ethanol alone or in combination with caffeine, as well as the group administered 40 mg/kg caffeine alone, spent significantly more time in the aversive arm and significantly less time in the non-aversive arm during testing than saline controls and the group administered 20 mg/kg caffeine alone ($p < 0.05$). In addition, only saline controls and the group administered 20 mg/kg caffeine alone spent significantly more time in the non-aversive arm than in the aversive arm. Finally, the groups administered caffeine and ethanol did not differ from the group administered ethanol alone in time in the aversive arm or in time in the non-aversive arm (Figure 3 A-B). Thus, both ethanol and caffeine impaired learning, and there was no reversal of the learning deficits after co-administration of the drugs.

Percent time in the open arms—There was a significant effect of ethanol on percent time in the open arms during training, $F(1,34) = 43.67$, $p < 0.001$. There was also a significant effect of caffeine on percent time in the open arms during training, $F(2,33) = 36.31$, $p < 0.001$, and an interactive effect of ethanol and caffeine on percent time in the open arms during training, $F(2,33) = 5.10$, $p < 0.05$. Tukey's post-hoc test revealed that the groups administered caffeine alone spent significantly less time in the open arms at training than saline controls and the groups administered ethanol alone or ethanol with caffeine ($p < 0.05$). There was a significant effect of ethanol, $F(1,34) = 7.84$, $p < 0.01$, but not caffeine, on percent time in the open arms during testing; there was no significant interaction of ethanol and caffeine on percent time in the open arms during testing. Tukey's post-hoc tests revealed that the group administered ethanol alone spent significantly more time in the open arms at testing than saline controls ($p < 0.05$) but there were no other group differences (Figure 3 C-D). Thus, ethanol reversed the anxiogenic effect of caffeine on training day and caffeine blocked ethanol-induced anxiolysis observed on testing day when drugs were administered on training day.

Total entries into all arms—There was a significant effect of group, $F(5,53) = 4.22$, $p < 0.01$, and a significant effect of session on entries per minute, $F(1,57) = 12.05$, $p < 0.001$, as well as an interactive effect of group and session, $F(5,53) = 2.87$, $p < 0.05$, on entries per minute at training. Tukey's post-hoc tests revealed that only the 40 mg/kg caffeine group had significantly fewer entries than the control group at training ($p < 0.05$). There were no other group differences. However, there were significant differences in entries per minute between the training and testing sessions; the ethanol-alone and both 20 mg/kg caffeine groups all made significantly fewer entries per minute during testing compared to training ($p < 0.05$), while there

was no significant difference between sessions for the groups administered saline or 40 mg/kg caffeine (Figure 3 E-F).

Interactions of 1.4 g/kg Ethanol and 40 mg/kg Caffeine in the PMDAT

Percent time in the aversive arm—Finally, we examined how the 40 mg/kg dose of caffeine interacted with the 1.4 g/kg dose of ethanol to determine whether co-administration of an anxiogenic dose of caffeine and an anxiolytic dose of ethanol (as shown in previous plus-maze studies: Gulick & Gould, 2009b; Silva & Frussa-Filho, 2000) would alter learning. Caffeine (40 mg/kg) and ethanol (1.4 g/kg) were administered before training. For training, two-way ANOVA revealed a significant effect of arm, $F(1,22) = 74.41$, $p < 0.001$, but no significant effect of group and no interaction (Figure 4 A). Duncan's post-hoc tests revealed that both ethanol groups spent less time in the non-aversive arm than saline controls ($p < 0.05$), and that all groups spent more time in the non-aversive arm than the aversive arm. For testing, two-way ANOVA revealed no effect of group but a significant effect of arm, $F(1,22) = 35.96$, $p < 0.001$, and a significant interaction, $F(1,22) = 17.30$, $p < 0.001$ (Figure 4 B). Duncan's post-hoc tests revealed that all experimental groups spent significantly more time in the aversive arm and less time in the non-aversive arm than controls at testing ($p < 0.05$), and only saline controls spent significantly more time in the non-aversive arm than the aversive arm ($p < 0.05$). Thus, both an anxiogenic dose of caffeine and an anxiolytic dose of ethanol impaired learning in the PMDAT, and learning deficits were not reversed by co-administration of the drugs.

