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Corticosterone protects against memory impairments and reduced hippocampal BDNF levels induced by a chronic low dose of ethanol in C57BL/6J mice

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Abstract

Acute low doses of ethanol can produce reversible memory deficits, but it is unknown whether they persist upon chronic use. We investigated whether the chronic intake of a low dose of ethanol induces memory impairments in the ethanol-preferring C57BL/6J mouse strain. Because stress precipitates alcohol abuse and the stress hormone corticosterone contributes to memory processes, ethanol consumption and toxic effects, we also determined the impact of co-treatment with corticosterone on these effects. BDNF contributes to memory function and toxic effects of ethanol, therefore its levels were quantified in the hippocampus and frontal cortex. Ethanol (1% in drinking water) and corticosterone (250 µg/mL) were administered using the two-bottle choice test to monitor their appetitive properties. Spatial and non-spatial memory performance was assessed using the spontaneous alternation, object recognition and object location tests. The chronic exposure to a low dose of ethanol caused spatial and non-spatial memory deficits after withdrawal associated with a reduction in hippocampal BDNF levels, which were prevented by co-treatment with corticosterone (~21 mg/kg/day). The protective effect of corticosterone on memory was no longer observed at higher doses (~41 mg/kg/day), but persisted for hippocampal BDNF levels. C57BL/6J mice did not develop an appetence for 1% ethanol, but the addition of corticosterone increased voluntary consumption of and preference for the ethanol+corticosterone solutions. Although acute low doses of corticosterone (1 mg/kg) were found to rescue established memory impairments, this is the first report of a protective effect of chronic doses of corticosterone in the range of 20–32 mg/kg, and particularly against memory deficits induced by alcohol.

Keywords: ethanol, corticosterone, memory, BDNF, C57BL/6J mice.

Introduction

Acute low to moderate doses of ethanol produce reversible cognitive deficits affecting specifically the use of spatial information as the hippocampus is particularly vulnerable to the deleterious effects of ethanol [1]. To our knowledge, the lowest acute dose of ethanol able to impair memory of adult mice is 0.5 g/kg [2]. This is, however, not a consistent finding and memory impairments are more likely to be observed at minimal doses ranging from 1–2 g/kg depending on the task and strain of mice used [3–5]. It is, however, unknown whether the chronic consumption of a low dose of ethanol, at the threshold for inducing acute cognitive deficits, will be well tolerated or whether it will produce lasting memory impairments. Indeed, amnesia induced by an acute pre-training dose of ethanol (0.5 and 1 g/kg) was found to be reversed by a second ethanol challenge administered prior to the memory retrieval phase [3, 6], but higher doses (*e.g.*, 1.5 g/kg) failed to rescue amnesic effects [7].

The first goal of the present study was, therefore, to investigate whether the chronic intake of a low dose of ethanol (~0.5 g/kg/day) induces memory impairments in the ethanol-preferring C57BL/6J mouse strain, which is also less sensitive to the memory disrupting effects of acute low to moderate doses of ethanol than non-preferring strains [5]. The second goal was to investigate whether co-administration of the stress hormone corticosterone impacts the cognitive effects of ethanol.

Corticosterone, the major glucocorticoid in rodents, plays a critical role in the regulation of the hypothalamic–pituitary–adrenal (HPA) axis, the major neuroendocrine system regulating stress responses, modulates learning and memory processes and is involved in ethanol consumption and toxic effects. Plasma corticosterone levels rise in response to acute ethanol challenges, but this response dampens with repeated exposure [8]. Stress or corticosterone administration facilitates ethanol self-administration and relapse after withdrawal [9, 10] whilst HPA axis function is compromised during ethanol dependence and after withdrawal [11]. Corticosterone also promotes spatial learning and memory in non-stressed rodents [12, 13] and under certain stressful test conditions [14, 15] whilst opposite effects are seen with long-term administration of stress levels of corticosterone [16]. Recently, ethanol has been reported to attenuate stress-induced memory impairment [17] but it is, however, unknown whether corticosterone modulates the cognitive effects of ethanol.

We therefore assessed the effects of a low dose of ethanol, supplemented or not with corticosterone, on spatial and non-spatial memory performance in C57BL/6J mice. Ethanol and corticosterone were administered in drinking water to avoid the stress of repeated injections, which, on its own, can produce memory impairments [18]. The concentration of corticosterone used (250 µg/mL) has previously been found to be sufficient to induce

memory impairments in mice [19] and the concentration of ethanol used (1%) results in a daily intake of ~0.5 g/kg based on the average fluid intake. Although this concentration of ethanol is below the range used for voluntary consumption and self-administration studies, corticosterone enhances the reinforcing properties of ethanol [20]. We, therefore, used the two-bottle choice test to monitor the possible development of a preference for the 1% ethanol ± corticosterone solutions. Because brain-derived neurotrophic factor (BDNF) signaling is thought to modulate cognitive function [21, 22] and ethanol-induced damage to the brain [23], levels of BDNF protein and its downstream effector extracellular-signal-regulated kinase 2 (ERK2) were quantified in the frontal cortex and hippocampus, two brain areas critical for learning and memory.

Materials and Methods

Animals

Forty-six 12-week-old C57BL/6J male mice (Charles River, UK) were individually caged under standard conditions: 12:12 light dark cycle with lights on at 7:00 a.m., food and drinking solutions *ad libitum*, and their body weights were recorded weekly. Five mice were excluded during the course of the study because of ill health or spontaneous death. Veterinary advice and post-mortem analysis excluded corticosterone treatment as the underlying cause. All procedures followed the ARRIVE guidelines [24] and were carried out in compliance with the *UK Animal Scientific Procedures Act* under project license 40/2715.

Two-bottle choice test

Drinking solutions

Ethanol 99% (Sigma-Aldrich UK) was dissolved in tap water to a final concentration of 1%. Corticosterone (Sigma-Aldrich, UK) was dissolved in 100% ethanol and then diluted in HPLC grade water to a final concentration of 250 μg/mL in 1% ethanol. Corticosterone administered *via* this route at doses ranging from 0.25 to 300 μg/mL elevates plasma levels of the hormone, primarily at night-time when most fluid is consumed [10]. Fluid (mL), corticosterone (mg/kg) and ethanol (g/kg) intake were calculated daily. Corticosterone and 1% ethanol are tasteless and cannot be discriminated easily in a two-bottle choice test. Inosine-5'-monophosphate (IMP, Sigma-Aldrich, UK) was, therefore, used to flavor some of the drinking solutions at a concentration of 696 μg/mL (2 mM). IMP has an umami taste [25] and this concentration was found to be discriminated by mice in a two-bottle choice test without altering total fluid intake [26]. In our experimental conditions, IMP was found to have a mildly aversive taste, thus also enabling us to influence the consumption of 1% ethanol+corticosterone and assess dose-dependent effects without the confounding effects of the stress of injection. Drinking solutions were freshly made three times per week and drinking tubes filled with 1% ethanol+corticosterone and/or IMP solutions were covered with aluminum foil to prevent light-induced degradation.

Experimental groups

The following combinations of control (water or IMP) or test (containing flavored or unflavored, 1% ethanol ± corticosterone) solutions were offered to the five experimental groups:

1 – Water *vs.* IMP (control – flavor group);

2 – Water *vs.* 1% ethanol;

3 – Water *vs.* IMP-flavored 1% ethanol+corticosterone (low consumers);

4 – Water *vs.* unflavored 1% ethanol+corticosterone (medium consumers);

5 – IMP *vs.* unflavored 1% ethanol+corticosterone (high consumers).

Side preference was prevented by alternating and counterbalancing the position of the two drinking tubes daily.

The experimental design showing the timeline of the two-bottle choice test is fully described in Figure 1.

