# Key Role of Ethanol-Derived Acetaldehyde in the Motivational Properties Induced by Intragastric Ethanol: A Conditioned Place Preference Study in the Rat

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**Background:** Acetaldehyde (ACD), the first metabolite of ethanol (EtOH), is produced peripherally by gastric and hepatic alcohol dehydrogenase (ADH) and centrally by brain catalase. In spite of the aversive properties classically ascribed to ACD, it has recently been suggested that ACD might mediate some of the motivational effects of EtOH. Accordingly, the relative role of ACD in the positive motivational properties of EtOH ingested is increasingly becoming the matter of debate. Thus, we studied the ability of intragastrically administered EtOH, ACD and EtOH-derived ACD to induce conditioned place preference (cpp) in rats.

**Methods:** Wistar rats were pretreated intraperitoneally with saline, the peripheral competitive inhibitor of ADH, 4-methylpyrazole (4-MP, 22.5, 45 or 67.5 mg/kg) or with the selective ACD-sequestrating agent, D-penicillamine (DP, 25 or 50 mg/kg), before the intragastric administration of saline, EtOH (0.5, 1 or 2 g/kg) or ACD (10, 20, or 40 mg/kg). The specificity of 4-MP and DP effects was addressed using morphine-induced cpp (2.5 mg/kg).

**Results:** Both, EtOH and ACD dose-dependently induced cpp; further, while EtOH-induced cpp was prevented by the administration of 4-MP and by DP, ACD-induced cpp was unaltered by 4-MP administration and prevented by DP. Both pretreatments did not interfere with morphine-induced cpp indicating that 4-MP and DP specifically modulate the motivational properties of EtOH and ACD.

**Conclusion:** The ability of 4-MP and DP to decrease EtOH-induced cpp suggests that a reduction of ACD levels is crucial in depriving EtOH from its motivational properties as indexed by the cpp procedure. In addition, this conclusion is supported by the inefficacy of 4-MP in preventing ACD-induced cpp, and by its blockade observed after administration of the selective ACD sequestrating agent DP. The present results underscore the role of EtOH-derived ACD in EtOH-induced motivational properties as well as its abuse liability.

**Key Words:** Ethanol, Acetaldehyde, Conditioned Place Preference, 4-Methylpyrazole, D-Penicillamine.

ACETALDEHYDE (ACD), THE FIRST metabolite of ethanol (EtOH), is produced peripherally by gastric and hepatic alcohol dehydrogenase (ADH) and centrally by brain catalase. Moreover, ACD is a potent volatile flavor compound found in several foods and drinks. It is usually produced by wine yeast during fermentation and in winery, ACD is regarded as a key component of wine, being one of the most important sensory carbonyl compounds that provide pleasant fruity aromas to the beverage (Liu and Pilone, 2000). Although very high levels of ACD in wine are regarded as a defect, white wine and fine sherry may easily contain up to 500 mg of ACD per liter (Liu and Pilone, 2000).

It has long been thought that ACD is primarily aversive and therapeutically useful in the management of alcoholics as a consequence of its blood accumulation after administration of the aldehyde-dehydrogenase (ALDH) inhibitors, such as disulfiram (Suh et al., 2006) and/or calcium carbimide (Brown et al., 1983) leading ultimately to an adverse toxic reaction (*flushing reaction*), which should discourage alcoholics from drinking (Suh et al., 2006). In sharp contrast with this notion, it has also been reported that ALDH inhibitors may potentiate the euphoric and pleasurable effects of low doses of EtOH (Brown et al., 1983) and that patients may actually benefit by taking low doses of EtOH when under disulfiram treatment (Chevens, 1953).