Percent time in the open arms—There was a significant effect of ethanol on time in the open arms during training, $F(1,23) = 19.9$, $p < 0.001$. There was also a significant effect of caffeine on time in the open arms during training, $F(1,23) = 6.68$, $p < 0.05$, but no interactive effect of ethanol and caffeine on time in the open arms during training (Figure 4 C). Tukey's post-hoc tests revealed that the groups administered ethanol spent significantly more time in the open arms than saline controls at training ($p < 0.05$), but there were no other group differences. There was a significant effect of ethanol, $F(1,23) = 7.68$, $p < 0.05$, but no effect of caffeine, on time in the open arms during testing, nor was there a significant interaction of ethanol and caffeine on time in the open arms during testing (Figure 4 D). Tukey's post-hoc tests revealed that the group administered ethanol alone spent more time in the open arms at testing ($p < 0.05$), but there were no other group differences.

Total entries into all arms—There was a significant effect of group on entries per minute, $F(4,45) = 14.50$, $p < 0.001$ and a significant effect of session on entries per minute, $F(1,47) = 21.14$, $p < 0.001$, but there was no interactive effect of group and session on entries per minute (Figure 4 E-F). Tukey's post-hoc tests revealed that the ethanol increased entries compared to saline controls at training and testing ($p < 0.05$), and caffeine blocked this effect at training ($p < 0.05$) but not at testing. Thus, 1.4 g/kg ethanol increased locomotor behavior, and this effect was not reversed by caffeine co-administration. In addition, the control group and the ethanol-alone group made significantly fewer entries per minute during testing compared to training ($p < 0.05$), suggesting that habituation occurred in these groups.

Discussion

The current study demonstrates that caffeine increases and ethanol decreases anxiety, but that caffeine does not reverse ethanol-induced learning impairments. Ethanol impairs learning across a number of tasks in both rats and mice (Bammer & Chesher, 1982; Gibson, 1985; Gould, 2003; Higgins *et al.*, 1992; Weitemier & Ryabinin, 2003), while caffeine may enhance learning in some tasks in rats and mice (Hauber & Bareiss, 2001; Kopf, Melani, Pedata, & Pepeu, 1999) and may reverse the learning deficits associated with cognitive decline (Takahashi, Pamplona, & Prediger, 2008). Although a number of studies have demonstrated

that caffeine reverses ethanol-induced behavioral deficits in tasks that measure sedation and attention, such as simulated driving and sleep latency tests (Drake *et al.*, 2003; Liguori & Robinson, 2001; Marczinski & Fillmore, 2003), none of these studies have directly examined the effect of caffeine on ethanol-induced learning deficits. In addition, we demonstrate that caffeine alone can impair learning and increase anxiety across a range of doses that do not significantly alter locomotion. Furthermore, caffeine produced only borderline effects on locomotion even at the highest dose tested. Our results for caffeine alone replicate research by Silva and Frussa-Filho (2000) examining the effects of caffeine in the PMDAT, as well as research demonstrating that caffeine increases anxiety across a wide variety of tasks in rats (Bhattacharya, Satyan, & Chakrabarti, 1997) and impairs other learning tasks such as fear conditioning in rats (Corodimas, Pruitt, & Stieg, 2000).