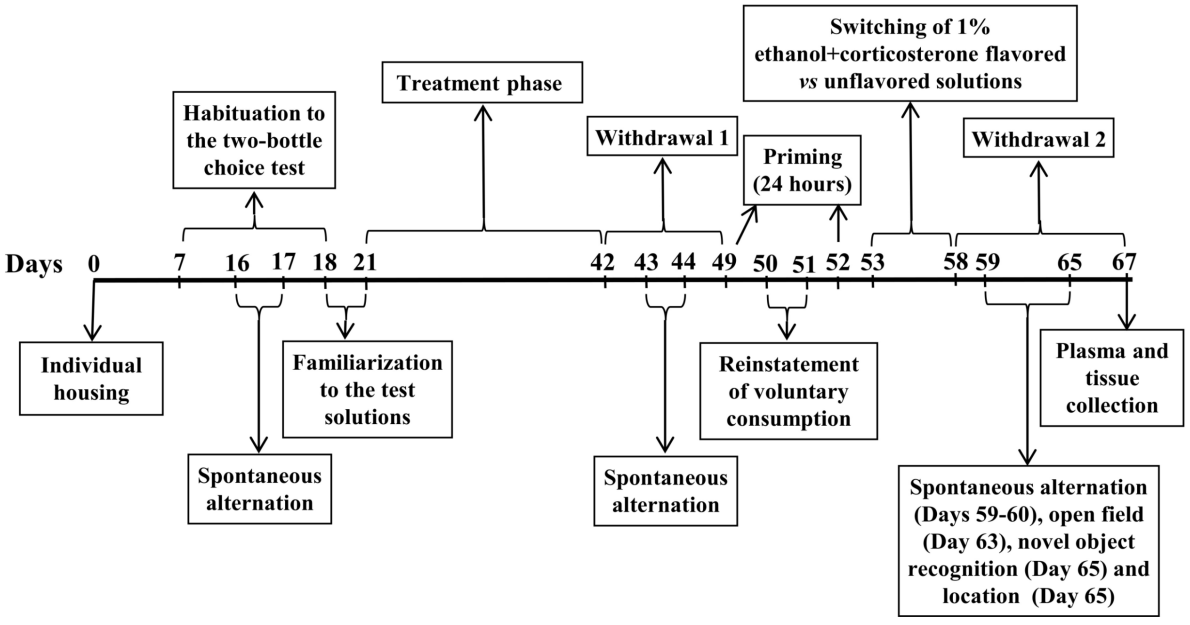

Figure 1 – ***Timeline of the experiment.***

Following one week of acclimatization to the animal facility, mice were offered two drinking tubes of water for 10 days to monitor normal fluid intake and tube preference. On days 16 and 17, mice were subjected to the spatial alternation test to establish baseline spatial working memory performance. Then, mice were assigned to the five experimental conditions and were exclusively offered the test solution for three days (familiarization phase, days 18–21) prior to being offered both the test and control solutions for three weeks (treatment phase, days 21–42). To test whether the mice developed an appetence to the test solutions, this was followed by one week of forced abstinence to induce reinstatement of voluntary consumption [27] during which only water was offered (withdrawal 1, days 42–49) and the mice were assessed again for spontaneous alternation performance after one day of washout (days 43 and 44). Primed reinstatement of voluntary consumption of ethanol with or without corticosterone was then examined by exposing the mice to the same test solutions for 24 hours (priming, day 49) followed by the same combination of drinking solutions as in the treatment phase for 48 hours (days 50–51). The flavoring agent influenced the development of a preference for the 1% ethanol+corticosterone solutions. To test whether a preference for the 1% ethanol + corticosterone solutions would persist independent of IMP, we therefore added a five-day switching phase (days 53–57) during which mice which initially had access to IMP *vs.* unflavored 1% ethanol+corticosterone were offered water *vs.* IMP-flavored 1% ethanol+corticosterone and *vice versa* (switching phase, see Table 1 for the schedule of test and control solutions). This followed a 24-hour priming to the test solutions as the sole source of fluid intake (day 52). Drinking solutions were withdrawn again from the mice (withdrawal 2) for 10 days (58–67) during which they were subjected to the battery of behavioral tests, after a one-day washout period (days 59–65). The mice were culled two days after the last behavioral test (day 67) for collection of trunk blood and brain tissue. The hippocampus and frontal cortex were rapidly dissected on ice, snap frozen and stored at -80^{0}C for further analysis.

Table 1 – ***Control and test solutions offered to each of the five experimental groups during the treatment, reinstatement and switching phases of the two-bottle choice test. Solutions that were exchanged during the switching phase are highlighted in bold***

	Treatment		Reinstatement		Switching	
	Control	***Test***	***Control***	***Test***	***Control***	***Test***
Control – Flavor (*n*=8)	Water	IMP	Water	IMP	Water	IMP
1% Ethanol (*n*=8)	Water	1% Ethanol	Water	1% Ethanol	Water	1% Ethanol
Low consumers Water *vs.* Flavored 1% ethanol + corticosterone (*n*=9)	Water	Flavored 1% ethanol + corticosterone	Water	Flavored 1% ethanol + corticosterone	**IMP**	**Unflavored 1% ethanol + corticosterone**
Medium consumers Water *vs.* Unflavored 1% ethanol + corticosterone (*n*=9)	Water	Unflavored 1% ethanol + corticosterone	Water	Unflavored 1% ethanol + corticosterone	Water	Unflavored 1% ethanol + corticosterone
High consumers IMP *vs.* Unflavored 1% ethanol + corticosterone (*n*=7)	IMP	Unflavored 1% ethanol + corticosterone	IMP	Unflavored 1% ethanol + corticosterone	**Water**	**Flavored 1% ethanol + corticosterone**

Behavioral testing

Spontaneous alternation

Spontaneous alternation was carried out in a T-maze consisting of three arms of equal dimensions (41.5 cm long, 6 cm wide in grey Plexiglas surrounded by 15 cm high walls in transparent Plexiglas) as described previously [28]. After one-minute of habituation, mice underwent nine consecutive trials. Each mouse was placed in the starting box (7.5 cm long) at the beginning of the central path for five seconds, and once the animal entered an arm, the doors to both arms were closed for 15 seconds. The mouse was then allowed to return to the starting box. At each time point, performance was assessed over two successive days and the data averaged. The percentage of correct alternations was calculated and the criterion for successful spatial working memory performance was an alternation rate above chance level (50%).

Novel object recognition and object location tests

A Perspex arena (30×35×25 cm) with transparent sides and a grey floor was used. The behavior was recorded by a camera positioned directly above the arena and subsequently analyzed using EthoVision Software (Noldus, Wageningen, Netherlands). Two sets of objects were used, triangular and circular wooden blocks or octagonal and rectangular wooden blocks, counterbalanced between mice to avoid preference bias.

Mice were first individually habituated to the empty arena for 30 minutes, during which their distance traveled was automatically recorded and the percentage of activity occurring in the center of the arena (defined as a 21×8 cm area) was also determined to ensure that any differences in object exploration levels between treatment groups were due to differences in memory and not activity and/or anxiety levels.

Twenty-four hours later, each mouse was subjected to the two trials of the object recognition test. Mice were first allowed to explore a single object for 10 minutes and four hours later, they could freely explore two objects, the familiar object from the acquisition trial and a novel object (different shape) for a further 10 minutes, as described previously [29].

Twenty-four hours later, mice were subjected to the two 10-minute trials of the object location test, also separated by four hours. Mice lose the ability to discriminate the spatial location after two hours [30]. We chose a longer inter-trial interval enabling us to detect improvement in performance. Each mouse was first left to explore two identical objects. The location of one object was changed during a second trial to another corner of

the arena, so that the two objects were diametrically opposed.

Object exploration, defined as the animal's nose pointing towards the object at a distance ≤0.5 cm, was assessed by eye, scored twice by an observer blind to the experimental groupings and averaged for each animal. If the two values differed by more than 10%, a third scoring was performed and the two closest values were used for statistical analyses.

The apparatus and objects were cleaned with 20% ethanol between tests to remove olfactory cues.

Western immunoblotting

Mice were killed by cervical dislocation two days after the last behavioral test and the hippocampus and frontal cortex rapidly dissected then lysed in ice-cold lysis buffer (5 mM Tris, 2 mM EGTA, 80 mM b-glycerophosphate, 1 mM sodium fluoride and protease inhibitor cocktail (Roche Diagnostics, Germany) and their protein concentration was determined using the Lowry assay [31] to ensure equal loading onto gels. Proteins (10 µg) were resolved by SDS-polyacrylamide gel electrophoresis onto 10 or 12% gels and transferred to nitrocellulose membranes (Amersham Biosciences). The membranes were probed with primary antibodies: anti-ERK2 (1:500) and anti-pERK1/2 (1:500) from Cell Signaling Technology, UK; and anti-GAPDH as a loading control (1:10.000; Sigma-Aldrich, UK), blocked with 1.5% fish skin gelatin and then probed with IRDye 800CW goat anti-mouse and/or anti-rabbit, IRDye 680CW goat anti-mouse or anti-rabbit secondary antibodies (LI-COR, Biosciences), as appropriate. The Snap ID protein detection system (Millipore, UK, WBAVDBASE) was used to block and incubate the membranes with ERK2 and pERK1/2 antibodies. For BDNF, membranes were blocked with 3% fish skin gelatin, incubated overnight at 4^0C with anti-BDNF (1:750; Sigma-Aldrich, UK) and anti-GAPDH antibodies (1:15.000; Sigma-Aldrich, UK) followed by 30 minutes incubation at 37^0C with appropriate secondary antibodies. Blotted proteins were detected and quantified using the Odyssey infrared imaging system (LI-COR).