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Experimental grounds to support the suggestion that ACD has positive motivational properties (Hunt 1996) were provided by early studies reporting that ACD is self-administered intracerebroventricularly (i.c.v.) in Wistar rats (Amit, 1977; Brown et al., 1979, 1980), intravenously (i.v.) in Long-Evans rats (Myers et al., 1984), and, more recently, even into the ventral tegmental area (VTA) of EtOH-preferring rats (Rodd-Henricks et al., 2002). Likewise, ACD causes conditioned place preference (cpp) after ICV infusion in Sprague–Dawley rats (Smith et al., 1984) or after intraperitoneal (i.p.) administration in Wistar rats (Quertemont and De Witte, 2001). In addition, ACD inactivation by peripheral administration of D-penicillamine (DP), an effective ACD-sequestering agent, blocks behavioral activation produced by ACD and EtOH (Font et al., 2005), and prevents the positive, but not aversive, effects of i.p. EtOH-induced cpp in mice (Font et al., 2006a). Further, DP administration reduces spontaneous EtOH intake in Long-Evans unselected rats (Font et al., 2006b).

In line with a primary role of ACD in the positive motivational properties of EtOH, we recently reported that ACD dose-dependently stimulates electrophysiological activity of VTA, DA-containing neurons whereas EtOH-induced effects are prevented by pharmacological blockade of EtOH metabolism with 4-MP (Foddai et al., 2004), thus providing additional support to the hypothesis that EtOH-derived ACD may play a critical role in EtOH-induced positive motivational effects. In line with these evidences, the aim of the present study was to determine the role of EtOH-derived ACD on EtOH-induced cpp, given intragastrically (by gavage) to mimic the oral route of administration commonly used by humans. To this end, we studied in Wistar rats, both intragastric (i.g.) EtOH and ACD reinforcing properties as measured by cpp and the relationship between the motivational properties of EtOH and the metabolic production of ACD by determining whether 4-MP, a peripheral competitive inhibitor of ADH, could prevent EtOH-induced cpp. Further, in order to better interpret the role of ACD in the reinforcing effect of EtOH ingested, we also studied the effect of DP on EtOH and ACD-induced cpp. Lastly, to analyze the specificity of two strategies employed (i.e. 4-MP or DP), we also investigated the effect on morphine-induced cpp. We decided to use a slightly biased place conditioning paradigm in agreement with Biala and Kotlińska (1999), Roma and Riley (2005) and Cunningham et al. (2003) which reported that place conditioning was apparent only when EtOH was paired with the initially less preferred cue, and not when paired with the preferred cue (Cunningham et al., 2003).

## MATERIALS AND METHODS

The study was carried out in accordance with Italian law D.L. 116, 1992, which allows experiments on laboratory animals only after submission and approval of a research project to the competent authorities, and in strict accordance with the "Principles of laboratory animal care" (NIH publications no. 80–23, revised 1996). All possible efforts were made to minimize animal pain and discomfort and to reduce the number of experimental subjects.

### *Animals*

Male Wistar rats (Harlan, Udine, Italy) weighting between 180 and 250 g were used for cpp procedure. Rats were housed in groups of 3 to 4 per cage and maintained under controlled environmental conditions (temperature 22 ± 2°C; humidity 60 to 65%; 12-h light/-dark cycle, light on at 08:00 AM). All animals were given a standard laboratory diet and tap water *ad libitum*. To minimize stress, subjects were habituated to the experimental procedures (handling, gavage) for at least 3 days before experimental procedures. Experiments were conducted during the light phase of the light/dark cycle.

### *Conditioned Place Preference*

The apparatus consisted of two rectangular steel boxes (48L × 33W × 30H cm) separated by a guillotine door. Distinctive visual and tactile cues distinguished the two compartments: the wall and floor coloring (one dark gray and the other clear gray), and the floor texture, smooth or grille. The apparatus was placed in a soundproof room with constant light provided by a 40 W lamp placed above each compartment.

