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# Effects of Ethanol and Caffeine on Behavior in C57BL/6 Mice in the Plus-Maze Discriminative Avoidance Task

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***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 5 min, 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 3 min. 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

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 coconsumption of ethanol and caffeine is attributable to the ability of caffeine to reverse ethanol-induced cognitive and motor deficits. Support 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; Marczinski & 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 Frussa-Filho (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 with time in all arms, is used as a measure of anxiety. Time in the aversive enclosed arm compared with time in the nonaversive 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 coadministered; 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.

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## Method

### *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 a.m.) was maintained, with all testing done between 9:00 a.m. and 5:00 p.m. 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 min before training and ethanol (1.0 or 1.4 g/kg) was administered 15 min 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 gray Plexiglas floors and walls with no top. The entire maze was 36″ off the ground, with two opposing enclosed (walled) arms (12 × 3 × 6 in, L × W × H, 5 lx) and two opposing open arms (12 × 3 in, L × W, 10 lx). A 75-W 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 5 min, 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-W 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 3 min, 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 versus percent time in the nonaversive 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 nonaversive 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 (nonhomogeneous variability) post hoc tests were run at the level of $p < .05$. Statistics were calculated with SPSS (Version 14; SPSS, Chicago).

### *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. Thirty minutes before training in the PMDAT (doses and timing based on Silva & 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 coadministered 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. Thirty minutes before training and 1.0 g/kg ethanol (Gulick & Gould, 2009b) or saline was administered i.p. Fifteen minutes before training in the plus-maze. Caffeine failed to reverse the learning deficit associated with the low, nonanxiolytic dose of ethanol. Thus, 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, 2009b; 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, $F(1, 35) = 88.56$, $p < .001$, but no effect of drug and no significant interaction. Tukey's post hoc tests revealed that all groups spent more time in the nonaversive arm than the aversive arm ($p < .05$). At testing, two-way ANOVA revealed no significant effect of drug treatment, but a significant effect of arm, $F(1, 35) = 48.27$, $p < .001$, as well as a significant interaction, $F(5, 31) = 25.53$, $p < .001$. Tukey's post hoc tests revealed that all groups except the group administered 40 mg/kg caffeine spent significantly more time in the nonaversive arm than the aversive arm ($p < .05$) 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 nonaversive arm than saline controls ($p < .05$) (Figure 1A–B). Thus, a high dose of caffeine administered at training produced a learning deficit at the test session.

To determine whether 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, $F(1, 17) = 90.12$, $p < .001$, as well as a significant interaction, $F(2, 16) = 6.89$, $p < .01$ (Figure 2A). Duncan's post hoc tests revealed that all groups spent significantly more time in the nonaversive arm than the aversive arm ($p < .05$). In addition, the group administered 40 mg/kg caffeine at training only spent less time in the aversive arm than saline controls ($p < .05$). There was no significant effect of arm or drug on percent time in the aversive arm at testing, but there was a significant interaction, $F(2, 16) = 24.54$, $p < .001$ (Figure 2B). Tukey's post hoc tests demonstrated that both groups administered caffeine spent significantly more time in the aversive arm and less time in the nonaversive arm than saline controls at testing ($p < .05$). However, there were no differences between the groups administered caffeine at train-

