

# Alcohol-induced retrograde memory impairment in rats: prevention by caffeine

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## Abstract

**Rationale** Ethanol and caffeine are two of the most widely consumed drugs in the world, often used in the same setting. Animal models may help to understand the conditions under which incidental memories formed just before ethanol intoxication might be lost or become difficult to retrieve.

**Objectives** Ethanol-induced retrograde amnesia was investigated using a new odor-recognition test.

**Materials and methods** Rats thoroughly explored a wood bead taken from the cage of another rat, and habituated to this novel odor (N1) over three trials. Immediately following habituation, rats received saline, 25 mg/kg pentylentetrazol (a seizure-producing agent known to cause retrograde

amnesia) to validate the test, 1.0 g/kg ethanol, or 3.0 g/kg ethanol. The next day, they were presented again with N1 and also a bead from a new rat's cage (N2).

**Results** Rats receiving saline or the lower dose of ethanol showed overnight memory for N1, indicated by preferential exploration of N2 over N1. Rats receiving pentylentetrazol or the higher dose of ethanol appeared not to remember N1, in that they showed equal exploration of N1 and N2. Caffeine (5 mg/kg), delivered either 1 h after the higher dose of ethanol or 20 min prior to habituation to N1, negated ethanol-induced impairment of memory for N1. A combination of a phosphodiesterase-5 inhibitor and an adenosine A<sub>2A</sub> antagonist, mimicking two major mechanisms of action of caffeine, likewise prevented the memory impairment, though either drug alone had no such effect. Binge alcohol can induce retrograde, caffeine-reversible disruption of social odor memory storage or recall.

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## Introduction

High doses of ethanol can cause amnesia, or impaired retrieval of memory, evidenced after the drug wears off (Goodwin 1995; Hartzler and Fromme 2003; Wixted 2005). Complete or partial memory impairment occurs commonly in both alcoholics and nonalcoholics and usually results from episodes of binge drinking (White 2003). This memory impairment may reflect a disruption of encoding, storage, consolidation, and/or retrieval capability (Birnbaum et al. 1978; Melia et al. 1996; Gold 2006; Matthews et al. 1999; Weitemier and Ryabinin 2003; Matthews and Silvers 2004;

Miller and Matzel 2006; Sara and Hars 2006). However, the impairment might also be due to ethanol's effects on attention, sensorimotor function, emotion, or motivation, the disruption of which could affect information handling, learning, and encoding efficiency (Ryabinin et al. 2002; Nader and Wang 2006). It is difficult to isolate the relative contribution of these processes in part because memory-degrading doses of ethanol might interfere with multiple brain mechanisms essential for optimal encoding or for recall after brain levels of alcohol recede.

One approach to this problem is to administer ethanol after learning. Hindrances to encoding are ruled out because the animal is not intoxicated during learning or when tests of memory are later conducted. In humans (Parker et al. 1980; Bruce and Pihl 1997) and animals (Alkana and Parker 1979; Prediger and Takahashi 2003; Prediger et al. 2004; Manrique et al. 2005), ethanol delivered after learning generally enhances or has little effect on memory examined the next day. However, the doses of ethanol in these studies were typically low. Also, the types of memories examined had high emotional impact (e.g., fear learning), which may have rendered them relatively resistant to retrograde disruption.

We investigated the retrograde amnesic effects of ethanol on a more incidental form of memory, the type most vulnerable to ethanol in humans. We used a simple and sensitive olfactory learning and memory test modified from related procedures (e.g., Tillerson et al. 2006; Crews et al. 2007; Ennaceur et al. 1997; Felt et al. 2006; Steckler et al. 1998; Mumby 2005). Following learning, we exposed rats to a high dose of ethanol (3.0 g/kg). One day later, they showed retrograde memory impairment.

We also investigated whether caffeine, or a combination of two agents with mechanisms of action shared by caffeine, might prevent retrograde memory impairment when delivered 1 h after postlearning exposure to ethanol. Caffeine is often consumed before, during, or shortly after ethanol in humans. In animals, caffeine has been reported to enhance memory (Angelucci et al. 1999; Prediger et al. 2005a, c; McLean et al. 2005), possibly due to phosphodiesterase (PDE) inhibition, blockade of adenosine receptors (Howell et al. 1997; Fredholm et al. 1999; Prediger et al. 2005b), or a combination of these and other mechanisms (Simola et al. 2008).

## Materials and methods

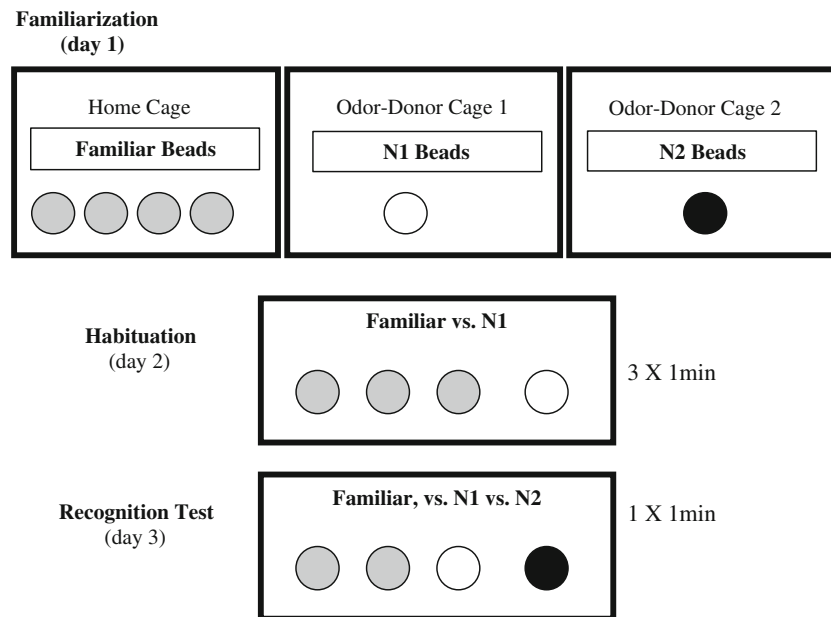
**Animals** Male Long–Evans rats (weighing approximately 200–400 g), obtained either from an on-site animal colony or from Harlan or Charles River Laboratories, were used. In a follow-up experiment to confirm that ethanol can cause retrograde memory impairment in another strain of rats, locally bred Sprague–Dawley rats (200–400 g) were used.