### *Procedure and Experimental Design*

Each experiment consisted of three phases. During the first phase (day 1, preconditioning phase) the guillotine door was kept lifted and each rat was placed in the center of the opening, with access to both compartments of the apparatus for 30 min. The time spent by each rat in the compartments was recorded to indicate the "unconditioned preference" for each compartment. During the second phase, conditioning phase, (days 2 to 16 for EtOH; days 2 to 9 for ACD and days 2 to 5 for morphine) the rats were administered with the drugs and placed for 30 minutes in the less preferred compartment. On alternate days, the rats were administered with saline and placed in the preferred compartment. As a result of this conditioning schedule, EtOH, ACD, morphine or saline were paired eight (EtOH), four (ACD) and two (morphine) times to the less preferred compartment. Doses and schedules for conditioning with EtOH (Bozarth, 1990) and ACD (Quertemont and De Witte, 2001) were selected in agreement with previously published data (Bozarth, 1990; Quertemont and De Witte, 2001). During the third phase (postconditioning phase), 24 h after the last treatment, the guillotine door was removed and the time spent by each rat in the drug-paired compartment was recorded during 30 minutes (1800 seconds) of observation. The conditions of the postconditioning test were identical to those of the preconditioning test. The time spent in the drug-paired compartment during the postconditioning phase with respect to that spent during the pre-conditioning phase is a measure of the degree of place conditioning induced by the drug (Carr et al., 1989). Thus, a statistically significant difference between the time spent during pre- and postconditioning phase as well as the time spent during postconditioning phase with respect to that of saline/saline group indicates the development of cpp.

### *Drugs*

EtOH (Zedda-Piras, Alghero, Italy) (0.5, 1, and 2 g/kg) and ACD (Sigma–Aldrich, Milano, Italy) (10, 20, and 40 mg/kg) were dissolved in saline (0.9% NaCl) to a final volume of 1 ml and administered by gavage (i.g.). EtOH solutions (20% v/v) were obtained by dilution of EtOH (95%) (Medicamenta, 1991-1992). 4-MP (Sigma–Aldrich, Milano, Italy) (22.5, 45, and 67.5 mg/kg) and DP (Sigma–Aldrich, Milano, Italy) (25 and 50 mg/kg) were dissolved in saline and administered by i.p. injection. Morphine hydrochloride

(morphine) (S.A.L.A.R.S., Camerlata, Como) (2.5 mg/kg) was dissolved in saline and administered by i.p. injection.

All drug dilutions where freshly prepared before every experiment. The gavage infusion rate was rapid (about 5 seconds) and given immediately before each conditioning session. All experiments were performed between 8:30 AM and 1:00 PM.

4-MP was administered at the doses of 22.5, 45, and 67.5 mg/kg every other day, just after the conditioning session with saline to the preferred compartment, approximately 24 hours before the saline, EtOH, ACD, or morphine -pairing to the less preferred compartment.

DP was administered at the doses of 25 and 50 mg/kg (Font et al., 2006a,b) 30 minutes before the conditioning session with saline, EtOH, ACD, or morphine-pairing to the less preferred compartment. Care was taken to balance the daily order of treatments (saline/saline, 4-MP/saline, DP/saline, saline/EtOH, saline/ACD, 4-MP/EtOH, 4-MP/ACD, DP/EtOH, DP/ACD, saline/morphine, 4-MP/morphine or DP/morphine) as well as the daily order of exposures to each compartment. Control animals were administered the same volume of saline (vehicle).

### *Blood EtOH and ACD Levels*

A 1000 $\mu$l aliquot of whole blood was collected from the right atrium and rapidly transferred in 10 ml HS-vials (Hewlett-Packard, Palo Alto, CA) for analysis. Thus, deproteinization which could give rise to ACD (Stowell et al., 1977), was not part of procedure. The vial to be analyzed for EtOH and ACD was placed in a heating block at 45°C for 10 min. The samples were analyzed on a HS-GC-FID system with a Dani 86.50 HSS-autosampler, and a Hewlett-Packard gas chromatography HP 6890 Plus. The capillary column used was an Econo CAP EC-5 (Alltech, Milan, Italy) (30 m, 0.53 mm i.d., 1.2 $\mu$m d.f.). The injection port temperature was maintained at 250°C. The GC oven temperature was maintained at 45°C in isothermal for 8 minutes. The flow rate of the carrier gas (helium) was 6.1 ml/min. The FID temperature was maintained at 250°C. The HS parameters were: 75°C manifold temperature, 150°C transferline temperature, 1.57 psi carrier gas pressure, 1 minutes vial pressurization time, 1 ml injection volume.