Corticosterone levels

Plasma was isolated by centrifugation at 3000×g for 10 minutes at 4^0C immediately after trunk blood collection and was kept at -80^0C. Plasma corticosterone levels were quantified in duplicate using an enzyme-linked immunoassay (ADI-900-097, Enzo Life, USA) according to the manufacturer's instructions.

Statistical analysis

Data, presented as mean ± SEM, were analyzed using InVivoStat [32]. Two-way ANOVAs with bottle (control *vs.* test) and group (five modalities according to choice offered) as between-subject factors were used to analyze fluid intake data for each week of the treatment and each day of the reinstatement and switching phases of the two-bottle choice test. Fluid intake data were SQRT transformed to normalize the distribution. Two-way ANOVAs with group as a between-subject factor and repeated measure over time were used to compare fluid intake during the withdrawal phases, ethanol and corticosterone doses, changes in body weight, percentage of correct alternations and total object exploration times in the novel object discrimination and location tests. One-way ANOVAs were applied to compare the experimental groups for all the other variables. *Post-hoc* planned comparisons and paired sample *t*-tests were used when appropriate. One-sample *t*-tests were used to compare the alternation rate and object preference index to 50%. *P*-values ≤0.05 were considered statistically significant.

Results

Body weight

Body weight was recorded weekly, but the data presented Table 2 focus on beginning and end of each experimental phase: prior to habituation to the two-bottle choice test (day 7) and after three days of familiarization to the test solutions (day 21), after the treatment phase (day 42), after withdrawal 1 (day 49), after reinstatement and switching (day 56) and during withdrawal 2 (day 63). Weight gain or loss was calculated as the difference in the body weight between two successive phases of the experiment. Two-way ANOVAs with repeated measures over time showed no significant main effects of groups on either body weight or weight gain, but there were significant time ($F_{5,180}$=165.00, p<0.001 and $F_{4,144}$=130.93, p<0.001 for body weight and weight gain, respectively) and time × group interaction ($F_{20,180}$=7.04, p<0.001 and $F_{16,144}$=14.44, p<0.001 for body weight and weight gain, respectively) effects. Overall, these data show that the intake of 1% ethanol+corticosterone is associated with a significant increase in body weight followed by weight loss during the withdrawal phases.

Table 2 – ***Body weight and weight gain [g] throughout the different phases of the two-bottle choice test***

	Water *vs.* IMP	Water *vs.* 1% ethanol	*Low consumers* Water *vs.* flavored 1% ethanol + corticosterone #	*Medium consumers* Water *vs.* unflavored 1% ethanol + corticosterone	*High consumers* IMP *vs.* unflavored 1% ethanol + corticosterone #
Body weight [g]					
Day 7	25.57 ± 0.63	26.15 ± 0.39	25.2 ± 0.62	25.65 ± 0.54	25.46 ± 0.49
Day 21	26.06 ± 0.48	27.00 ± 0.25*	25.81 ± 0.67	25.99 ± 0.54*	26.48 ± 0.56*
Day 42	26.77 ± 0.47	27.97 ± 0.32*	29.08 ± 0.81^{a,b,*}	30.25 ± 0.76^{a,*}	30.54 ± 0.64^{a,b,*}
Day 49	27.15 ± 0.45	28.21 ± 0.40	27.60 ± 0.88*	28.55 ± 0.95*	28.51 ± 0.75*
Day 56	27.71 ± 0.54	29.05 ± 0.47*	30.20 ± 0.97^{a,*}	30.62 ± 0.81^{a,*}	31.20 ± 0.77^{a,b,*}
Day 63	28.09 ± 0.52	29.34 ± 0.56	28.22 ± 0.93*	29.12 ± 1.07*	29.18 ± 0.81*

	Water *vs.* IMP	Water *vs.* 1% ethanol	*Low consumers* Water *vs.* flavored 1% ethanol + corticosterone #	*Medium consumers* Water *vs.* unflavored 1% ethanol + corticosterone	*High consumers* IMP *vs.* unflavored 1% ethanol + corticosterone #
Weight gain [g]					
Day 21–Day 7	-0.49 ± 0.23	-0.85 ± 0.18	-0.61 ± 0.21	-0.33 ± 0.20	-1.03 ± 0.19
Day 42–Day 21	0.71 ± 0.09*	0.97 ± 0.25*	3.27 ± 0.42^{a,b,*}	4.26 ± 0.44^{a,b,*}	4.05 ± 0.38^{a,b,*}
Day 49–Day 42	0.37 ± 0.10	0.24 ± 0.18	-1.48 ± 0.41^{a,b,*}	-1.70 ± 0.57^{a,b,*}	-2.03 ± 0.47^{a,b,*}
Day 56–Day 49	0.56 ± 0.15	0.84 ± 0.13	2.60 ± 0.28^{a,b,*}	2.07 ± 0.28^{a,b,*}	2.68 ± 0.51^{a,b,*}
Day 63–Day 56	0.37 ± 0.08	0.29 ± 0.13	-1.98 ± 0.26^{a,b,*}	-1.50 ± 0.43^{a,b,*}	-2.01 ± 0.43^{a,b,*}

Data are presented as mean ± SEM. [a], $p<0.05$ compared to water *vs.* IMP (control – flavor group); [b], $p<0.05$ compared to water *vs.* 1% ethanol.

Fluid intake

Total fluid intake is presented in Table 3. During the familiarization and two priming phases, there were significant effects of the choice offered on total fluid intake ($F_{4,36}$=4.90, p=0.003; $F_{4,36}$=4.39, p=0.005 and $F_{4,36}$=4.09, p=0.008, respectively), which was usually higher in the 1% ethanol+corticosterone-treated groups. Two-way repeated measure ANOVAs revealed that the total fluid intake differed significantly as a function of the choice offered during the treatment ($F_{4,36}$=11.16, p<0.001), reinstatement ($F_{4,36}$=11.44, p<0.001) and switching ($F_{4,36}$=8.40, p<0.001) phases.

During the treatment phase, total fluid intake of 1% ethanol+corticosterone-treated groups rose gradually (week effect: $F_{2,72}$=6.85, p=0.002; week × group interaction: $F_{8,72}$=3.48, p=0.002). Fluid intake of 1% ethanol+corticosterone treated groups also fluctuated with repeated days during switching ($F_{4,144}$=5.85, p<0.001). Total fluid intake of 1% ethanol+corticosterone-treated groups, however, decreased significantly with time during both withdrawal periods days (days effect: $F_{4,144}$=3.96, p=0.004 and $F_{2,72}$= 21.15, p<0.001 for withdrawal 1 and 2, respectively; day × group interaction effect: $F_{16,144}$=2.63, p=0.001 and $F_{8,72}$=2.99, p=0.006 for withdrawal 1 and 2, respectively).

Overall, the data show that the presence of the IMP-flavor modulated intake of 1% ethanol+corticosterone solutions. Mice offered water *vs.* IMP-flavored 1% ethanol+corticosterone were the lowest consumers; mice offered water *vs.* unflavored 1% ethanol+corticosterone were medium consumers whilst mice offered IMP *vs.* unflavored 1% ethanol+corticosterone were the highest consumers. For the sake of clarity, these three groups are referred to as low, medium and high consumers throughout the rest of the manuscript.