The effects of ethanol and caffeine on time in the aversive arm most likely represent changes in the processes underlying learning rather than associated changes in arm preference or anxiety. We previously demonstrated that animals presented with neither light or tone aversive stimulus at training spend a similar amount of time in the (to be) aversive and non-aversive arms at testing, suggesting that avoidance of the aversive arm in trained animals reflects a learned association between the aversive stimuli and the arm in which they were presented (Gulick & Gould, 2009b). In addition, of the doses of caffeine that increased anxiety, only the 40 mg/kg dose also impaired learning, suggesting that the learning deficits associated with caffeine were not due to changes in anxiety. Thus, the effects of caffeine and ethanol on learning may not be mediated by changes in anxiety or arm preference, but rather by changes in the substrates of learning. Furthermore, ethanol and caffeine may yield selective effects on learning. Both ethanol and caffeine produced deficits in learning to associate the aversive arm with the cues presented in it, but neither drug blocked habituation to the plus-maze, as demonstrated by a decrease in arm entries per minute of the maze at testing compared to training. Studies from another paradigm which models habituation learning, the novel object recognition task, support our finding that the doses of ethanol and caffeine used in the current study do not impair habituation. In mice, higher doses of ethanol than those used in the current study are required to impair novel object recognition (Ryabinin, Miller, & Durrant, 2002), and one study suggests that caffeine may produce beneficial or null effects on novel object recognition depending on the administration paradigm and time of testing (Costa *et al.*, 2008).

In the current study, caffeine failed to reverse ethanol-induced learning deficits. On the surface, this finding contradicts work by Silva and Frussa-Filho (2000), who found that caffeine reversed memory deficits in mice when co-administered with a memory-impairing dose of the benzodiazepine chlordiazepoxide; they interpreted their findings as evidence that the antagonistic actions of the anxiolytic chlordiazepoxide and anxiogenic caffeine produced a normalized level of anxiety and arousal that allowed animals to learn to avoid the aversive arm. In the current study, however, the moderate doses of caffeine (10-20 mg/kg) increased anxiety without altering learning, and although caffeine and ethanol interacted to alter anxiety, this interaction was not associated with a rescue of learning, suggesting that the learning deficit does not depend on changes in anxiety. Thus, some mechanism other than neutralization of changes in anxiety may underlie the interactive effects of caffeine with ethanol and benzodiazepines on learning.

Both ethanol and chlordiazepoxide have amnesic and anxiolytic actions via GABA receptors (Grobin, Matthews, Devaud, & Morrow, 1998; Hevers & Luddens, 1998; Stackman & Walsh, 1992; White, Simson, & Best, 1997), but the differential effects of caffeine on the learning-impairing effects of each drug may be due to changes in other processes. Benzodiazepines primarily work in the central nervous system to alter the function of GABA receptors (Richter, 1981), whereas ethanol alters receptor function for a variety of neurotransmitter systems,

including glutamate, GABA, and acetylcholine (Nevo & Hamon, 1995). Caffeine may not be able to antagonize all of the functional changes that ethanol produces at these diverse receptors. This is in contrast with the effects of nicotine on ethanol-induced learning deficits in the PMDAT and fear conditioning, as nicotine reversed learning deficits associated with ethanol administration (Gulick & Gould, 2008, 2009a). Thus, it appears that the reversal of ethanol-induced learning deficits is due to actions at specific receptors targeted by both ethanol and nicotine, rather than some general property of drugs classified as stimulants; in the current study, there was no interactive effect of caffeine and ethanol on learning that could explain the frequent co-use of these drugs.

Although caffeine did not block the learning deficits associated with ethanol treatment, ethanol did block the anxiogenic effect of caffeine. Caffeine also reverses ethanol-induced sedation and decreases self-reports of intoxication in humans (Drake *et al.*, 2003; Liguori & Robinson, 2001; Marczinski & Fillmore, 2003, 2006). Thus, one reason for the popularity of drinks combining alcohol and caffeine may be the interactive effects of these drugs on sedation and anxiety.

In conclusion, caffeine has been shown to reverse some of the behavioral effects of ethanol, including sedation and deficits in attention, but the current study demonstrates that caffeine was unable to reverse ethanol-induced deficits in avoidance learning. However, ethanol reduced caffeine-induced anxiogenesis. Although co-consumption of ethanol and caffeine may increase alertness during intoxication, and decrease the awareness of intoxication, there may be no equivalent rescue of learning. Thus, drinkers may consume more alcohol when they are also consuming caffeine (O'Brien, McCoy, Rhodes, Wagoner, & Wolfson, 2008), producing greater intoxication and leading to greater decrements in learning.