### *Statistical Analysis*

Data are expressed as mean ± SEM of time in seconds spent during 1800 seconds of observation in the drug-paired compartment during the postconditioning phase with respect to that spent during the pre-conditioning phase. To analyze the spontaneous preference during pre-conditioning phase, data (time spent in each compartment) were analyzed by one-way analysis of variance (ANOVA). To determine the effect of EtOH, ACD, or morphine, 4-MP and DP on EtOH, ACD, or morphine -induced cpp effect, data were analyzed by repeated measures, two ways ANOVA. Post hoc comparisons were undertaken if a significant effect of the interaction was found ($p < 0.05$). The comparisons were carried out using Newman–Keuls tests or least significant difference test for blood EtOH or ACD concentrations.

## RESULTS

### *Conditioned Place Preference*

*Preconditioning Phase*. As expected, during the pre-conditioning phase, the spontaneous preference for the two compartments was slightly uneven [$F(1,90) = 10,24$, $p < 0.05$] (data from EtOH and ACD control groups); the rank order of preference being clear-gray walls and grille floor (985.46 ± 47.7 seconds); dark-gray walls and smooth floor (819,49 ± 47.7 seconds) during 1800 seconds of observation.

*Ethanol-Induced cpp*. Two-way ANOVA yielded a significant effect of group [$F(3,70) = 4.22$, $p < 0.01$], of conditioning group [$F(1,70) = 21.64$, $p < 0.00005$] and group × conditioning group interaction [$F(3,70) = 8.63$, $p < 0.0001$]. The effect of EtOH-induced cpp is shown in Fig. 1. Rats receiving EtOH (1 g/kg, i.g.; $n = 20$) during the conditioning sessions (15 days) spent more time in the drug paired compartment of the apparatus (1276.0 ± 43.1) with respect to the pre-conditioning phase (728.3 ± 59.5, $p < 0.001$) and with respect to the postconditioning phase of control group ($n = 23$; $p < 0.001$). This effect is dose-dependent and was not observed at the lower and higher EtOH doses (0.5 and 2 g/kg, i.g.; $n = 8$ and 21, respectively).

*Acetaldehyde-Induced cpp*. Two-way ANOVA of ACD on cpp revealed significant effects of conditioning group [$F(1,55) = 20.85$, $p < 0.00005$] and group × conditioning group interaction [$F(3,55) = 5.35$, $p < 0.005$]. The effect following ACD administration on cpp is shown in Fig. 2. Rats receiving ACD (20 mg/kg, i.g.; $n = 20$) during the conditioning sessions (8 days) spent more time (1197.6 ± 59.3 seconds) in the drug-paired compartment of the apparatus as compared to the pre-conditioning phase (664.8 ± 62.7 seconds, $p < 0.001$) and with respect to the postconditioning phase of control group (892.2 ± 42.8, $n = 20$; $p < 0.001$). Reminiscent of the EtOH-induced cpp the effect is dose-dependent and was not observed at the lower and higher doses of ACD (10 and 40 mg/kg, i.g.; $n = 10$ and 9, respectively).