Table 3 – ***Total fluid intake [mL] during the two-bottle choice test***

				Low consumers	*Medium consumers*	*High consumers*
		Water *vs.* IMP	Water *vs.* 1% ethanol	Water *vs.* flavored 1% ethanol + corticosterone	Water *vs.* unflavored 1% ethanol + corticosterone	IMP *vs.* unflavored 1% ethanol + corticosterone
Familiarization	*Days 18–21*	2.80 ± 0.16	3.23 ± 0.14	3.37 ± 0.23^{a}	3.96 ± 0.25^{a,b,c}	3.77 ± 0.24^{a}
Treatment	*Week 1*	3.10 ± 0.17	3.05 ± 0.15	3.61 ± 0.28	4.12 ± 0.32	4.39 ± 0.35^{b}
	Week 2	3.03 ± 0.18	3.14 ± 0.21	3.96 ± 0.29	4.94 ± 0.51^{b}	6.13 ± 0.81^{a,b,c,*}
	Week 3	3.02 ± 0.14	3.11 ± 0.18	3.93 ± 0.22	4.89 ± 0.71^{b}	7.55 ± 1.20^{a,b,c,*}
Withdrawal 1	*Day 42*	2.08 ± 0.36	2.99 ± 0.15	3.11 ± 0.37^{b}	2.84 ± 0.44	3.20 ± 0.54^{a}
	Day 43	2.98 ± 0.44*	2.37 ± 0.20*	2.32 ± 0.29*	2.40 ± 0.24	2.04 ± 0.19^{a,*}
	Day 44	2.64 ± 0.18	2.71 ± 0.14	2.41 ± 0.28*	1.79 ± 0.24^{b,*}	2.24 ± 0.19*
	Day 45	2.79 ± 0.17*	3.03 ± 0.16	2.32 ± 0.32*	2.22 ± 0.14^{b,*}	2.40 ± 0.08*
	Days 46–48	2.83 ± 0.11*	2.97 ± 0.10	2.43 ± 0.28*	2.17 ± 0.15^{b,*}	2.37 ± 0.12*
Priming	*Day 49*	2.67 ± 0.16	2.97 ± 0.13	3.32 ± 0.22	4.01 ± 0.46^{a,b}	4.23 ± 040^{a,b,c}
Reinstatement	*Day 50*	2.70 ± 0.22	2.80 ± 0.20	4.13 ± 0.37	5.55 ± 0.57^{b}	6.54 ± 0.76^{a,b,c}
	Day 51	2.43 ± 0.10	2.43 ± 0.10	4.34 ± 0.34^{a,b}	7.09 ± 1.32^{b,c,*}	6.30 ± 0.82^{a,b,c}
		Water *vs.* IMP	**Water *vs.* 1% ethanol**	**IMP *vs.* unflavored 1% ethanol + corticosterone**	**Water *vs.* unflavored 1% ethanol + corticosterone**	**Water *vs.* flavored 1% ethanol + corticosterone**
Priming	*Day 52*	2.65 ± 0.09	2.83 ± 0.12	4.81 ± 0.34	8.23 ± 2.25^{a,b,c}	5.25 ± 0.50
Switching	*Day 53*	3.00 ± 0.11	3.08 ± 0.16	4.20 ± 0.17	8.86 ± 2.07^{b,c}	6.61 ± 1.28^{a,b}
	Day 54	2.84 ± 0.15	2.79 ± 0.12	4.06 ± 0.19	8.64 ± 1.71^{b,c}	6.33 ± 1.02^{a,b}
	Day 55	3.09 ± 0.16	3.25 ± 0.13	5.10 ± 0.35*	8.34 ± 1.20^{b,c}	7.23 ± 1.23^{a,b}
	Day 56	2.60 ± 0.19	2.58 ± 0.14	4.15 ± 0.26	6.19 ± 1.07*	5.04 ± 0.65*
	Day 57	2.93 ± 0.24	2.96 ± 0.12	4.79 ± 0.62^{a,b}	7.91 ± 1.34^{b,c}	6.40 ± 0.84^{a,b}
Withdrawal 2	*Day 58*	2.57 ± 0.32	2.92 ± 0.27	3.43 ± 0.28	3.71 ± 0.67	4.19 ± 0.47^{a,b}
	Day 59	2.65 ± 0.06	2.88 ± 0.10	2.89 ± 0.32	2.86 ± 0.58*	3.01 ± 0.21*
	Day 60–62	2.57 ± 0.13	2.70 ± 0.12	2.18 ± 0.20*	2.29 ± 0.15*	2.49 ± 0.12*

Data are presented as mean ± SEM mL ingested. [a], $p<0.05$ compared to water *vs.* IMP (control – flavor group); [b], $p<0.05$ compared to water *vs.* 1% ethanol; [c], $p<0.05$, compared to low consumers; *$p<0.05$ compared to week 1 (treatment), day 50 (reinstatement), day 53 (switching).

Doses of ethanol and corticosterone ingested

During the familiarization and two priming phases, the averaged dose of ethanol and corticosterone ingested did not differ between the treated groups (Table 4).

Treatment phase

Two-way ANOVAs with repeated measures detected significant main effects of group for both ethanol ($F_{3,29}$= 17.70, p=0.001) and corticosterone ($F_{2,22}$=16.14, p<0.001) intake and time ($F_{2,58}$=5.11, p=0.009) for ethanol intake. Compared to 1% ethanol-treated mice, ethanol intake was significantly higher in the high consumer group (p<0.01 for all three weeks), but not in the low and medium consumer groups. The high consumer group also ingested significantly more ethanol and corticosterone than the low (p<0.001 for all three weeks) and medium (ethanol: p<0.01 for weeks 2 and 3; corticosterone: p<0.05 for all three weeks) consumers groups. Medium consumers also ingested more ethanol than low consumers on weeks 2 and 3 (p<0.05) and more corticosterone on week 2 (p<0.05).

Reinstatement

During the reinstatement phase, ethanol and corticosterone intake varied significantly as a function of the choices offered ($F_{3,29}$=8.13, p<0.001 and $F_{2,22}$=5.42, p=0.012, respectively), the repeated days ($F_{1,29}$=6.39, p=0.017 and $F_{1,22}$=5.87, p=0.02, respectively), and a significant interaction between these two factors was found for ethanol intake ($F_{3,29}$=3.04, p<0.05). The high and medium consumer groups ingested significantly more ethanol than 1% ethanol-treated mice (p<0.01 on both days) and low consumer group mice (p<0.05 on both days). Corticosterone intake was also significantly lower in the low consumer group compared to the high and medium consumer group (p<0.05 for both days).

Switching phase

In this phase, high consumer were offered IMP-flavored 1% ethanol+corticosterone to assess whether they retain high intake of the cocktail solution in presence of IMP, while low consumers were presented with unflavored 1% ethanol+corticosterone to assess whether they will increase their intake of cocktail solution in the absence the flavor. During the switching phase, ethanol intake also differed significantly between groups ($F_{3,29}$=3.36, p=0.03) while both ethanol and corticosterone intake varied significantly over the five days ($F_{4,116}$=4.93, p=0.001 and $F_{4,88}$=4.44, p=0.003, respectively). Compared to 1% ethanol-treated mice, ethanol intake was higher in the high consumer group on day 55 (p<0.05) and in the medium consumer group on days 53, 54 and 55 (p<0.01). Medium consumer mice also ingested more ethanol than low consumer mice on day 53 (p<0.01). Corticosterone intake, however, was only higher in the high consumer group compared to the low consumer group on day 53 (p<0.05).

Ethanol intake averaged across all phases of the experiment differed significantly between the three groups ($F_{3,28}$=10.27, p<0.001) with the 1% ethanol-treated group, and low consumer group ingesting less ethanol than both the medium (p<0.01 in both cases) and high (p<0.001 in both cases) consumer groups. Corticosterone intake averaged across all phases of the experiment differed significantly between the three groups ($F_{2,22}$=9.10, p=0.001) with the low consumer groups ingesting significantly less corticosterone than both the medium (p<0.02) and high (p<0.001) consumer group.

Table 4 – ***Doses of ethanol [g/kg/day] and corticosterone [mg/kg/day] throughout all phases of the two-bottle choice test***