Acknowledgments

This work was supported by National Institute on Alcohol Abuse and Alcoholism (NIAAA) Grant AA015515 (T.J.G.). All research complies with current US laws for the care and use of laboratory animals.

References

- Bammer G, Chesher GB. An analysis of some effects of ethanol on performance in a passive avoidance task. *Psychopharmacology (Berl)* 1982;77(1):66–73. [PubMed: 6812120]
- Bhattacharya SK, Satyan KS, Chakrabarti A. Anxiogenic action of caffeine: an experimental study in rats. *J Psychopharmacol* 1997;11(3):219–224. [PubMed: 9305413]
- Cohen C, Welzl H, Battig K. Effects of nicotine, caffeine, and their combination on locomotor activity in rats. *Pharmacol Biochem Behav* 1991;40(1):121–123. [PubMed: 1780334]
- Corodimas KP, Pruitt JC, Stieg JM. Acute exposure to caffeine selectively disrupts context conditioning in rats. *Psychopharmacology (Berl)* 2000;152(4):376–382. [PubMed: 11140329]
- Costa MS, Botton PH, Mioranza S, Ardais AP, Moreira JD, Souza DO, Porciuncula LO. Caffeine improves adult mice performance in the object recognition task and increases BDNF and TrkB independent on phospho-CREB immunocontent in the hippocampus. *Neurochem Int* 2008;53(3-4): 89–94. [PubMed: 18620014]
- Drake CL, Roehrs T, Turner L, Scofield HM, Roth T. Caffeine reversal of ethanol effects on the multiple sleep latency test, memory, and psychomotor performance. *Neuropsychopharmacology* 2003;28(2): 371–378. [PubMed: 12589390]
- Fillmore MT. Alcohol tolerance in humans is enhanced by prior caffeine antagonism of alcohol-induced impairment. *Exp Clin Psychopharmacol* 2003;11(1):9–17. [PubMed: 12622339]
- Franks HM, Hagedorn H, Hensley VR, Hensley WJ, Starmer GA. The effect of caffeine on human performance, alone and in combination with ethanol. *Psychopharmacologia* 1975;45(2):177–181. [PubMed: 1215448]