*Effect of 4-MP-Pretreatment on EtOH-Induced cpp*. Two-way ANOVA of different doses of 4-MP-pretreatment (22.5, 45, 67.5 mg/kg, i.p.) on EtOH-induced cpp revealed signifi-

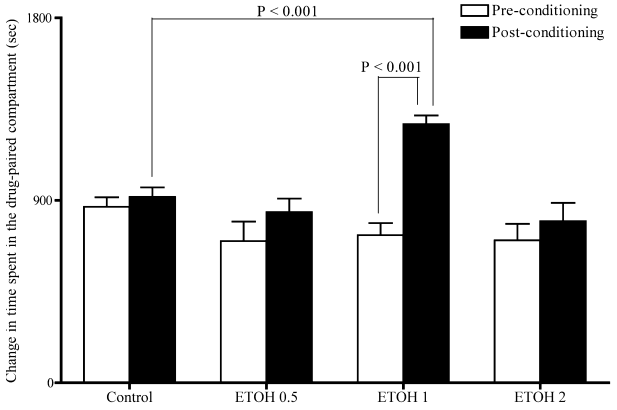


**Fig. 1.** Effect of different doses of EtOH (0.5, 1, and 2 g/kg, i.g.) on cpp, paired with the less preferred compartment. Data are shown as time in seconds (±SEM; $n = 8$–23). Significant differences between time spent during postconditioning phase compared to preconditioning or postconditioning phase of control group are indicated (two-way ANOVA followed by Newman–Keuls, post hoc test).

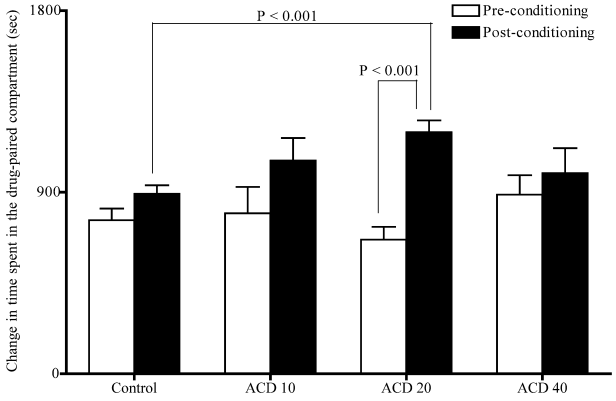


**Fig. 2.** Effect of different doses of ACD (10, 20, and 40 mg/kg, i.g.) on cpp, paired with the less preferred compartment. Data are shown as time in seconds (±SEM; $n$ = 9–20). Significant differences between time spent during postconditioning phase compared to preconditioning or postconditioning phase of control group are indicated (two-way ANOVA followed by Newman–Keuls, post hoc test).

cant effects of group [$F(7,73) = 1.87, p < 0.05$], conditioning group [$F(1,73) = 19.10$, $p < 0.00005$] and an interaction group × conditioning group [$F(7,73) = 4.27$, $p < 0.001$]. 4-MP prevented EtOH-induced cpp (1 g/kg, i.g.); its effect is shown in Fig. 3. Rats pretreated with 4-MP (45 or 67.5 mg/kg, i.p.; $n = 9$ in both cases) spent less time (857.0 ± 78.1 seconds and 920.1 ± 48.5 seconds, respectively) in the compartment paired with 4-MP/EtOH, as compared to the group paired with saline/EtOH-induced cpp (as reported above, 1276.0 ± 43.1; $p < 0.001$ in both cases). This effect on EtOH-induced cpp was not observed at the lower 4-MP dose ($n = 7$, 22.5 mg/kg, i.p.).

Since 4-MP administration produces an increase in EtOH blood levels (Table 1) (see also Waller et al., 1982), we decided to further evaluate the role of high blood levels of EtOH by administering 4-MP at a dose of EtOH (0.5 g/kg) ineffacious in inducing cpp. The 45 mg/kg dose of 4-MP was chosen because was found to be the minimal dose that prevented EtOH-induced cpp, without altering animals gross behavior (as reported above). Two-way ANOVA yielded a significant effect of group [$F(5,64) = 3.03$, $p < 0.05$], of conditioning group [$F(1,64) = 21.52$, $p < 0.00005$] and group × conditioning group interaction [$F(5,64) = 6.65$, $p < 0.00005$]. As shown in Fig. 4, pretreatment with 4-MP on lower dose of EtOH (0.5 mg/kg, i.g.; $n = 9$) did not reveal any cpp effect while it prevented EtOH-induced cpp effect at the dose of 1 g/kg, i.g. (as reported above, $n = 9$; $p < 0.001$).