		Ethanol intake [g/kg/day]				Corticosterone dose [mg/kg/day]		
		Water *vs.* 1% ethanol	Water *vs.* flavored 1% ethanol + corticosterone	Water *vs.* unflavored 1% ethanol + corticosterone	IMP *vs.* unflavored 1% ethanol + corticosterone	Water *vs.* flavored 1% ethanol + corticosterone	Water *vs.* unflavored 1% ethanol + corticosterone	IMP *vs.* unflavored 1% ethanol + corticosterone
			Low consumers	*Medium consumers*	*High consumers*	*Low consumers*	*Medium consumers*	*High consumers*
Familiarization	*Days 18–21*	0.94 ± 0.04	1.04 ± 0.08	1.20 ± 0.06	1.13 ± 0.09	32.95 ± 2.42	37.90 ± 1.89	35.81 ± 2.73
Treatment	*Week 1*	0.46 ± 0.09	0.30 ± 0.06[b]	0.70 ± 0.11	1.16 ± 0.10[c]	9.24 ± 2.01[b]	22.24 ± 3.40	39.99 ± 4.22[a]
	Week 2	0.51 ± 0.08	0.48 ± 0.17[a,b]	0.96 ± 0.19[b]	1.61 ± 0.21[a,c,*]	15.05 ± 5.48[a,b]	29.02 ± 5.24	45.21 ± 3.82[a]
	Week 3	0.54 ± 0.07	0.45 ± 0.14[a,b]	0.92 ± 0.25[b]	1.97 ± 0.31[a,c,*]	20.67 ± 6.62[b]	26.38 ± 6.49	43.20 ± 2.74[a]
Priming	*Day 49*	0.83 ± 0.03	0.97 ± 0.09	1.11 ± 0.12	1.17 ± 0.011	30.68 ± 2.77	35.18 ± 3.81	37.18 ± 3.62
Reinstatement	*Day 50*	0.45 ± 0.08	0.61 ± 0.19[a,b]	1.25 ± 0.21[c]	1.57 ± 0.24[c]	19.43 ± 5.92[a,b]	39.70 ± 6.63	49.62 ± 7.53
	Day 51	0.45 ± 0.09	0.82 ± 0.16[a,b]	1.98 ± 0.43[c]	1.57 ± 0.17[c]	25.86 ± 5.02[a,b]	58.06 ± 10.85[*]	47.72 ± 4.66
		Water *vs.* 1% ethanol	IMP *vs.* unflavored 1% ethanol + corticosterone	Water *vs.* unflavored 1% ethanol + corticosterone	Water *vs.* flavored 1% ethanol + corticosterone	IMP *vs.* unflavored 1% ethanol + corticosterone	Water *vs.* unflavored 1% ethanol + corticosterone	Water *vs.* flavored 1% ethanol + corticosterone
Priming	*Day 52*	0.79 ± 0.03	1.39 ± 0.12	2.35 ± 0.72	1.46 ± 0.15	44.12 ± 3.71	74.50 ± 22.83	46.33 ± 4.76
Switching	*Day 53*	0.39 ± 0.10	0.94 ± 0.16[a]	2.28 ± 0.71[c]	1.45 ± 0.47	29.90 ± 4.99[b]	58.27 ± 13.18	45.88 ± 15.00
	Day 54	0.54 ± 0.05	1.00 ± 0.12	1.94 ± 0.64[c]	1.37 ± 0.44	31.81 ± 3.70	43.73 ± 10.62	36.65 ± 11.09
	Day 55	0.55 ± 0.10	1.37 ± 0.14	2.03 ± 0.47[c]	1.70 ± 0.52[c]	43.33 ± 4.43	48.58 ± 8.46	42.69 ± 11.80
	Day 56	0.34 ± 0.06	0.71 ± 0.15	1.17 ± 0.34[*]	1.02 ± 0.22	22.60 ± 4.63	25.07 ± 4.97[*]	26.73 ± 4.80
	Day 57	0.52 ± 0.10	0.92 ± 0.25	1.47 ± 0.48[*]	1.35 ± 0.36	29.24 ± 7.99	37.24 ± 11.86[*]	40.73 ± 10.68
Averaged	*All days*	0.55 ± 0.04	0.62 ± 0.08[a,b]	1.13 ± 0.17[c]	1.51 ± 0.19[c]	20.93 ± 2.52[a,b]	32.65 ± 3.87	41.81. ± 3.74

Data are presented as mean ± SE. [a], p<0.05 compared to medium consumer group (water *vs.* unflavored corticosterone); [b], p<0.05 compared to high consumers (IMP *vs.* unflavored corticosterone); [c], p<0.05 compared to water *vs.* 1% ethanol; *, p<0.05 compared to week 1 (treatment phase), day 50 (reinstatement) or day 53 (switching).

C57BL/6J mice did not develop a preference for 1% ethanol in the absence of corticosterone

The data presented above clearly indicate that the presence of IMP in the 1% ethanol+corticosterone solutions influenced both the total fluid intake and the doses of ethanol and corticosterone ingested. The data presented below indicates whether the mice consume preferentially the test or control solutions. Main statistical effects of the choice offered, bottle preference and time on fluid intake during the treatment, reinstatement and switching phases of the two-bottle choice test are presented in Table 5. Figure 2 describes the intake of the two solutions offered to each experimental group.

Water vs. IMP (control-flavor group)

As represented Figure 2A, mice significantly disliked IMP during the first and third (p<0.05) week of treatment but significantly preferred IMP on the second day of reinstatement (p<0.02). No aversion or preference was seen during the switching phase.

Water vs. 1% ethanol

Mice did not significantly prefer either drinking solution throughout the experiment (Figure 2B).

Water vs. flavored 1% ethanol+corticosterone (low consumer group)

The significant aversion towards the flavored 1% ethanol+corticosterone solution seen during the first week of treatment (p<0.05 *vs.* water) gradually disappeared and a preference for this solution was observed on the second day of reinstatement (p<0.05 *vs.* water, Figure 2C). During the switching phase, the 1% ethanol+corticosterone solution was presented unflavored against IMP and remained preferred for the first three days (p<0.05 *vs.* IMP, Figure 2C), despite the fact that IMP was no longer aversive to the mice.

Water vs. unflavored 1% ethanol+corticosterone (medium consumer group)

The 1% ethanol+corticosterone solution was only preferred during the reinstatement (p<0.01 *vs.* water for both days) and switching phases on days 53, 54, 55 and 56 (all p<0.05 *vs.* water, Figure 2D).

IMP vs. unflavored 1% ethanol+corticosterone

Mice drank almost exclusively from the 1% ethanol + corticosterone solution during the treatment (p<0.001 *vs.* IMP for all three weeks) and reinstatement (p<0.01 *vs.* IMP for both days) phases (Figure 2E). During the switching phase, the 1% ethanol+corticosterone solution was IMP-flavored and offered against water, and remained significantly preferred on days 53, 55, 56 and 57 (all p<0.04 *vs.* water, Figure 2E).

Behavioral performance

Impairment of spatial working memory induced by voluntary consumption of 1% ethanol was prevented by co-administration of corticosterone

There was a significant group × time interaction ($F_{8,72}$=2.58, p=0.016) on spontaneous alternation performance. Baseline spatial working memory performance was similar in all experimental groups and significantly above chance level (p<0.05 compared to 50% in all cases, Figure 3A). After three weeks of treatment, the performance of all groups was above chance level (p<0.05 compared to 50% in all cases, Figure 3A), but the low consumers performed worse than the control-flavor group (p=0.02, Figure 3A). The spontaneous alternation rate of all three 1% ethanol+corticosterone-treated groups significantly increased between withdrawal 1 and withdrawal 2 (p<0.05 in all cases, Figure 3A) whilst the performance of 1% ethanol-treated mice decreased (p=0.02) and was significantly lower than all three 1% ethanol+corticosterone-treated groups (p<0.05 in all cases, Figure 3A). All groups except the 1% ethanol, alternated above chance levels (p<0.05 compared to 50%, Figure 3A) indicating that the chronic exposure to 1% ethanol impaired spatial working memory but the co-administration of corticosterone prevented this memory deficit.

Table 5 – *Main statistical effects for data acquired during the two-bottle choice test*

		Group	Bottle	Group × Bottle
Treatment	*Week 1*	$F_{4,36}$=2.51, p=0.059	$F_{1,36}$=2.51, p=0.75	$F_{4,36}$=11.93, p<0.001
	Week 2	$F_{4,36}$=4.99, p=0.003	$F_{1,36}$=4.83, p=0.034	$F_{4,36}$=6.35, p<0.001
	Week 3	$F_{4,36}$=4.12, p=0.008	$F_{1,36}$=6.13, p=0.018	$F_{4,36}$=11.75, p<0.001
Reinstatement	*Day 50*	$F_{4,36}$=15.11, p<0.001	$F_{1,36}$=7.73, p=0.009	$F_{4,36}$=3.83, p=0.011
	Day 51	$F_{4,36}$=6.33, p<0.001	$F_{1,36}$=66.16, p<0.001	$F_{4,36}$=5.81, p=0.001
Switching	*Day 53*	$F_{4,36}$=5.83, p=0.001	$F_{1,36}$=16.82, p<0.001	$F_{4,36}$=3.29, p=0.021
	Day 54	$F_{4,36}$=6.63, p<0.001	$F_{1,36}$=12.21, p=0.001	$F_{4,36}$=2.35, p=0.073
	Day 55	$F_{4,36}$=5.27, p=0.002	$F_{1,36}$=28.26, p<0.001	$F_{4,36}$=2.37, p=0.071
	Day 56	$F_{4,36}$=6.85, p<0.001	$F_{1,36}$=6.03, p=0.019	$F_{4,36}$=2.01, p=0.11
	Day 57	$F_{4,36}$=7.66, p<0.001	$F_{1,36}$=7.59, p=0.009	$F_{4,36}$=0.64, p=0.64

Significant main effects of group and bottle were observed during the second and third week of treatment. The group × bottle interaction was, however, significant for each of the three weeks indicating that preference for the test solution developed as a function of the choice offered. There were significant effects of group, bottle and group × bottle interaction for each of the two days of reinstatement and first day of switching, and significant group and bottle effects for the other four days of the switching phase.