- Fredholm BB, Battig K, Holmen J, Nehlig A, Zwartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev* 1999;51(1):83–133. [PubMed: 10049999]
- Gibson WE. Effects of alcohol on radial maze performance in rats. *Physiol Behav* 1985;35(6):1003–1005. [PubMed: 4095174]
- Gould TJ. Ethanol disrupts fear conditioning in C57BL/6 mice. *J Psychopharmacol* 2003;17(1):77–81. [PubMed: 12680742]
- Grobin AC, Matthews DB, Devaud LL, Morrow AL. The role of GABA(A) receptors in the acute and chronic effects of ethanol. *Psychopharmacology (Berl)* 1998;139(1-2):2–19. [PubMed: 9768538]
- Gulick, Gould. Interactive effects of ethanol and nicotine on learning in C57BL/6J mice depend on both dose and duration of treatment. *Psychopharmacology (Berl)* 2008;196(3):483–495. [PubMed: 17968532]
- Gulick, Gould. The hippocampus and cingulate cortex differentially mediate the effects of nicotine on learning versus on ethanol-induced learning deficits via different effects at nicotinic receptors. *Neuropsychopharmacology*. 2009a In Press.
- Gulick, Gould. Interactive effects of ethanol and nicotine on learning, anxiety, and locomotion in C57BL/6 mice in the plus-maze discriminative avoidance task. *Neuropharmacology*. 2009b In Press.
- Gulick, Gould. Interactive effects of ethanol and nicotine on learning, anxiety, and locomotion in C57BL/6 mice in the plus-maze discriminative avoidance task. *Neuropharmacology*. 2009c
- Hauber W, Bareiss A. Facilitative effects of an adenosine A1/A2 receptor blockade on spatial memory performance of rats: selective enhancement of reference memory retention during the light period. *Behav Brain Res* 2001;118(1):43–52. [PubMed: 11163632]
- Hevers W, Luddens H. The diversity of GABAA receptors. Pharmacological and electrophysiological properties of GABAA channel subtypes. *Mol Neurobiol* 1998;18(1):35–86. [PubMed: 9824848]
- Higgins ST, Rush CR, Hughes JR, Bickel WK, Lynn M, Capeless MA. Effects of cocaine and alcohol, alone and in combination, on human learning and performance. *J Exp Anal Behav* 1992;58(1):87–105. [PubMed: 1645103]
- Kameda SR, Frussa-Filho R, Carvalho RC, Takatsu-Coleman AL, Ricardo VP, Patti CL, Calzavara MB, Lopez GB, Araujo NP, Abilio VC, Ribeiro Rde A, D'Almeida V, Silva RH. Dissociation of the effects of ethanol on memory, anxiety, and motor behavior in mice tested in the plus-maze discriminative avoidance task. *Psychopharmacology (Berl)* 2007;192(1):39–48. [PubMed: 17242924]
- Kopf SR, Melani A, Pedata F, Pepeu G. Adenosine and memory storage: effect of A(1) and A(2) receptor antagonists. *Psychopharmacology (Berl)* 1999;146(2):214–219. [PubMed: 10525758]
- Liguori A, Robinson JH. Caffeine antagonism of alcohol-induced driving impairment. *Drug Alcohol Depend* 2001;63(2):123–129. [PubMed: 11376916]
- Marczinski CA, Fillmore MT. Dissociative antagonistic effects of caffeine on alcohol-induced impairment of behavioral control. *Exp Clin Psychopharmacol* 2003;11(3):228–236. [PubMed: 12940502]
- Marczinski CA, Fillmore MT. Clubgoers and their trendy cocktails: implications of mixing caffeine into alcohol on information processing and subjective reports of intoxication. *Exp Clin Psychopharmacol* 2006;14(4):450–458. [PubMed: 17115872]
- Nehlig A, Debry G. Potential teratogenic and neurodevelopmental consequences of coffee and caffeine exposure: a review on human and animal data. *Neurotoxicol Teratol* 1994;16(6):531–543. [PubMed: 7862054]
- Nevo I, Hamon M. Neurotransmitter and neuromodulatory mechanisms involved in alcohol abuse and alcoholism. *Neurochem Int* 1995;26(4):305–336. discussion 337–342. [PubMed: 7633325]
- O'Brien MC, McCoy TP, Rhodes SD, Wagoner A, Wolfson M. Caffeinated cocktails: energy drink consumption, high-risk drinking, and alcohol-related consequences among college students. *Acad Emerg Med* 2008;15(5):453–460. [PubMed: 18439201]
- Richter JJ. Current theories about the mechanisms of benzodiazepines and neuroleptic drugs. *Anesthesiology* 1981;54(1):66–72. [PubMed: 6109471]
- Ryabinin AE, Miller MN, Durrant S. Effects of acute alcohol administration on object recognition learning in C57BL/6J mice. *Pharmacol Biochem Behav* 2002;71(1-2):307–312. [PubMed: 11812537]

- Ryback RS. The continuum and specificity of the effects of alcohol on memory. A review. *Q J Stud Alcohol* 1971;32(4):995–1016. [PubMed: 4944697]
- Silva RH, Frussa-Filho R. The plus-maze discriminative avoidance task: a new model to study memory-anxiety interactions. Effects of chlordiazepoxide and caffeine. *J Neurosci Methods* 2000;102(2):117–125. [PubMed: 11040408]
- Stackman RW, Walsh TJ. Chlordiazepoxide-induced working memory impairments: site specificity and reversal by flumazenil (RO15-1788). *Behav Neural Biol* 1992;57(3):233–243. [PubMed: 1319705]
- Takahashi RN, Pamplona FA, Prediger RD. Adenosine receptor antagonists for cognitive dysfunction: a review of animal studies. *Front Biosci* 2008;13:2614–2632. [PubMed: 17981738]
- Weitemier AZ, Ryabinin AE. Alcohol-induced memory impairment in trace fear conditioning: a hippocampus-specific effect. *Hippocampus* 2003;13(3):305–315. [PubMed: 12722971]
- White AM, Simson PE, Best PJ. Comparison between the effects of ethanol and diazepam on spatial working memory in the rat. *Psychopharmacology (Berl)* 1997;133(3):256–261. [PubMed: 9361331]
- Wilkinson DA, Poulos CX. The chronic effects of alcohol on memory. A contrast between a unitary and dual system approach. *Recent Dev Alcohol* 1987;5:5–26. [PubMed: 3550917]