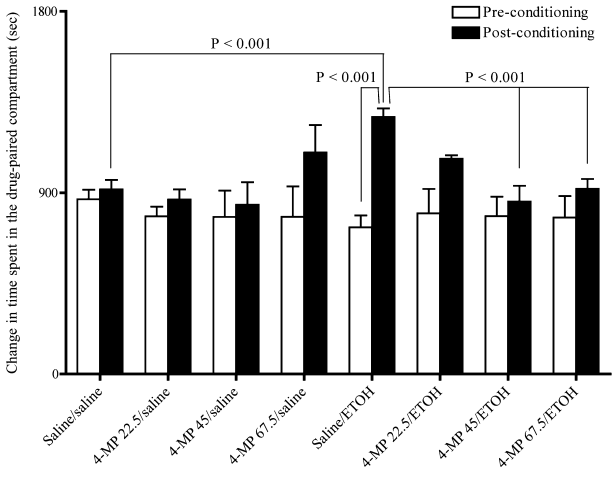


**Fig. 3.** Effect of 4-MP-pretreatment (22.5, 45, and 67.5 mg/kg, i.p.) on EtOH-induced cpp (1 g/kg, i.g.). Data are shown as time in seconds (±SEM; $n$ = 8–16). Significant differences between time spent during postconditioning phase compared to saline/saline group, to preconditioning or postconditioning phase of EtOH group are indicated (two-ways ANOVA followed by Newman–Keuls, post hoc test).

*Effect of 4-MP-Pretreatment on ACD-Induced cpp.* The effect of pretreatment with 4-MP (45 mg/kg, i.p.) was then tested on ACD-induced cpp (20 mg/kg, i.g.), and is shown in Fig. 5. Two-way ANOVA of ACD-induced cpp values in response to 4-MP-pretreatment yielded a significant effect of conditioning group [$F(1,15) = 18.02$, $p < 0.0001$] and an interaction group × conditioning group [$F(3,15) = 3.02$, $p < 0.05$]. As already shown, treatment with saline/ACD induced an increase of time spent in the less preferred compartment during the postconditioning phase ($n = 8$, 1245.0 ± 80.9 seconds) with respect to its pre-conditioning phase (752.0 ± 65.4 seconds; $p < 0.05$) and with respect to postconditioning phase of saline/saline group ($n = 7$, 867.8 ± 40.2 seconds; $p < 0.05$). Pre-treatment with 45 mg/kg of 4-MP, before i.g. ACD, failed to reduce ACD-induced cpp. This is because the animals spent equal time in the compartment paired with saline/ACD than in the compartment paired with 4-MP/ACD-induced cpp. (4-MP/ACD postconditioning phase vs. saline/saline group $p < 0.05$; vs. its pre-conditioning phase: $p < 0.05$).

*Effect of 4-MP-Pretreatment on Morphine-Induced cpp.* Two-way ANOVA (group × conditioning group) yielded a significant effect of group [$F(3,31) = 2.31$, $p < 0.05$], of conditioning group [$F(1,31) = 47.76$, $p < 0.0000001$] and group × conditioning group interaction [$F(3,31) = 16.56$, $p < 0.00005$]. As shown in Fig. 6, panel *A*, rats conditioned for 4 days with morphine (as hydrochloride, 2.5 mg/kg, i.p) showed cpp for the drug-paired compartment (1210.0 ± 49.1 seconds) with respect to its pre-conditioning phase (733.4 ± 79.2 seconds, $n = 9$; $p < 0.001$) and with respect to the postconditioning phase of saline/saline group (788.7 ± 38.3 seconds, $n = 10$; $p < 0.001$). Pretreatment with 45 mg/kg of 4-MP before morphine, did not interfere with the effect of morphine-induced cpp. Indeed, rats showed preference for the 4-MP/morphine-paired compartment (1172.8 ± 67.5 seconds) with respect to its pre-conditioning phase (639.2 ± 102. 3 seconds, $n = 9$; $p < 0.001$) and with respect to the postconditioning phase in saline/saline group (788.7 ± 38.3 seconds, $n = 10$; $p < 0.001$).