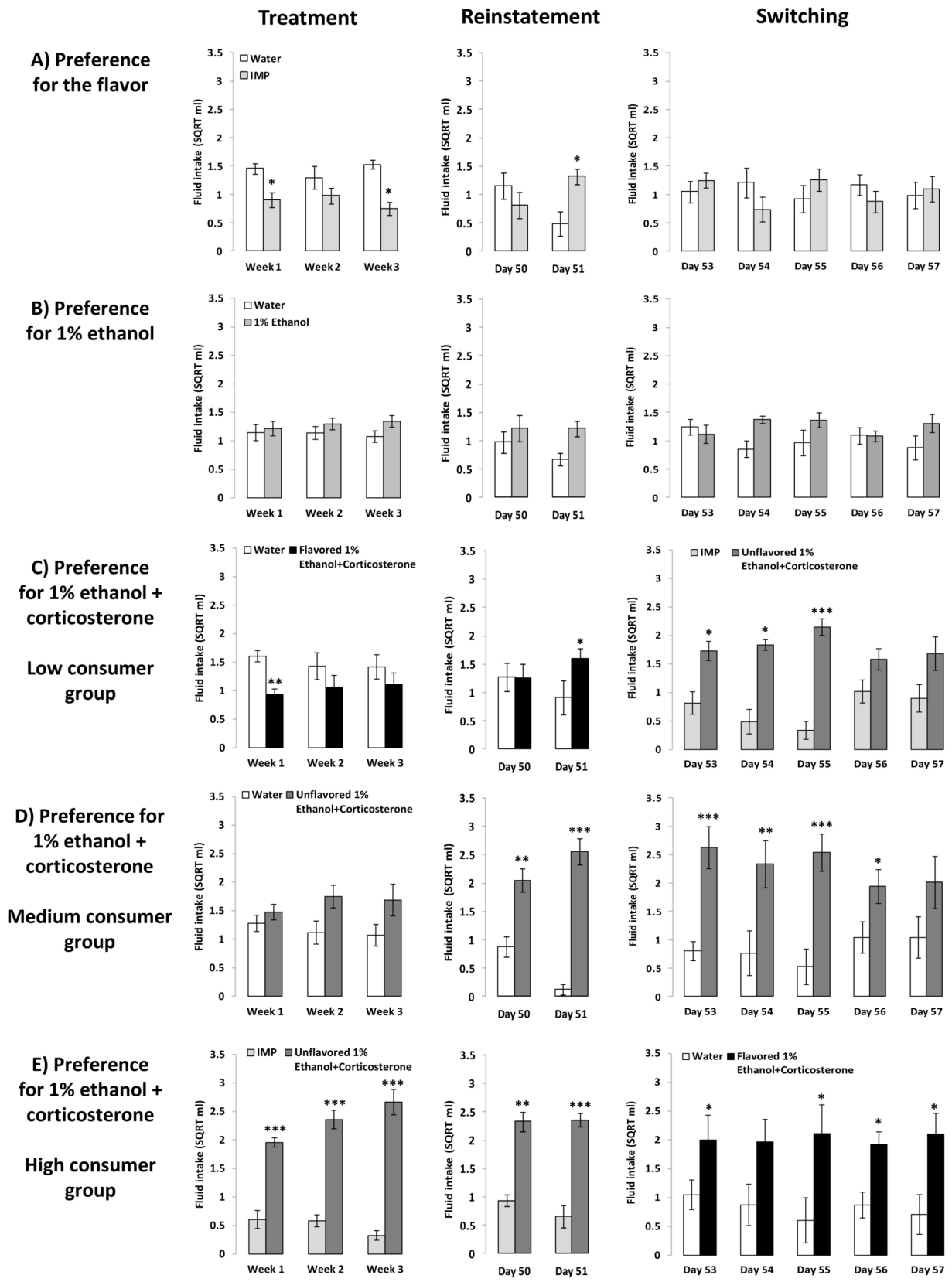

Figure 2 – ***Preference for 1% ethanol ± corticosterone in the two-bottle choice test. Mean ± SE SQRT (mL) drunk from the test and control solutions during the three phases of the two-bottle choice test. Mice were offered (A) water vs. IMP (control – flavor); (B) water vs. 1% ethanol; (C) Low consumers: water vs. flavored 1% ethanol+corticosterone during the acquisition and reinstatement phases followed by IMP vs. unflavored 1% ethanol+corticosterone during the switching phase; (D) Medium consumers: water vs. unflavored 1% ethanol+corticosterone; and (E) high consumers: IMP vs. unflavored 1% ethanol+corticosterone during the acquisition and reinstatement phases of corticosterone self-administration followed by water vs. flavored 1% ethanol+corticosterone during the switching phase. For each time point, the left bar represents the control solution and the right bar, the test solution. *, p<0.05; **, p<0.01 and ***, p<0.001 compared to the control solution for each individual time point.***

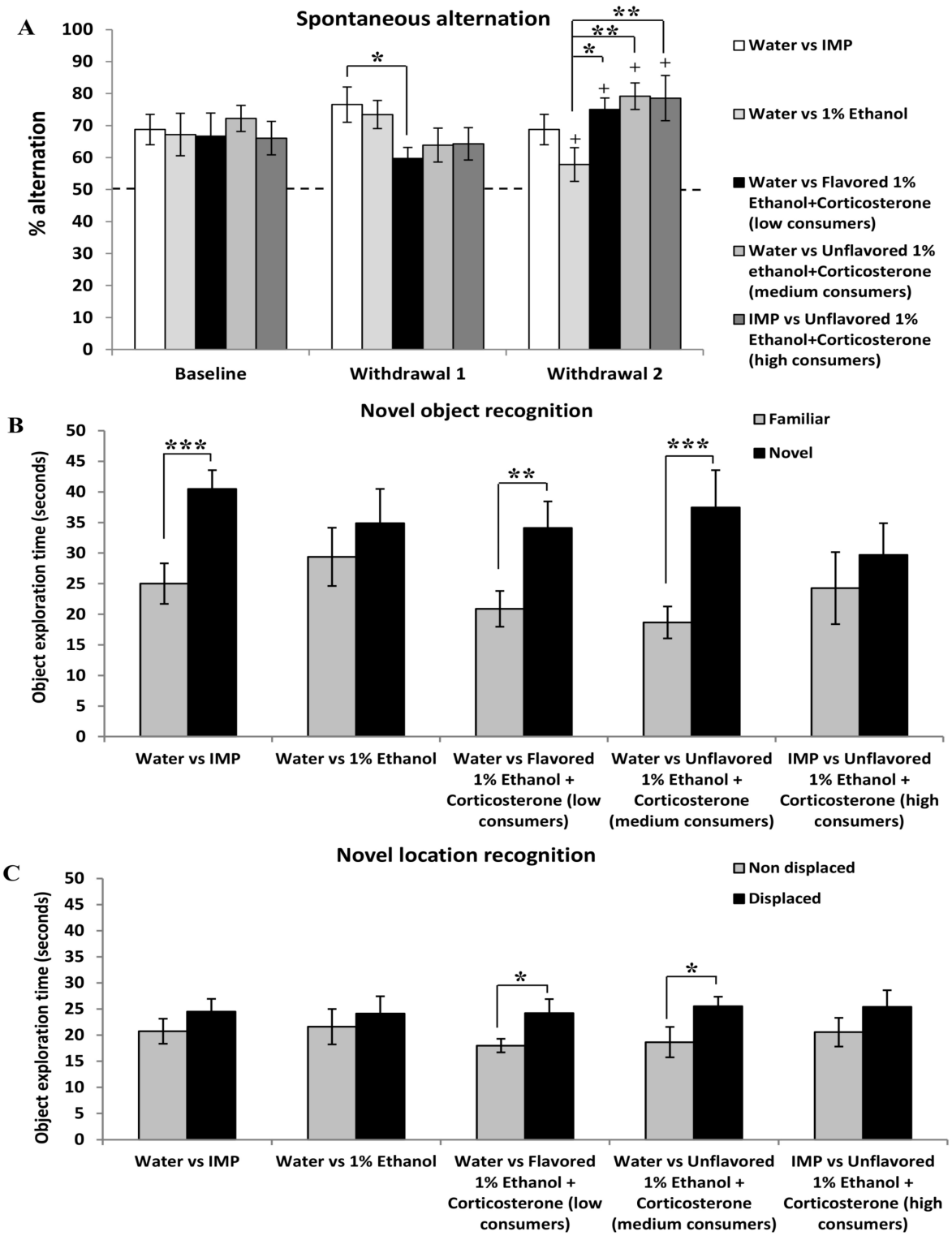

Figure 3 – ***Impact of voluntary consumption of 1% ethanol ± corticosterone on memory performance. Data are presented as mean ± SE. (A) Spontaneous alternation performance expressed as percentage of correct alternations. Following the treatment phase (withdrawal 1), the low consumer group showed a significantly reduced alternation rate compared to the control – flavor group. Following the reinstatement phase and switching of flavored and unflavored corticosterone solutions (withdrawal 2), all three 1% ethanol+corticosterone-treated groups showed improved alternation rates compared to withdrawal 1 whilst the performance of 1% ethanol-treated mice was impaired compared to all 1% ethanol+corticosterone-treated groups and withdrawal 1. One sample t-test: #, p<0.05; ##, p<0.01; ###, p<0.001, compared to 50% (random choices). (B) Novel object recognition. Both the 1% ethanol-treated and high consumer groups were unable to discriminate the novel and familiar object with a four-hour inter-trial interval. (C) Novel object location. Using a four-hour inter-trial interval, the control-flavor group which received neither corticosterone nor ethanol was unable to discriminate between the novel and familiar object location. This was also the case for the 1% ethanol-treated mice and the high consumer group, but spatial discrimination performance was improved in the low and medium consumer groups. *, p<0.05; **, p<0.01 and ***, p<0.001 compared to familiar or non-displaced objects. +, p≤ 0.05 compared to withdrawal 1.***