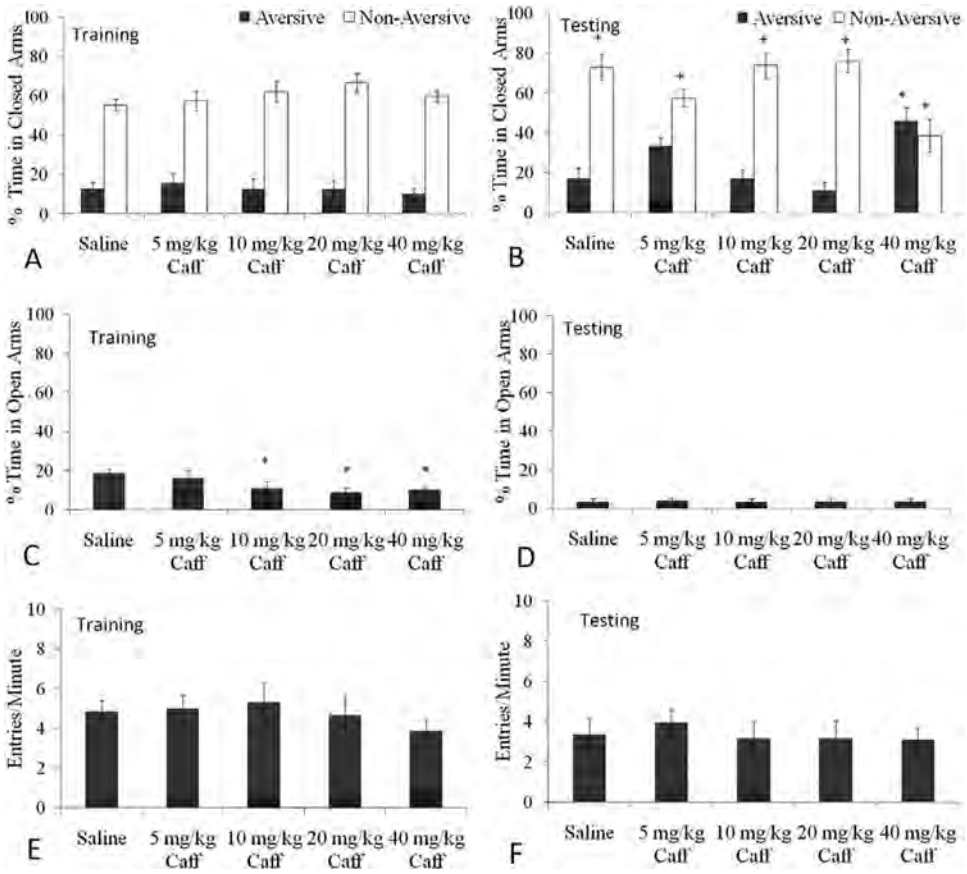



*Figure 1.* Effects of caffeine administered at training. (A–B) Effects on learning (percent time in the aversive and nonaversive enclosed arms): (A) Caffeine did not alter time in the aversive arm or nonaversive arm at training. (B) Caffeine (40 mg/kg) increased time in the aversive arm and decreased time in the nonaversive arm at testing ($n = 8–10$; mean ± *SEM*; $^{*}$ = significantly different from controls and $^{+}$ = significant difference between arms at $p < .05$). (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 ± *SEM*; $^{*}$ = significantly different from controls at $p < .05$). (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 ± *SEM*).

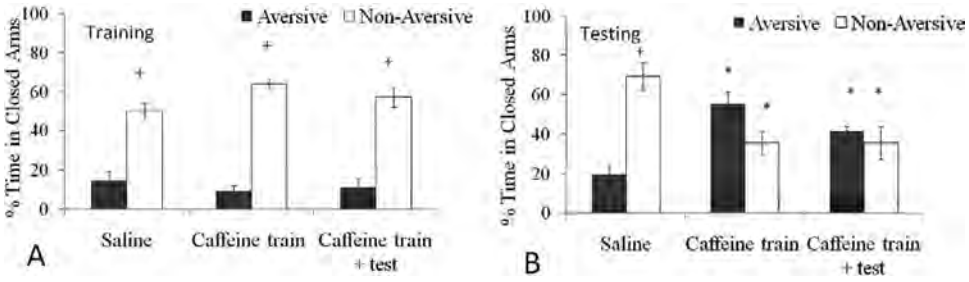



*Figure 2.* Effects of caffeine (40 mg/kg) administered at training only versus at training and testing on learning (percent time in the aversive and nonaversive arms). (A) There were no differences between groups at training. (B) Both caffeine-treated groups spent more time in the aversive arm and less time in the nonaversive arm than saline controls at testing ($n = 6$; mean ± *SEM*; * = significantly different from controls and + = significant difference between arms at $p < .05$).