Animals were housed three per cage in clear polycarbonate cages with wood shavings, maintained under a 12:12-h light/dark cycle, and given access to food and water ad libitum. One week after arriving in the laboratory animal colony, rats were handled and familiarized to the researchers and to the testing conditions, including transient social isolation. Behavioral testing occurred over a 3-day period, during the dark cycle, in the laboratory colony room where the animals were housed. All animal care and experimental procedures were approved by the University of Texas at Austin's Institutional Animal Care and Use Committee.

**Prehabitation procedures** Animals were removed from group-housing cages, weighed, and rehoused singly in identical cages with sawdust bedding and removable wire tops. Once singly housed, animals remained in these test cages for the duration of the experiment. During the initial 24-h familiarization period, four 2.5-cm round wooden beads, each with a small hole bored through its diameter (<http://www.craftworks.com>) were introduced into the test cages in order to acquire the odor of the animal and to serve as familiar odors for subsequent use in the experiment. Housing the animals in the test cages with the beads for 24 h allowed for familiarization to both the testing environment and the presence of the beads.

Several beads were also introduced into the cages of three previously selected odor-donor groups (housed three rats per cage), whose cages had not been changed for 1 week to allow for a build-up of animal-specific novel odors. Wood beads incubated in these odor-donor cages provided equally salient novel odors for the upcoming task (Fig. 1). The hole channeled through the bead was found in pilot work to enhance exploration (possibly due to increased focal odor absorption). Because they were round and large, little or no gnawing took place during incubation or testing; however, only intact beads were used. The cages designated to provide donor odor beads were counter-balanced, so that any one odor served as either a recently novel odor (N1) or a brand new novel odor (N2) during memory assessment for different experimental rats.

**Habituation to the first novel odor** During the habituation phase of the task, after 24 h of familiarization to the presence of four beads in the testing environment, the four now-familiar beads were removed for 1 h. After this 1-h period, a novel-odor wood bead (N1), taken from an odor-donor cage, and three familiar beads that had been taken from their own cages 1 h previously were introduced into the cage. They were exposed to these four beads for three 1-min trials with 1-min intertrial intervals during which the beads were removed from the testing enclosure (Fig. 1). This procedure produces habituation to N1 while minimizing olfactory adaptation.



**Fig. 1** Representation of the experimental procedure (circles correspond to odor beads and are color coded to match the figures)

For each 1-min trial, the three familiar-odor beads and the N1 bead were placed in the middle of the testing cage, and the rats were allowed 1 min to actively explore the beads. The first approach to a bead made during this period initiated the timing of the 1-min trial. Exploration time for each of the four beads was recorded by experimenters blind to which beads were familiar or novel (beads were number-coded). The spatial arrangement of the beads in the middle of the cage was randomly altered between trials. To maximize the sensitivity of the test, one novel (N1) and three familiar-odor beads were used during habituation trials rather than N1 only. Similarly, during memory retention assessment (below), four beads were used (N1, N2, and two familiar beads) rather than N1 and N2 only. The four-choice procedure for assessing relative odor preference greatly increases power (sensitivity and reliability) compared to two-choice procedures typically used in recognition memory tests.

**Odor-recognition memory assessment** Twenty-four hours after the novel-odor habituation phase, the odor-recognition test was conducted. For this phase of the task, rats were presented with the odor N1 bead (which it had thoroughly explored on the previous day) in the presence of one unfamiliar novel-odor bead (N2) taken from a different odor-donor cage and two familiar (own-cage) odor beads, following the same procedure outlined for the habituation phase (see Fig. 1). To dismiss scent marking as a confound, the N1 bead was discarded after habituation and replaced by another N1 bead taken from the same odor-donor cage for the recognition memory phase. Movies of rat behavior during the habituation and odor-recognition test phases can be viewed on our website at <http://www.schallertlab.org>.

**Pharmacological treatments** Using the above-described behavioral procedures, PTZ or ethanol was delivered immediately after habituation to N1. Memory recall or retrograde memory impairment was assessed the following day by examining significant preference or nonpreference for exploring N2 over N1.

**Pentylentetrazol** To demonstrate that retrograde memory impairment could be evaluated using the new social odor-recognition test, rats were treated with the established seizure-producing drug and amnesic agent, PTZ (Sigma; dissolved in saline and administered at 25 mg/kg, intraperitoneally [i.p.]) immediately following the last habituation trial with N1. PTZ has been used previously to cause retrograde amnesia (Baratti 1987). This dose is sufficient to produce a brief, mild seizure within minutes of the injection but low enough that the seizures were not long-lasting (Hernandez and Schallert 1988). PTZ-treated rats were pooled ( $n=26$ ) from separate experiments carried out either alone or in tandem with experiments examining ethanol's effects. A small group of matched controls ( $n=7$ ) were administered equivalent volumes (1 ml/kg, i.p.) of saline vehicle. Assessment of memory for N1 was carried out 24 h after PTZ was administered, as outlined above and in Fig. 1.

**Lower-dose ethanol** Immediately following the last N1 habituation trial, animals ( $n=13$ ) were given i.p. injections of 20% ( $w/v$ ) ethanol, a concentration that was determined based on an extensive pilot work and a review of the literature, at a dose of 1.0 g/kg ethanol with matched controls ( $n=7$ ) receiving equivalent volumes of saline i.p. The next day, overnight memory for N1 was assessed.