Corticosterone self-administration depressed locomotor activity

Open field data are presented in Table 6. There were significant differences between groups for the total distance traveled ($F_{4,36}$=5.74, p=0.001), with all 1% ethanol +corticosterone-treated groups being less active than the 1% ethanol-treated group (p≤0.001 in all cases) while the low and high consumer groups were significantly less active than the control-flavor group (p<0.05 in both cases). Also, there was a significant group effect for the percentage of ambulation in the center of the open field ($F_{4,36}$ =6.05, p<0.001) with high consumer mice showing a slight increase in anxiety-like behavior and/or reduced exploratory activity, manifested by a reduced exploration of the center of the open-field (p<0.05 in all cases). The number of defecations was not different between the groups.

Table 6 – ***Behavioral and corticosterone data are presented as mean ± SE***

	Water *vs.* IMP (control – flavor)	Water *vs.* 1% ethanol	*Low consumers*	*Medium consumers*	*High consumers*
			Water *vs.* flavored 1% ethanol + corticosterone[#]	Water *vs.* unflavored 1% ethanol + corticosterone	IMP *vs.* unflavored 1% ethanol + corticosterone[#]
Open-field					
Distance traveled [m]	95.35 ± 4.28	101.85 ± 7.09	79.51 ± 2.93[a,b]	80.03 ± 3.69[b]	78.71 ± 3.22[a,b]
% Distance traveled in the center	23.73 ± 0.78	22.99 ± 1.30	20.57 ± 1.44	23.04 ± 1.13	12.87 ± 3.49[a,b,c,d]
No. of defecations	5.38 ± 1.09	6.38 ± 0.65	5.00 ± 0.85	5.33 ± 0.75	5.43 ± 0.81
Novel object recognition					
Trial 1. Total exploration time [s]	74.12 ± 6.37	60.00 ± 4.39	54.22 ± 5.95	57.11 ± 8.15	36.86 ± 4.36
Trial 2. Total exploration time [s]	65.5 ± 4.73	64.25 ± 9.99	55.00 ± 6.39	56.11 ± 8.55	54.00 ± 9.32
Novel object location					
Trial 1. Total exploration time [s]	48.12 ± 7.17	45.87 ± 4.86	44.44 ± 5.21	39.55 ± 5.13	35.00 ± 7.43
Trial 2. Total exploration time [s]	45.25 ± 3.98	45.75 ± 5.87	42.22 ± 3.43	44.22 ± 3.42	46.00 ± 5.36
Plasma corticosterone levels [ng/mL]	68.94 ± 4.98	73.67 ± 11.40	77.68 ± 8.36	69.98 ± 3.59	64.71 ± 4.38

[a], p<0.05 compared to water *vs.* IMP (control – flavor group); [b], p<0.05 compared to water *vs.* 1% ethanol; [c], p<0.05, compared to low consumers; [d], p<0.05 compared to medium consumers.

Chronic intake of 1% ethanol induced a novel object recognition deficit, prevented by co-administration of corticosterone

Total object exploration times are given in Table 6 and were not significantly altered. In the retention trial, there was a significant overall object effect ($F_{1,36}$=42.32, p<0.001). Mice from the 1% ethanol-treated and high consumers groups did not preferentially explore the novel object in contrast to the control-flavor, low consumers and medium consumers groups (p<0.01 *vs.* familiar object in all cases, Figure 3B), indicating that corticosterone was able to protect against 1% ethanol-induced recognition memory deficits within a certain dose range.

Voluntary consumption of 1% ethanol+corticosterone improved object location memory

Total object exploration times are given in Table 6 and were not significantly altered. A significant preference for the novel location was observed overall ($F_{1,36}$ =13.99, p<0.001) but only mice from the low and medium consumer groups significantly discriminated the novel location (p<0.05 compared to non-displaced object, Figure 3C) consistent with the preserved recognition memory seen following lower consumed doses of 1% ethanol+corticosterone.

Chronic intake of 1% ethanol induced a reduction in hippocampal BDNF levels, prevented by co-administration of corticosterone

Protein levels, expressed as a ratio of GAPDH levels, were calculated as a percentage of the control-flavor group.

BDNF

Hippocampal BDNF levels differed between groups ($F_{4,36}$=4.88, p=0.003) and were reduced in 1% ethanol-treated mice compared to the control-flavor, low and high 1% ethanol+corticosterone consumer groups (all p<0.01, Figure 4A).

ERK2

Total ERK2 levels in the hippocampus differed between groups ($F_{1,36}$=2.79, p=0.04) and were higher in 1% ethanol-treated mice compared to the control-flavor, low and high consumer groups (all p<0.05, Figure 4B).

Phosphorylated (p)ERK2/ERK2

Hippocampal pERK2 levels differed between the groups ($F_{4,35}$=3.95, p=0.01) and were reduced in high consumer mice compared to the control-flavor and 1% ethanol-treated groups (both p<0.05, Figure 4C). Low consumer mice also had lower pERK2 levels than 1% ethanol-treated mice (p<0.01, Figure 4C). Fronto-cortical pERK2 levels differed between the groups ($F_{4,35}$=3.61, p=0.01) and were significantly reduced in the low consumer group compared to the control-flavor group (p=0.04, Figure 4C). All 1% ethanol+corticosterone-treated groups had significantly lower pERK2 levels than 1% ethanol-treated mice (p<0.05 in all cases, Figure 4C).

Corticosterone levels

There were no differences in circulating levels of corticosterone between groups (Table 6), 10 days after the last self-administration session. Despite high levels of self-administered corticosterone, circulating levels of the hormone were unaltered 10 days after access to corticosterone ceased, suggesting that it did not result in long-lasting alterations of HPA axis activity.