*Effect of* D*-Penicillamine-Pretreatment on EtOH-Induced cpp.* The effect of pretreatment with DP (25 or 50 mg/kg, i.p.), 30 minutes before EtOH-induced cpp in rats (1 g/kg,

**Table 1.** Blood EtOH and ACD Levels 30, 60, and 120 Minutes After Gavage With EtOH (1 g/kg) and i.p. Pretreated With 4-MP (45 mg/kg)

| | EtOH | | | ACD | | |
|---|---|---|---|---|---|---|
| Treatment | 30 minutes | 60 minutes | 120 minutes | 30 minutes | 60 minutes | 120 minutes |
| Saline /EtOH | 0.1407 ± 0.0175 | 0.1383 ± 0.0363 | 0.1163 ± 0.0196 | 0.0027 ± 0.0004 | 0.0040 ± 0.0012 | 0.0035 ± 0.0006 |
| 4-MP/EtOH | 0.1873 ± 0.0086 * | 0.1795 ± 0.0250 * | 0.1402 ± 0.0273 * | 0.0014 ± 0.0009 ** | 0.0015 ± 0.0002 *** | 0.0009 ± 0.00007 *** |

Each data point is the mean (mg/ml ± SEM; $n = 6$). EtOH and ACD levels were measured as described in "Materials and Methods". Significant differences with respect to EtOH group (*) are indicated (two-way ANOVA followed by LSD, post hoc test).

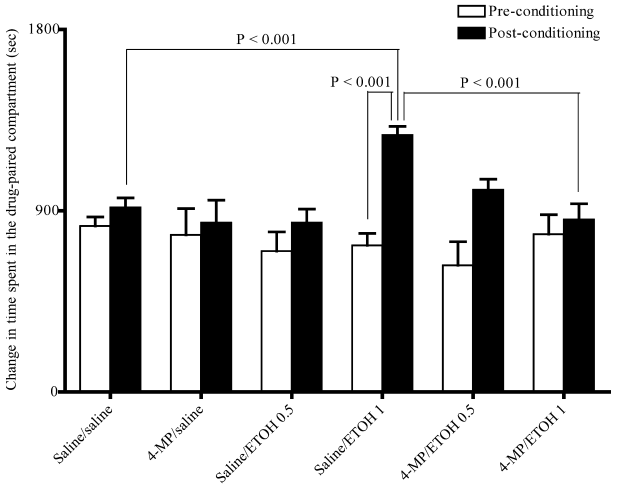


**Fig. 4.** Effect of 4-MP-pretreatment (45 mg/kg, i.p.) on lower dose of EtOH (0.5 mg/kg, i.g.). Data are shown as time in seconds (±SEM; $n = 9$–24). Significant differences between time spent during postconditioning phase compared to saline/saline group, to preconditioning or postconditioning phase of EtOH group are indicated (two-way ANOVA followed by Newman–Keuls, post hoc test).