**Higher-dose ethanol** Immediately following the last habituation trial, Long–Evans rats ( $n=25$ ) were given i.p. injections of 20% ( $w/v$ ) ethanol at a dose of 3.0 g/kg ethanol with matched controls ( $n=11$ ) receiving equivalent volumes of saline. Sprague–Dawley rats were treated identically in a second experiment to determine whether another strain would show retrograde memory impairment with a high dose of ethanol (ethanol group  $n=13$ ; saline group  $n=13$ ). In both strains, assessment of memory for N1 was carried out 24 h after ethanol or saline was administered, as outlined above and in Fig. 1. Pilot work examining the effects of 2.0 g/kg ethanol suggested that this intermediate dose causes retrograde amnesia in some animals and no memory impairment in others.

**Veisalgia (“hangover”) control** Rats were given i.p. injections of either 20% ( $w/v$ ) ethanol at a dose of 3.0 g/kg ( $n=6$ ) or saline ( $n=6$ ) at 24 h, rather than immediately, after habituation. Twenty-four hours after this, all rats were tested for recognition memory. In this procedure, the longer time-span between learning and ethanol administration was sufficient for memory consolidation to occur without disruption, yet comparable veisalgia signs would presumably still be present during the recognition test, which was carried out 24 h after administration of high-dose ethanol, as in the other experiments.

**Caffeine and related drugs** To examine whether caffeine might negate retrograde memory impairment produced by the higher dose of ethanol, this drug was delivered, in separate experiments, either 1 h after ethanol or 20 min before habituation to N1. We also investigated whether a PDE5 inhibitor, an  $A_{2A}$  antagonist, or their combination could likewise negate ethanol-induced retrograde memory impairment when delivered 1 h after ethanol exposure.

**Caffeine postethanol** Rats ( $n=9$ ) were given injections of 3.0 g/kg ethanol with matched saline-injected controls ( $n=9$ ) immediately following the last N1 habituation trial. Then, after a 1-h delay, these rats (all 18) received i.p. injections of 5 mg/kg caffeine dissolved in saline. They were then left alone until odor-recognition testing the following day. This dose of caffeine was based on previous studies indicating that it was a dose comparable to human caffeine consumption (Fredholm et al. 1999). Also, pilot work indicated that this was consistently effective in preventing ethanol-induced retrograde memory impairment but does not prevent or reverse the ethanol-induced loss of righting or, in the case of the preethanol study, cause hyperactivity that might interfere with exploration of the novel odors.

**Caffeine preethanol** Twenty minutes before the first novel-odor (N1) habituation trial, rats were given i.p. injections of

5 mg/kg caffeine (MP Biomedicals; dissolved at 5 mg/ml in saline). Then, following the last N1 habituation trial, subgroups of these caffeine-treated animals were given either 20% ( $w/v$ ) ethanol at a dose of 3.0 g/kg, i.p. ( $n=23$ ) or equivalent volumes of saline ( $n=20$ ). Assessment of memory for N1 was carried out 24 h after ethanol was administered.

**PDE5 inhibitor postethanol** Rats ( $n=10$ ) were given injections of 3.0 g/kg ethanol with matched saline-injected controls ( $n=4$ ) immediately following the last N1 habituation trial. Then, after a 1-h delay, all of these rats received i.p. injections of the PDE5 inhibitor zaprinast (Tocris Bioscience; dissolved in 100% DMSO; 10 mg/0.1 ml/kg; Fredholm et al. 1999). They were then left alone until odor-recognition testing the following day. In a follow-up test, six additional rats received twice the dose of zaprinast (20 mg/kg) 1 h after habituation to N1 and ethanol and were tested the next day for recognition memory.

**$A_{2A}$  antagonist postethanol** Rats ( $n=11$ ) were given injections of 3.0 g/kg ethanol with matched saline-injected controls ( $n=6$ ) immediately following the last N1 habituation trial. Then, after a 1-h delay, these rats (all 17) received i.p. injections of 1 mg/0.1 ml/kg ZM241385 (Tocris Bioscience; dissolved in 100% DMSO; see Fredholm et al. 1999). The rats were then left alone until odor-recognition testing the following day.

**Combination PDE5 inhibitor and  $A_{2A}$  antagonist postethanol** Immediately following the last trial of habituation to N1, rats ( $n=8$ ) were given injections of 3.0 g/kg ethanol with matched saline-injected controls ( $n=7$ ). Then, after a 1-h delay, these rats (all 15) received i.p. injections of both 10 mg/kg zaprinast and 1 mg/kg ZM241385. They were then left alone until odor-recognition testing the following day.

**Analysis of N1 salience and habituation** More exploration of N1 than of familiar odors on the first habituation trial was regarded as evidence for novel-odor salience, and the statistical significance of this difference was assessed using a within-subjects  $t$  test. Similarly, a significant reduction in N1 exploration time between the first and last (third) habituation trials was regarded as evidence of adequate habituation to N1 (i.e., learning), and this difference was also assessed using a within-subjects  $t$  test.

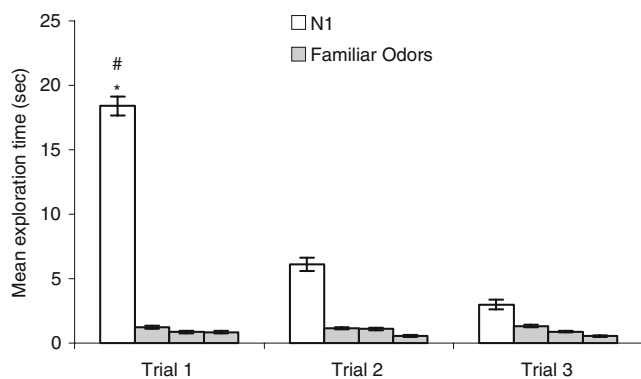
**Analysis of memory for recently novel odor N1** On the final day of the task (odor-recognition test), rats were allowed to explore the N1 bead, the N2 bead, and two own-cage “foil” beads (Fig. 1). The focus was to assess overnight memory for the N1 bead. Overnight memory for N1 was indicated

by significantly more time spent exploring N2 than N1 on the first trial of the test phase, as determined by within-subjects *t* tests. Cohen's *d* was also calculated as a measure of effect size for the difference in percent time spent exploring N1 versus N2.