ing and at training and testing. Thus, the learning deficit associated with the 40 mg/kg dose of caffeine is not because of 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, $F(4, 36) = 2.65$, $p < .05$. Duncan's post hoc tests revealed that the groups administered 10 to 40 mg/kg caffeine spent a significantly lower percentage of time in the open arms compared to saline controls ($p < .05$). There was no significant effect of caffeine dose on percent time in the open arms during testing (Figure 1C–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 1E–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, $F(1, 76) = 9.02$, $p < .001$, 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.* Because 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, $F(5, 30) = 4.73$, $p < .01$, and of arm, $F(1, 34) = 81.4$, $p < .001$, as well as a drug by arm interaction, $F(5, 30) = 6.95$, $p < .001$. Duncan's post hoc tests revealed that all groups spent more time in the nonaversive arm than in the aversive arm ($p < .05$), and the groups administered caffeine alone spent more time in the nonaversive arm than saline controls ($p < .05$). For testing, two-way ANOVA revealed no effect of group but a significant effect of arm, $F(5, 30) = 92.53$, $p < .001$ and a significant interaction, $F(5, 30) = 18.32$, $p < .001$. 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 nonaversive arm during testing than saline controls and the group administered 20 mg/kg caffeine alone ($p < .05$). In addition, only saline controls and the group administered 20 mg/kg caffeine alone spent significantly more time in the nonaversive 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 nonaversive arm (Figure 3A–B). Thus, both ethanol and caffeine impaired learning, and there was no reversal of the learning deficits after coadministration 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, $F(1, 34) = 43.67$, $p < .001$. There was also a significant effect of caffeine on percent time in the open arms during training, $F(2, 33) = 36.31$, $p < .001$, and an interactive effect of ethanol and caffeine on percent time in the open arms during training, $F(2, 33) = 5.10$, $p < .05$. 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 ($p < .05$). There was a significant effect of ethanol, $F(1, 34) = 7.84$, $p < .01$, 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 ($p < .05$) but there were no other group differences (Figure 3C–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, $F(5, 53) = 4.22$, $p < .01$, and a significant effect of session on entries per minute, $F(1, 57) = 12.05$, $p < .001$, as well as an interactive effect of group and session, $F(5, 53) = 2.87$, $p < .05$, 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 ($p < .05$). There were no other group differences. However, there were significant differences in entries per minute between the training and testing ses-