Figure 1.

Effects of caffeine administered at training. (A-B) Effects on learning [percent time in the aversive and non-aversive enclosed arms]. A) Caffeine did not alter time in the aversive arm or non-aversive arm at training. B) Caffeine (40 mg/kg) increased time in the aversive arm and decreased time in the non-aversive arm at testing ($n=8-10$; Mean $\pm$ SEM; * = significantly different from controls and + = significant difference between arms at $p<0.05$). (C-D) Effects on anxiety [percent time in the open arms]: C) Caffeine dose-dependently decreased time in the open arms at training. D) There was no change in anxiety at testing ($n=8-10$; Mean $\pm$ SEM; * = significantly different from controls at $p<0.05$). (E-F) Effects on locomotion [total arm entries]: There were no significant differences between groups at training (E) or testing (F) ($n=8-10$; Mean $\pm$ SEM).

Figure 2.

Effects of caffeine (40 mg/kg) administered at training only vs. at training and testing on learning [percent time in the aversive and non-aversive arms]. A) There were no differences between groups at training. B) Both caffeine-treated groups spent more time in the aversive arm and less time in the non-aversive arm than saline controls at testing ($n = 6$; Mean $\pm$ SEM; * = significantly different from controls and + = significant difference between arms at $p < 0.05$).

Figure 3.

Effects of 1.0 g/kg ethanol and caffeine (20-40 mg/kg) administered at training. (A-B) Effects on learning [percent time in the aversive and non-aversive enclosed arms]: A) Caffeine (20 and 40 mg/kg) increased time in the non-aversive arm at training, and ethanol blocked this effect. B) Both caffeine (40 mg/kg) and ethanol increased time in the aversive arm and decrease time in the non-aversive arm at testing ($n=6-7$; Mean $\pm$ SEM; * = significantly different from controls and + = significant difference between arms at $p<0.05$). (C-D) Effects on anxiety [percent time in the open arms]: C) Caffeine decreased time in the open arms at training, and ethanol reversed this effect. D) Ethanol administered at training increased time in the open arms at testing, but there were no other group effects ($n=6-7$; Mean $\pm$ SEM; * = significantly different from controls at $p<0.05$). (E-F) Effects on locomotion [total arm entries]: E) 40 mg/kg caffeine decreased locomotion at training. F) There were no differences between groups at testing ($n=8-10$; Mean $\pm$ SEM; * = significantly different from controls at $p<0.05$).

Figure 4.

Effects of caffeine (40 mg/kg) and ethanol (1.4 g/kg) administered at training. (A-B) Effects on learning [percent time in the aversive and non-aversive arms]: A) Both ethanol-treated groups spent less time in the non-aversive arm than saline controls at training. B) All experimental groups spent more time in the aversive arm and less time in the non-aversive arm than saline controls at testing (n = 6; Mean $\pm$ SEM; * = significantly different from controls at p<0.05). (C-D) Effects on anxiety [percent time in the open arms]: C) Ethanol increased time in the open arms at training. D) Ethanol increased time in the open arms and caffeine blocked this effect at testing (n=8-10; Mean $\pm$ SEM; * = significantly different from controls at p<0.05). (E-F) Effects on locomotion [total arm entries]: E) Ethanol increased arm entries at training and caffeine blocked this effect. F) Ethanol increased arm entries at testing. (n=8-10; Mean $\pm$ SEM; * = significantly different from controls at p<0.05).