## Results

**Habituation to N1** Figure 2 shows the habituation behavior for all groups receiving no drug treatment prior to habituation, behavior that was virtually identical in all experiments. Rats showed a significant reduction in the amount of time spent exploring N1 between trial 1 and trial 3, suggesting marked habituation as seen in Table 1. Initially, of course, N1 was much more salient than the familiar odors. A comparison of individual odor exploration within the first trial revealed that rats spent significantly more time exploring N1 than the familiar own-cage odor beads, as seen in Table 2. These animals persisted in exploring N1 more than the own-cage odors across habituation trials; however, as seen in Fig. 2, there was a dramatic reduction in the mean difference between the time spent exploring N1 and the average time spent exploring the three familiar odors on each subsequent trial (means±SE were 13.1±0.7 s in trial 1, 3.3±0.4 s in trial 2, and 0.9±0.3 s in trial 3).

**PTZ** The seizure/amnesia-inducing drug PTZ appeared, as expected, to cause retrograde memory impairment. Recognition memory test data for the saline- versus PTZ-treated rats are shown in Supplementary Fig. 1 and Table 3. Data are expressed as the mean percent of total exploration time, calculated by dividing the mean time spent exploring each individual odor by the total time spent exploring all odor beads during the first 1-min trial. Absolute mean explora-



**Fig. 2** Novel-odor preference and habituation to a novel odor across three trials. #*p*<0.01, significantly more time exploring the novel odor than familiar odors within the first trial; \**p*<0.01, significant reduction in time spent exploring the novel odor between trial 1 and trial 3. Data are the means±SEM

**Table 1** Habituation to N1

Group	N1 trial 1	N1 trial 3	<i>t</i> value	<i>p</i> value
No drug	18.3±0.73	3.0±0.37	21.6	<0.0001
Caffeine before habituation	13.3±1.3	3.6±0.66	7.4	<0.0001

Data reflect the mean (±SEM) exploration time (in seconds) of N1 on the trial indicated

tion times are summarized separately in Table 4. Control rats spent significantly more time exploring the new novel odor (N2) than the recently novel odor (N1) whereas PTZ-treated rats did not show a difference in exploration of N1 versus N2, which is consistent with an impairment of overnight memory for N1.

**Lower-dose ethanol** Recognition memory 24 h after habituation to N1 was not detectably affected by administration of the lower dose of ethanol after learning. Data for the saline- versus ethanol 1.0 g/kg-treated rats are shown in Supplementary Fig. 2 and Table 3. Both control and ethanol-treated rats spent significantly more time exploring N2 than N1, indicating retained memory for N1 in both groups.

**Higher-dose ethanol** The higher dose of ethanol led to an outcome consistent with severe retrograde memory impairment. Recognition memory was undetectable 24 h after ethanol, which had been delivered immediately after habituation to N1. Data for the saline- vs. ethanol 3.0 g/kg-treated rats are shown in Fig. 3 and Table 3. Control rats explored N2 significantly more than N1 whereas rats receiving 3.0 g/kg ethanol did not. This effect held in both strains of rats tested, Sprague–Dawley and Long–Evans.

**Veisalgia control** When habituation was followed 24 h later (rather than immediately) by high-dose ethanol and recognition memory was assessed 24 h after that, both control and ethanol-treated rats spent significantly more time exploring the novel odor N2 than N1, indicating retained memory for N1 as seen in Table 3. Thus, memory for N1 was detectable 48 h after habituation and there were no residual (veisalgia) effects of ethanol administered 24 h earlier that conceivably might have adversely affected

**Table 2** Novel-odor preference in the habituation phase

Group	N1	Familiar odors	<i>t</i> value	<i>p</i> value
No drug	18.3±0.73	1.2±0.12	22.9	<0.0001
Caffeine before habituation	13.3±1.3	0.72±0.1	9.5	<0.0001

Data reflect the mean (±SEM) exploration time (in seconds) of the odor indicated during the first habituation trial