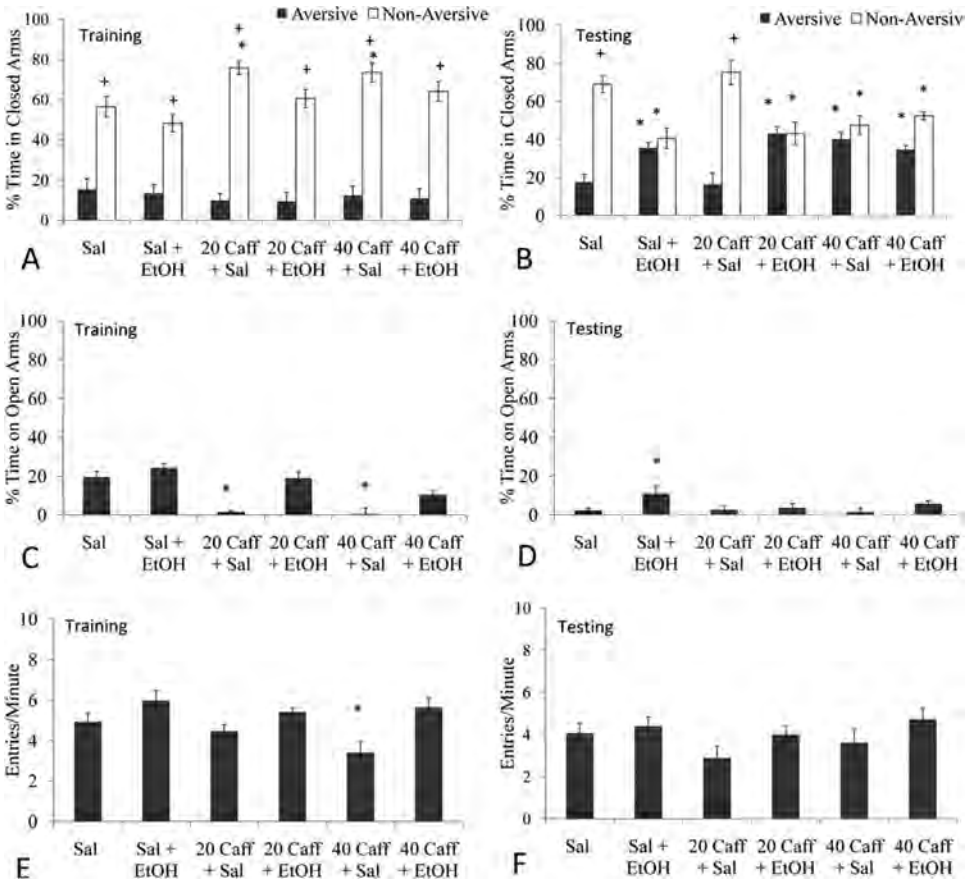



*Figure 3.* Effects of 1.0 g/kg ethanol and caffeine (20–40 mg/kg) administered at training. (A–B) Effects on learning (percent time in the aversive and nonaversive enclosed arms): (A) Caffeine (20 and 40 mg/kg) increased time in the nonaversive arm at training, and ethanol blocked this effect. (B) Both caffeine (40 mg/kg) and ethanol increased time in the aversive arm and decrease time in the nonaversive arm at testing ($n = 6–7$; mean ± *SEM*; * = significantly different from controls and + = significant difference between arms at $p < .05$). (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 ± *SEM*; * = significantly different from controls at $p < .05$). (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 ± *SEM*; * = significantly different from controls at $p < .05$).

sions; the ethanol-alone and both 20 mg/kg caffeine groups all made significantly fewer entries per minute during testing compared with training ($p < .05$), while there was no significant difference between sessions for the groups administered saline or 40 mg/kg caffeine (Figure 3E–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 coadministration 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, $F(1, 22) = 74.41$, $p < .001$, but no significant effect of group and no interaction (Figure 4A). Duncan's post hoc tests revealed that both ethanol groups spent less time in the nonaversive arm than saline controls ($p < .05$), and that all groups spent more time in the nonaversive arm than the aversive arm. For testing, two-way ANOVA revealed no effect of group but a significant effect of arm, $F(1, 22) = 35.96$, $p < .001$, and a significant interaction, $F(1, 22) = 17.30$, $p < .001$ (Figure 4B). Duncan's post hoc tests revealed that all experimental groups spent significantly more time in the aversive arm and less time in the nonaversive arm than controls at testing ($p < .05$), and only saline controls spent significantly more time in the nonaversive arm then the aversive arm ($p < .05$). 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 coadministration of the drugs.

*Percent time in the open arms.* There was a significant effect of ethanol on time in the open arms during training, $F(1, 23) =$