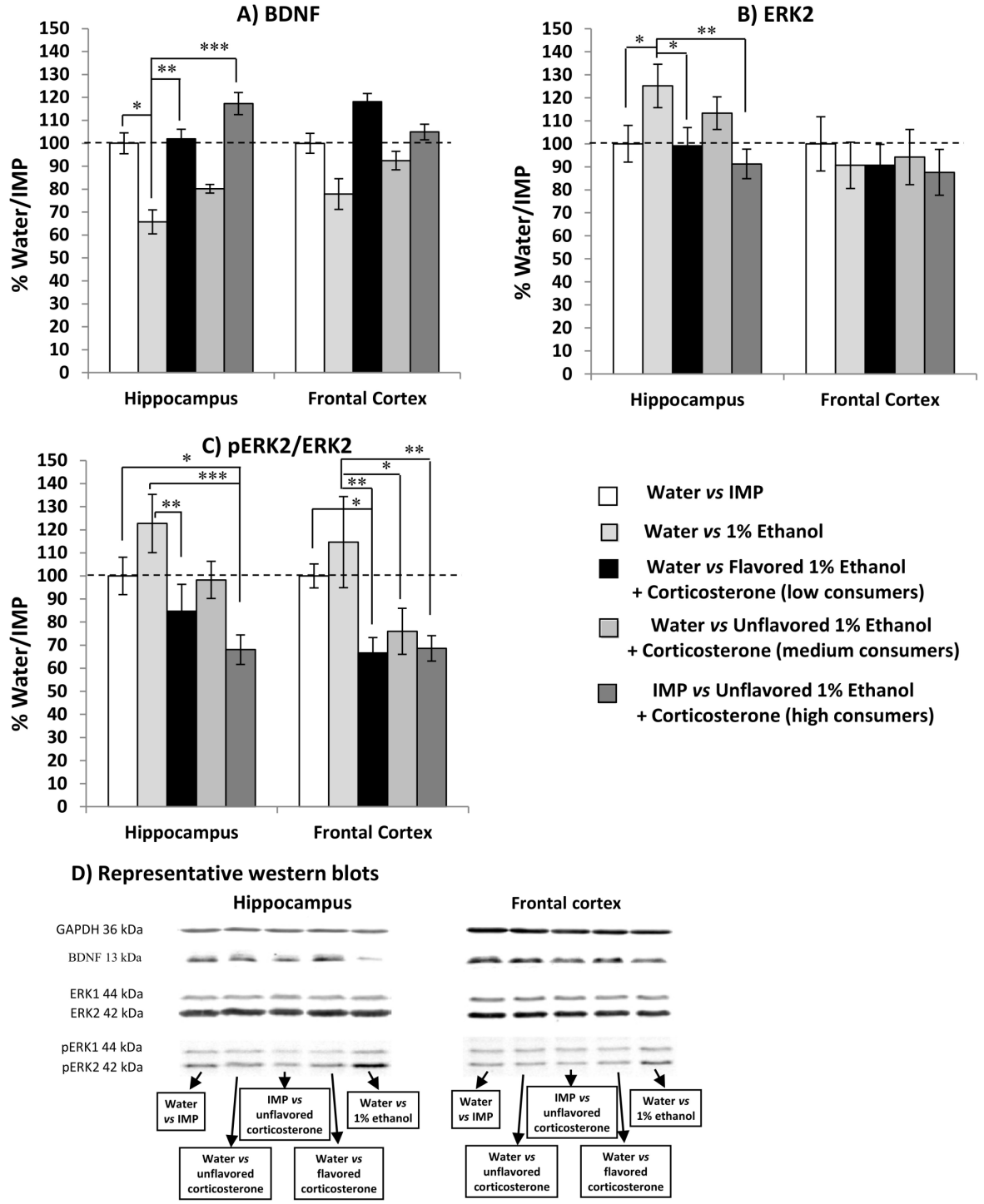

Figure 4 – ***Impact of 1% ethanol on BDNF signaling in the hippocampus and frontal cortex. Ratios of each protein of interest to GAPDH were then expressed as % of the control – flavor group. Data are presented as mean ± SE. (A) Hippocampal BDNF levels were significantly reduced by the chronic intake of 1% ethanol, and this was prevented by co-administration of corticosterone. (B) Total ERK1/2 levels in the hippocampus was increased by exposure to 1% ethanol, and this effect was prevented by co-administration of corticosterone. (C) Hippocampal ERK2 activity was reduced by treatment with 1% ethanol+corticosterone, but fronto-cortical pERK1/2 levels of all 1% ethanol+corticosterone-treated groups were significantly reduced compared to the 1% ethanol-treated group. *, p<0.05; **, p<0.01 and ***, p<0.001. (D) Representative western blots. Note that the order of the groups on the gels differ from their order on the figures.***

Discussion

Mice presented with a 1% ethanol solution had a chronic intake of ~0.55 g/kg/day. This low dose, at the threshold for inducing memory deficits after acute administration, caused persistent spatial and non-spatial memory deficits associated with a reduction in hippocampal BDNF levels, without altering fluid intake or inducing a preference over water. In contrast, co-administration of 1% ethanol and corticosterone enhanced consumption of the cocktail solution, which became significantly preferred to water after a period of with-

drawal, suggesting that it had the potential for being appetitive. This led to a higher intake of ethanol and corticosterone preventable by the addition of a mildly aversive flavor. Interestingly, the combination of corticosterone and 1% ethanol attenuated memory impairments due to 1% ethanol and improved object location memory regardless of the dose ingested while dose-dependently preventing the reduction in hippocampal BDNF levels seen in mice offered 1% ethanol *vs.* water.

A chronic low dose of ethanol induces memory deficits

Mice offered water *vs.* 1% ethanol, exhibited adverse cognitive effects with impaired novel object recognition and spontaneous alternation performance after the switching phase despite the low dose ingested (~0.55 g/kg/day). To our knowledge, there is no published report investigating the cognitive effects of chronic or intermittent self-exposure to 1% ethanol, but single acute i.p. injections of ethanol, at doses within the range ingested by our experimental groups, were found to impair memory performance of mice at doses of 0.5 g/kg; 1.0 and 1.5 but not 0.25 g/kg [2, 6]. These memory deficits could be mediated, at least in part, by the associated persistent decrease in BDNF levels, still visible 10 days after discontinuation of the treatment. Hippocampal-specific deletion of BDNF was found to impair novel object recognition and spatial memory [21] and recent work showed that hippocampal BDNF mRNA levels are reduced following an acute moderate dose of ethanol (1.25 g/kg), while doubling the dose exacerbated this decrease and also affected the frontal cortex [33]. This is in agreement with our finding of a greater decrease in hippocampal compared with fronto-cortical BDNF protein levels.

Adverse cognitive effects of a low dose of ethanol are prevented by corticosterone

The impairments in spatial working memory and object recognition memory induced by ethanol were completely rescued by co-administration of corticosterone in the two lowest consuming groups, and partially (spontaneous alternation performance only) in the highest consuming group. In the object location test, the low and medium ethanol consumers outperformed the control – flavor and ethanol-treated groups, in which, as expected, the long inter-interval trial used (four hours) prevented control mice from discriminating the novel location [30]. The reduced locomotor activity during the habituation in the open field, also reported in C57BL/6 mice offered a lower corticosterone concentration in drinking water (100 μg/mL in 1% ethanol) [34] was also unlikely to interfere with cognitive performance as it similarly affected all 1% ethanol+corticosterone-treated groups.

No significant decrease in hippocampal BDNF levels was observed in any of the 1% ethanol+corticosterone-treated groups, indicating that co-administration of corticosterone partially or completely prevented this adverse effect of ethanol, possibly contributing to the beneficial cognitive effects. This was, however, not dependent upon the dose ingested. The greater protection from the ethanol-induced reduction in hippocampal BDNF levels seen in the low and high consumer groups was also associated with a significant decrease in hippocampal ERK2 activity. Although acute high doses of ethanol (3 g/kg) were also found to decrease hippocampal pERK2 levels [35], the reduction in fronto-cortical pERK2 levels may be more related to direct effects of corticosterone as it was seen in all three 1% ethanol+corticosterone groups, consistent with the persistent reduction in hippocampal and fronto-cortical levels of pERK2 seen in mice chronically treated with corticosterone in drinking water 10 days after withdrawal [36].

Although acute low doses of corticosterone (1 mg/kg) are known to improve memory and to rescue established memory impairments [37], to our knowledge, this is the first report of a protective effect of chronic doses of corticosterone in the range of 20–32 mg/kg on cognitive impairments, and particularly against those induced by alcohol. Some caution is needed in concluding that stress, or corticosterone, has a universally protective effect against ethanol-induced cognitive impairment. Indeed, although the combination of ethanol with stress has been reported to be protective in male rats [17] restraint stress and ethanol appear to produce additive cognitive impairments in females [38].

Reinforcing potential of 1% ethanol in C57BL/6J mice

Bottle preference and fluid intake data indicate that the mice could not discriminate and/or did not develop an appetence for the 1% ethanol solution throughout all phases of the experiment. The addition of corticosterone, in the absence of flavoring agent, resulted in the slow development of a preference for the 1% ethanol + corticosterone solution, becoming evident during the reinstatement phase. The concomitant increase in fluid intake, however, suggests that the mice failed to discriminate the test solution by its taste and were seeking it. Voluntary consumption of 1% ethanol+corticosterone doubled during reinstatement, after forced abstinence, indicating a relapse-like state [27]. The highest voluntary consumption of 1% ethanol+corticosterone and strongest preference for this solution was, however, observed when it was offered against IMP, which had an aversive taste. During switching, the 1% ethanol+corticosterone solution was then presented as a flavored solution. The dose ingested did not decrease during priming to the flavored solution and the preference for, and high intake of, the 1% ethanol+corticosterone solution were retained in spite of the unpleasant flavor. Altogether, this suggests that the addition of corticosterone either enhanced the reinforcing properties of ethanol or added to it as corticosterone has been found to have dose-dependent reinforcing properties [39], but this hypothesis needs to be confirmed using operant self-administration procedures.

Conclusions

We found that a chronic intermittent intake of a low dose of ethanol impaired spatial and recognition memory and reduced hippocampal BDNF levels, and that these adverse effects can be prevented by co-administration of the stress hormone corticosterone.

Although acute low doses of corticosterone were found to have beneficial effects on memory function, we are the first to report a protective effect of chronic doses of corticosterone on cognitive impairments, and particularly against those induced by alcohol, but sex differences in the protective effect of stress and/or corticosterone against ethanol-induced cognitive impairment will need to be considered in future studies.

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