**Table 3** Mean proportion exploration time ( $\pm$ SEM) in the odor-recognition test with statistics

Group	n	Odors				t value	p value	Effect size
		N1	N2	Familiar	Familiar			
PTZ	26	<i>0.35<math>\pm</math>0.03</i>	<i>0.41<math>\pm</math>0.04</i>	<i>0.10<math>\pm</math>0.01</i>	<i>0.12<math>\pm</math>0.01</i>	0.99	0.325	0.27
Control (saline)	7	0.25 $\pm$ 0.03	0.60 $\pm$ 0.04	0.02 $\pm$ 0.007	0.11 $\pm$ 0.047	5.29	<0.0001	2.82
High-dose ethanol (Sprague–Dawley)	13	<i>0.45<math>\pm</math>0.08</i>	<i>0.47<math>\pm</math>0.08</i>	<i>0.04<math>\pm</math>0.01</i>	<i>0.02<math>\pm</math>0.006</i>	0.24	0.812	0.09
Control (saline; Sprague–Dawley)	13	0.23 $\pm$ 0.03	0.63 $\pm$ 0.04	0.07 $\pm$ 0.02	0.05 $\pm$ 0.02	6.97	<0.0001	2.7
High-dose ethanol (Long–Evans)	25	<i>0.47<math>\pm</math>0.03</i>	<i>0.45<math>\pm</math>0.03</i>	<i>0.04<math>\pm</math>0.01</i>	<i>0.02<math>\pm</math>0.004</i>	0.24	0.809	0.07
Control (saline; Long–Evans)	11	0.31 $\pm$ 0.05	0.61 $\pm$ 0.05	0.05 $\pm$ 0.01	0.01 $\pm$ 0.004	4.18	<0.0001	1.78
Ethanol (veisalgia)	6	0.27 $\pm$ 0.05	0.68 $\pm$ 0.05	0.01 $\pm$ 0.01	0.02 $\pm$ 0.004	5.23	0.0001	3.02
Saline controls (veisalgia)	6	0.29 $\pm$ 0.06	0.62 $\pm$ 0.07	0.03 $\pm$ 0.01	0.04 $\pm$ 0.01	3.43	0.006	1.98
Low-dose ethanol	13	0.26 $\pm$ 0.05	0.61 $\pm$ 0.06	0.08 $\pm$ 0.03	0.04 $\pm$ 0.009	4.23	<0.0001	1.65
Control (saline)	7	0.31 $\pm$ 0.07	0.57 $\pm$ 0.06	0.02 $\pm$ 0.007	0.07 $\pm$ 0.03	2.51	0.028	1.34
Caffeine before habituation+ethanol	23	0.27 $\pm$ 0.04	0.56 $\pm$ 0.04	0.06 $\pm$ 0.009	0.09 $\pm$ 0.02	4.75	<0.0001	1.4
Control (caffeine+saline)	20	0.33 $\pm$ 0.05	0.53 $\pm$ 0.05	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01	2.55	0.015	0.8
Ethanol+caffeine 1 h after	9	0.26 $\pm$ 0.04	0.62 $\pm$ 0.05	0.03 $\pm$ 0.007	0.07 $\pm$ 0.01	4.87	<0.0001	2.29
Control (saline+caffeine)	9	0.31 $\pm$ 0.07	0.58 $\pm$ 0.06	0.03 $\pm$ 0.01	0.05 $\pm$ 0.01	2.78	0.015	1.39
Ethanol+PDE5 inhibitor	16	<i>0.46<math>\pm</math>0.11</i>	<i>0.49<math>\pm</math>0.12</i>	<i>0.01<math>\pm</math>0.01</i>	<i>0.02<math>\pm</math>0.01</i>	0.41	0.686	0.18
Control (saline+PDE5 inhibitor)	4	0.18 $\pm$ 0.04	0.77 $\pm$ 0.04	0.01 $\pm$ 0.006	0.02 $\pm$ 0.008	8.76	<0.0001	6.19
Ethanol+A <sub>2A</sub> antagonist	11	<i>0.39<math>\pm</math>0.07</i>	<i>0.49<math>\pm</math>0.07</i>	<i>0.05<math>\pm</math>0.01</i>	<i>0.04<math>\pm</math>0.01</i>	0.98	0.338	0.41
Control (saline+A <sub>2A</sub> antagonist)	6	0.28 $\pm$ 0.11	0.64 $\pm$ 0.09	0.03 $\pm$ 0.01	0.03 $\pm$ 0.01	2.40	0.037	1.38
Ethanol+PDE5 inhibitor+A <sub>2A</sub> antagonist	8	0.27 $\pm$ 0.07	0.64 $\pm$ 0.07	0.02 $\pm$ 0.006	0.05 $\pm$ 0.01	3.63	0.003	1.81
Control (saline+both drugs)	7	0.21 $\pm$ 0.04	0.65 $\pm$ 0.04	0.08 $\pm$ 0.03	0.04 $\pm$ 0.009	6.19	<0.0001	3.31

Italicized values indicate groups where the comparison between N1 and N2 was not statistically significant, indicating a loss of overnight memory for N1

performance in some way. More importantly, pertinent to the previous experiment in which the high dose of ethanol was delivered immediately after habituation, it is not likely that veisalgia effects contributed to the lack of preferential exploration of N2 over N1 in the recognition memory task carried out the next day. It follows also that the ability of caffeine to negate ethanol-induced retrograde memory impairment (described below) is not likely due to the prevention of veisalgia-related retrieval problems.

**Caffeine postethanol** Caffeine delivered 1 h after exposure to N1 prevented retrograde recognition memory disruption by the higher dose of ethanol (Fig. 4 and Table 3). Both control and ethanol 3.0 g/kg-treated rats spent significantly more percent time exploring N2 than N1.

**Caffeine preethanol** Caffeine delivered 20 min before the first exposure to N1 prevented retrograde recognition memory disruption by a subsequent high dose of ethanol. Recognition of N1 appeared to be intact the next day despite the administration of 3.0 g/kg ethanol following habituation. The behavior of rats that received caffeine 20 min prior to habituation is shown in Supplementary Fig. 3 and Table 3. Both control and ethanol 3.0 g/kg-treated rats exposed to caffeine prehabitation spent

significantly more time exploring N2 than N1. Furthermore, caffeine administered prior to habituation trials did not affect habituation to N1 or absolute bead exploration times. Greater exploration of N1 than familiar own-cage odors was followed by normal habituation over trials, as shown in Supplementary Fig. 3 (left side).

**PDE5 inhibitor postethanol** Neither dose of the PDE5 inhibitor, delivered 1 h after exposure to N1, prevented ethanol-induced retrograde recognition memory disruption (Supplementary Fig. 4 and Table 3). There were no differences in outcome between the ethanol 3.0 g/kg-treated rats that received 10 or 20 mg/kg zaprinast; therefore, these groups were combined for analysis. Ethanol-treated rats showed no difference in percent time exploring N1 versus N2, whereas control rats spent significantly more time exploring N2 than N1.