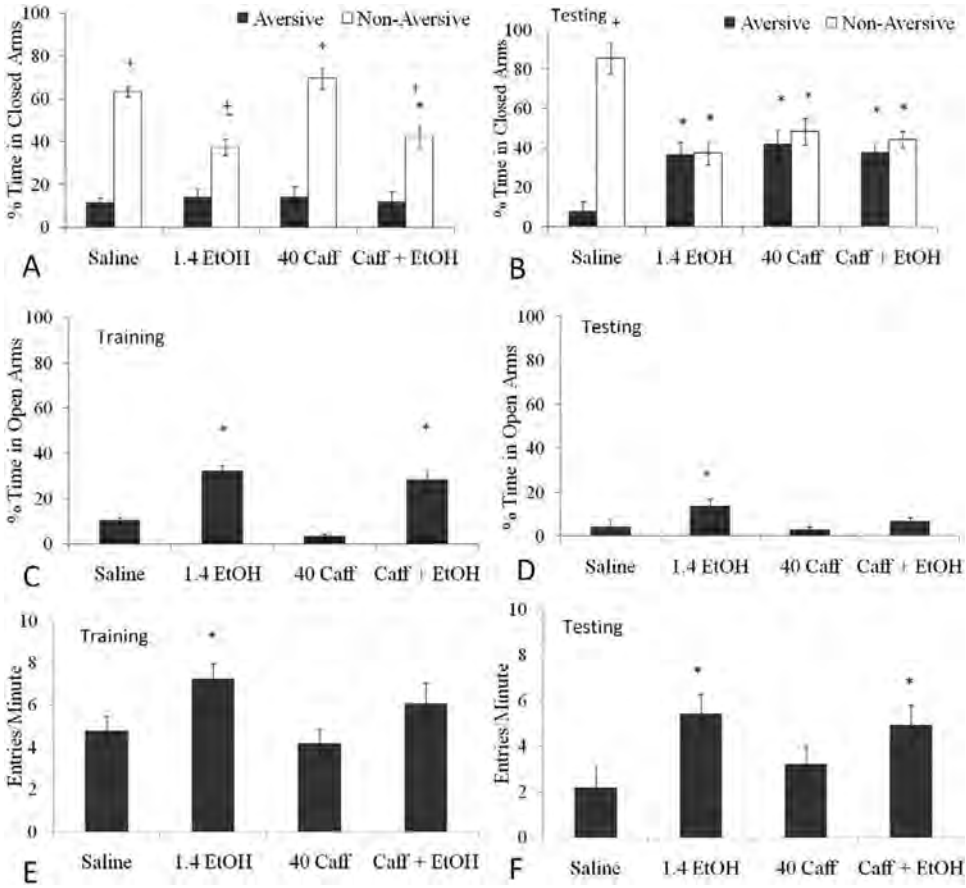



*Figure 4.* Effects of caffeine (40 mg/kg) and ethanol (1.4 g/kg) administered at training. (A–B) Effects on learning (percent time in the aversive and nonaversive arms): (A) Both ethanol-treated groups spent less time in the nonaversive arm than saline controls at training. (B) All experimental groups spent more time in the aversive arm and less time in the nonaversive arm than saline controls at testing ($n = 6$; mean ± *SEM*; * = significantly different from controls at $p < .05$). (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 ± *SEM*; * = significantly different from controls at $p < .05$). (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 ± *SEM*; * = significantly different from controls at $p < .05$).

19.9, $p < .001$. There was also a significant effect of caffeine on time in the open arms during training, $F(1, 23) = 6.68$, $p < .05$, but no interactive effect of ethanol and caffeine on time in the open arms during training (Figure 4C). Tukey's post hoc tests revealed that the groups administered ethanol spent significantly more time in the open arms than saline controls at training ($p < .05$), but there were no other group differences. There was a significant effect of ethanol, $F(1, 23) = 7.68$, $p < .05$, 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 4D). Tukey's post hoc tests revealed that the group administered ethanol alone spent more time in the open arms at testing ($p < .05$), but there were no other group differences.

*Total entries into all arms.* There was a significant effect of group on entries per minute, $F(4, 45) = 14.50$, $p < .001$ and a significant effect of session on entries per minute, $F(1, 47) = 21.14$, $p < .001$, but there was no interactive effect of group and session on entries per minute (Figure 4E–F). Tukey's post hoc tests revealed that ethanol increased entries compared to saline at training and testing ($p < .05$), and caffeine blocked this effect at training ($p < .05$) but not at testing. Thus, 1.4 g/kg ethanol increased locomotor behavior, and this effect was not fully reversed by caffeine coadministration. In addition, the control group and the ethanol-alone group made significantly fewer entries per minute during testing compared to training ($p < .05$), 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 nonaversive 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 coadministered 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 because of 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 coconsumption 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.

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