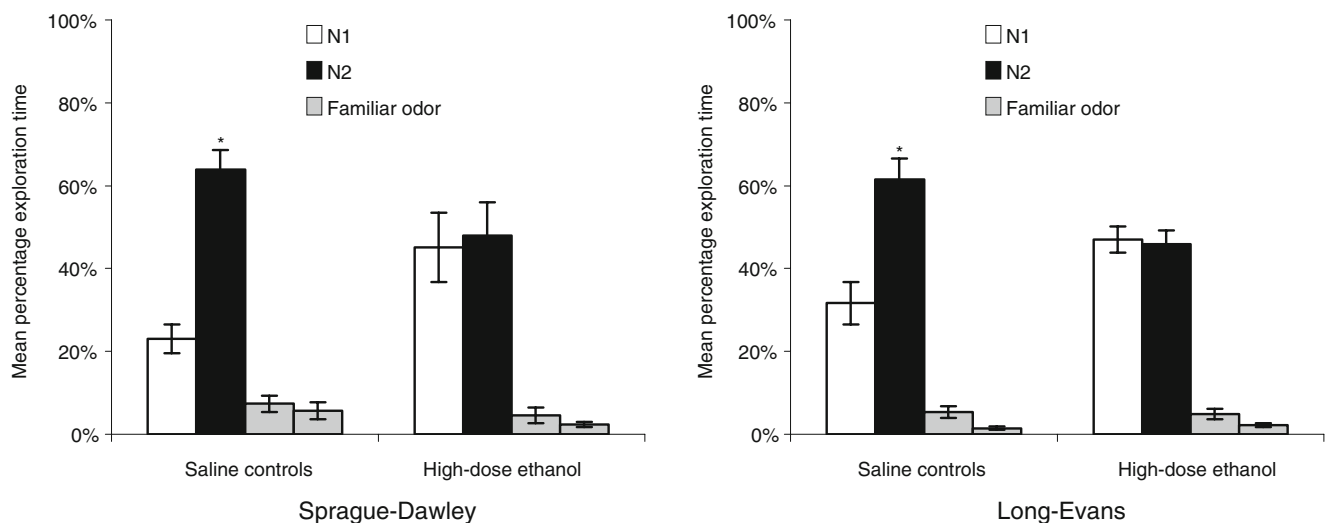
**A<sub>2A</sub> antagonist** The A<sub>2A</sub> antagonist, ZM241385, by itself did not negate ethanol-induced retrograde memory impairment. Supplementary Fig. 5 and Table 3 show that rats receiving this drug 1 h after habituation to N1 and subsequent exposure to a high dose of ethanol did not preferentially explore either N1 or N2 when tested for recognition memory 24 h later, in contrast to control rats which significantly preferred N2.

**Table 4** Mean ( $\pm$ SEM) absolute exploration times (in seconds) in the odor-recognition test

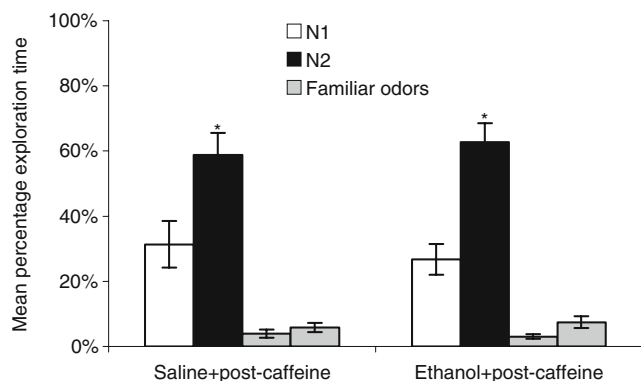
Group	Odors			
	N1	N2	Familiar	Familiar
PTZ	3.04 $\pm$ 0.55	3.46 $\pm$ 0.69	1.00 $\pm$ 0.18	0.78 $\pm$ 0.12
Control (saline)	3.59 $\pm$ 0.54	9.41 $\pm$ 1.50	0.30 $\pm$ 0.09	1.60 $\pm$ 0.67
High-dose ethanol (Sprague–Dawley)	5.57 $\pm$ 1.30	5.87 $\pm$ 1.40	0.37 $\pm$ 0.13	0.29 $\pm$ 0.08
Control (saline; Sprague–Dawley)	2.22 $\pm$ 0.61	6.65 $\pm$ 1.60	0.42 $\pm$ 0.08	0.29 $\pm$ 0.08
High-dose ethanol (Long–Evans)	8.09 $\pm$ 0.88	9.81 $\pm$ 1.80	0.72 $\pm$ 0.14	0.39 $\pm$ 0.09
Control (saline; Long–Evans)	5.19 $\pm$ 1.20	9.82 $\pm$ 1.60	0.85 $\pm$ 0.33	0.20 $\pm$ 0.06
Ethanol (veisalgia)	7.13 $\pm$ 1.80	17.65 $\pm$ 3.32	0.33 $\pm$ 0.16	0.47 $\pm$ 0.10
Saline control (veisalgia)	6.40 $\pm$ 1.87	13.68 $\pm$ 3.35	0.72 $\pm$ 0.36	0.87 $\pm$ 0.47
Low-dose ethanol	4.38 $\pm$ 0.92	11.40 $\pm$ 2.30	0.62 $\pm$ 0.12	1.06 $\pm$ 0.35
Control (saline)	5.71 $\pm$ 1.80	10.50 $\pm$ 2.90	0.62 $\pm$ 0.24	1.02 $\pm$ 0.45
Caffeine before habituation+ethanol	2.75 $\pm$ 0.52	5.76 $\pm$ 0.87	0.68 $\pm$ 0.09	0.56 $\pm$ 0.07
Control (caffeine+saline)	4.70 $\pm$ 0.81	8.80 $\pm$ 1.50	0.85 $\pm$ 0.16	0.79 $\pm$ 0.14
Ethanol+caffeine 1 h after	4.67 $\pm$ 1.50	11.40 $\pm$ 2.30	1.15 $\pm$ 0.34	0.47 $\pm$ 0.13
Control (saline+caffeine)	4.94 $\pm$ 1.30	9.18 $\pm$ 2.50	0.61 $\pm$ 0.21	0.50 $\pm$ 0.17
Ethanol+PDE5 inhibitor	8.66 $\pm$ 1.60	10.20 $\pm$ 1.90	0.25 $\pm$ 0.09	0.52 $\pm$ 0.21
Control (saline+PDE5 inhibitor)	3.95 $\pm$ 2.50	12.10 $\pm$ 4.00	0.18 $\pm$ 0.08	0.50 $\pm$ 0.25
Ethanol+A <sub>2A</sub> antagonist	7.24 $\pm$ 2.50	7.38 $\pm$ 1.90	0.64 $\pm$ 0.25	0.49 $\pm$ 0.10
Control (saline+A <sub>2A</sub> antagonist)	5.50 $\pm$ 2.30	9.40 $\pm$ 3.10	0.35 $\pm$ 0.04	0.38 $\pm$ 0.05
Ethanol+PDE5 inhibitor+A <sub>2A</sub> antagonist	3.67 $\pm$ 0.97	9.85 $\pm$ 2.50	0.35 $\pm$ 0.08	0.80 $\pm$ 0.17
Control (saline+both drugs)	4.74 $\pm$ 1.10	15.60 $\pm$ 3.70	1.60 $\pm$ 0.68	1.04 $\pm$ 0.21

**PDE5 inhibitor+A<sub>2A</sub> antagonist** A combination of the PDE5 inhibitor (10 mg/kg zaprinast) and the adenosine A<sub>2A</sub> antagonist (1 mg/kg ZM241385) administered 1 h following the higher dose of ethanol prevented ethanol-induced retrograde memory impairment (Fig. 5 and Table 3). Both control and ethanol 3.0 g/kg-treated rats spent significantly more time exploring N2 than N1, indicating retained overnight memory for N1.

Animals in the 3-g/kg ethanol group, as expected, lost the righting reflex and appeared behaviorally to be asleep within minutes, and remained so for several hours. When injected with caffeine or the combination of the PDE5 inhibitor and A<sub>2A</sub> antagonist 1 h after ethanol administration, the righting reflex was not regained and there was no overt sign of rescue from the sleep-like behavior.



**Fig. 3** Higher-dose ethanol disrupts odor memory.  $*p < 0.01$ , significantly more time spent exploring the novel odor than the recently novel odor. Data are the means $\pm$ SEM. *Left* Sprague–Dawley rats, *right* Long–Evans rats

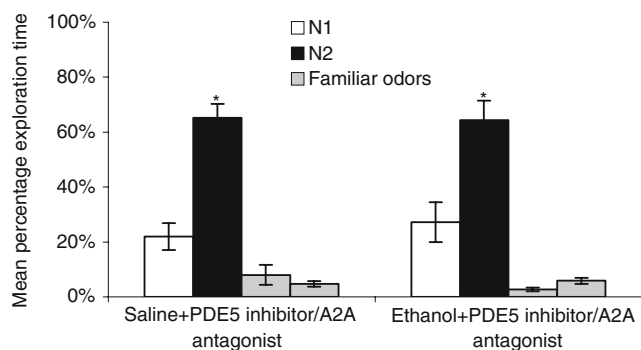


**Fig. 4** Caffeine administered after a postlearning high dose of ethanol also prevents memory disruption. \* $p < 0.05$ , significantly more time spent exploring the novel odor than the recently novel odor. Data are the means  $\pm$  SEM

## Discussion

Binge consumption of alcohol can cause memory impairments, but surprisingly little is known about these impairments even though they are common, potentially dangerous, socially and economically costly, and linked to alcohol abuse (Sweeney 1989; Anthenelli et al. 1994; Jennison and Johnson 1994; Buelow and Koeppl 1995; Buelow and Harbin 1996; Hartzler and Fromme 2003; Silvers et al. 2003; Matthews and Silvers 2004). To our knowledge, there are no studies in the literature indicating that these memory impairments might include retrograde mechanisms or that they might be preventable or their occurrence limited.

In the present study, it was found that a very high dose of ethanol, given immediately after exposure to a novel odor, led to retrograde memory impairment in a memory recall test conducted 1 day later. This memory impairment could be reversed with the administration of caffeine, a drug often contained in beverages consumed before, during, or after alcohol. Caffeine somehow prevented the ethanol-



**Fig. 5** The combination of a PDE5 inhibitor and an adenosine  $A_{2A}$  antagonist administered after a postlearning high dose of ethanol prevents disruption of odor memory. \* $p < 0.05$ , significantly more time spent exploring the novel odor than the recently novel odor. Data are the means  $\pm$  SEM

induced retrograde amnesia, not only when delivered just prior to learning and exposure to ethanol, but even more surprisingly also when delivered 1 h after ethanol.

Caffeine is both a phosphodiesterase inhibitor and an adenosine antagonist (Howell et al. 1997; Fredholm et al. 1999), although multiple mechanisms could be responsible for the observed reversal of ethanol's amnesic effects. As an adenosine antagonist, caffeine has equal affinity for  $A_1$  and  $A_{2A}$  subtypes of adenosine receptors (Prediger et al. 2005a). It has been shown that antagonists specific to the  $A_{2A}$  subtype (ZM241385; Yang et al. 2007), at doses of 1.0 mg/kg, and not antagonists specific to the  $A_1$  subtype of adenosine can improve memory in social odor-recognition tasks (Prediger et al. 2005b). Furthermore, as a phosphodiesterase inhibitor, caffeine is nonselective for multiple subtypes of phosphodiesterase while zaprinast, a potent inhibitor of the PDE5 subtype, has been shown to improve memory consolidation in an object recognition task and only at a dose of 10 mg/kg (Prickaerts et al. 1997, 2004; Blokland et al. 2006). Based on this literature, we used each of these drugs, zaprinast and ZM241385 separately and in unison, in a preliminary attempt to reverse ethanol-induced retrograde memory impairments.

Neither an adenosine  $A_{2A}$  antagonist nor a phosphodiesterase-5 inhibitor alone prevented retrograde amnesia when delivered 1 h after ethanol (unlike caffeine, which did prevent the amnesia). However, a combination of these two drugs was highly effective at the previously ineffective doses, which were selected on the basis of the dose of caffeine. Thus, it appears that mimicking two of caffeine's key mechanisms of action simultaneously with a PDE5 inhibitor and an  $A_{2A}$  antagonist is at least sufficient, if not necessarily required, for the prevention of retrograde amnesia by ethanol.

It remains possible that caffeine and related agents would not have negated memory impairments had the ethanol been administered during learning. However, to rule out nonspecific factors such as attention and sensorimotor function impairments that might influence how well or even whether a memory is laid down, the present study was designed to ensure that memory for N1 was established in a completely sober state. Binge alcohol-induced fragmentary blackouts in people may be primarily anterograde; however, the present study raises the possibility that at least some memory impairment could be retrograde. It remains, though, quite likely that emotionally charged, extremely salient memories would be resistant to retrograde disruption by ethanol. Indeed, we have found that highly salient memories are not reliably reversed retrogradely by a high dose of ethanol (data not shown).

It is unknown whether the ethanol-induced retrograde memory impairment was a consolidation deficit, a retrieval (memory accessibility) deficit, or both (Squire 2006).

Regardless, these data suggest that pharmacological manipulations might begin to shed new light on potential mechanisms of suppression and reversal of memory access.

It is unlikely that the effect of the higher dose of ethanol was related to reinforcement associated with its pairing with N1 because the lower dose of ethanol (which is still a substantial dose) did not enhance the salience of N1, in that 24 h later N1 was explored significantly less than N2 (indicating intact memory and no increase in interest in N1 relative to controls).

It is also unlikely that the effect of the high dose of ethanol was due to lingering effects that might influence performance, for several reasons. First, the total mean exploration times (combining N1 and N2) of the control and 3.0 g/kg ethanol-treated rats (see Table 4) were not very different between groups: control (11.94 s) and 3.0 g/kg ethanol (14.67 s). Rats exposed to the higher dose of ethanol 24 h earlier did not show reduced exploration. Secondly, the 3.0-g/kg dose of ethanol would be metabolized by the time the recognition test was performed 24 h after habituation. Third, a separate group of rats administered 3.0 g/kg ethanol 24 h after habituation, well past the time when memory consolidation should have been established and, therefore, would be resistant to disruption, showed apparently normal memory for N1 the following day, when any “veisalgia” effects would be assumed to be taking place. That is, on the day following the higher dose of ethanol, these rats had no problem distinguishing between N1 and N2, showing the greatest preference for N2 (the most novel of the two odors), while maintaining a preference for N1 relative to familiar home cage odors. Thus, in this follow-up veisalgia control experiment, memory recall for the N1 odor bead was intact 48 h after habituation to N1, which was 24 h after ethanol administration (matching the time-span from ethanol exposure to memory assessment in the experiment in which the high dose of ethanol was delivered immediately after habituation to N1). Fourth, rats treated with caffeine or the combination of the PDE5 inhibitor and the A<sub>2A</sub> antagonist 1 h after exposure to the higher dose of ethanol (ethanol delivered immediately after habituation to N1) showed memory retrieval comparable to control animals. Together, these four lines of evidence suggest that lingering veisalgia was not masking memory retrieval capacity.

Note that ethanol was always delivered acutely via i.p. injection. This delivery method, as well as repeated intermittent doses of ethanol, may produce some degree of stress (Zhang et al. 2007). However, the acute injection of the low dose of ethanol should have produced stress but did not produce retrograde memory impairment. Memory effects of the combination of injection stress or distraction and high-dose ethanol cannot be ruled out as a factor and should be further explored.

Ethanol has many different mechanisms of action. For example, ethanol at high doses is known to interfere with glutamatergic action at memory-linked NMDA, AMPA, and kainate receptors while it also enhances GABAergic synaptic transmission with a surprising degree of specificity in memory-related areas of the brain such as the hippocampus (White et al. 2000). NMDA antagonists are known to disrupt overnight memory (Creeley et al. 2006; Miserendino et al. 1990; Nilsson et al. 2007). Relevant to the effects of caffeine on ethanol-induced memory impairment, it has also been reported that ethanol can greatly increase extracellular levels of adenosine, leading to dysfunction of memory-linked cellular cascades (Dohrman and Diamond 1997; Mailliard and Diamond 2004).

It has been argued that for new learning to undergo consolidation (i.e., the transfer from a labile to stable state), protein synthesis may be critically involved (Flexner and Stellar 1965; Schafe and LeDoux 2000; Kandel 2001), and there is growing evidence that the activation of NMDA receptors is a crucial step in this process (Miserendino et al. 1990; Rodrigues et al. 2001; Riedel et al. 2003). Acute ethanol exposure can inhibit critical steps in at least some protein synthesis pathways, possibly through its antagonism of NMDA receptors (Chandler and Sutton 2005).

Ethanol's effects are widespread throughout the brain, and it will be difficult to learn which specific brain structures and mechanisms of action are necessary or sufficient to cause retrograde amnesia. However, Matthews and Silvers (2004) reviewed their own work and that of others and argue that ethanol's effects on memory may have remarkably specific action in the hippocampus, particularly by enhancing GABAergic potency at GABA-A receptors and by interfering with glutamate at NMDA receptors, to affect spatial memory. Memory-impairing effects of ethanol may influence GABAergic activity by increasing levels of allopregnanolone in the hippocampus. Indeed, finasteride (which reduces ethanol-induced allopregnanolone levels by almost 50%), when combined with ethanol, blocked ethanol-induced inhibition of hippocampal pyramidal neurons and prevented spatial memory deficits. These investigators go on to suggest that ethanol's potentiation of GABA-A receptor activity in the hippocampus may reduce hippocampal levels of acetylcholine. Anticholinergic drugs, like injury to the hippocampus directly or indirectly via traumatic brain injury or stroke, are known to interfere with hippocampal-dependent memory, particularly when the learning procedure requires strategy switching for optimal spatial performance (e.g., Day and Schallert 1996; Lindner and Schallert 1988; Choi et al. 2006).

In summary, we have refined a test for odor-recognition memory that can be carried out in the home cage, that rats can readily perform, and that results in an easily observable and relatively lasting form of memory that avoids some of

the problems associated with standard memory assessment models. Conspecific social odor memory was confirmed to be established in a sober state but was disrupted by subsequent ethanol intoxication. That is, ethanol appeared to cause impairment either of the capacity to adequately store a modestly salient memory or the ability to retrieve that memory, whereas caffeine and related agents appeared to prevent the memory impairment. These data may be useful for understanding how binge ethanol consumption can cause memory impairments in humans and how caffeine or other agents might ameliorate these impairments.

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