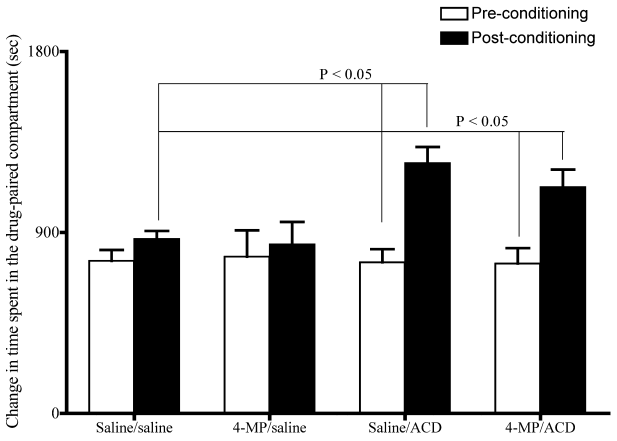


**Fig. 5.** Effect of 4-MP-pretreatment (45 mg/kg, i.p.) on ACD-induced cpp (20 mg/kg, i.g.). Data are shown as time in seconds (±SEM; $n = 6$-8). Significant differences between time spent during postconditioning phase compared to preconditioning or postconditioning phase of saline/saline group are indicated (two-way ANOVA followed by Newman–Keuls, post hoc test).

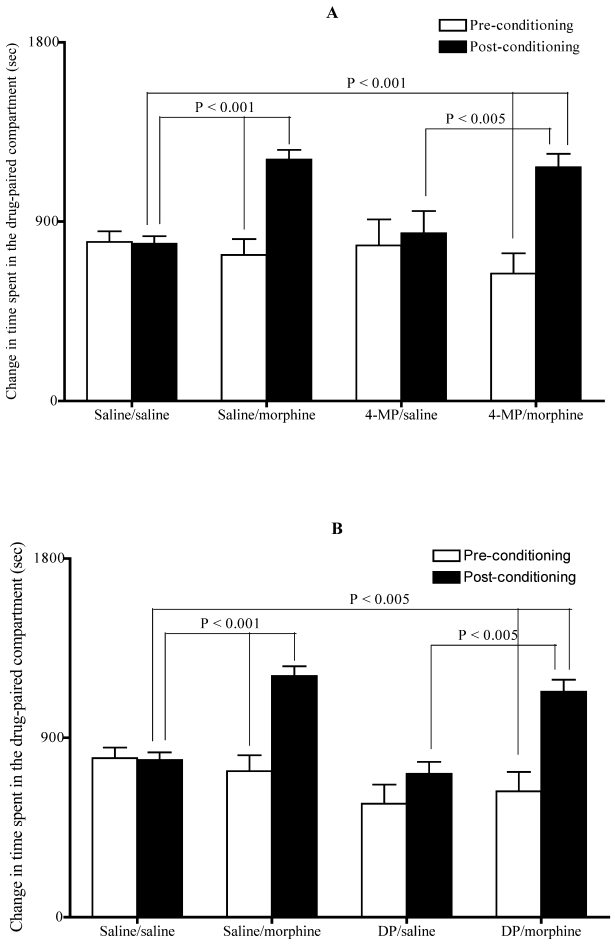


**Fig. 6.** Effect of 4-MP (45 mg/kg, i.p.), panel **A** and DP (50 mg/kg, i.p.), panel **B**: pretreatment on morphine-induced cpp (2.5 mg/kg, i.p.). Data are shown as time in seconds (±SEM; $n = 8$–10). Significant differences between time spent during postconditioning phase compared to preconditioning or postconditioning phase of saline/saline or 4-MP, DP/saline group are indicated (two-way ANOVA followed by Newman–Keuls, post hoc test).

i.g.), during conditioning session, is shown in Fig. 7. Two-way ANOVA of DP-pretreatment on EtOH-induced cpp values revealed significant effects of group [$F(5,94) = 5.70$, $p < 0.0005$], of conditioning group [$F(1,94) = 20.37$, $p < 0.00005$] and group × conditioning group interaction [$F(5,94) = 4.06$, $p < 0.005$]. The saline/saline-treated group ($n = 24$) versus DP (25 and 50 mg/kg; $n = 14$ in both cases)/saline groups spent equal time in the drug-paired compartments, indicating an absence of cpp. Treatment with saline/EtOH ($n = 29$, 1 g/kg, i.g.; $n = 29$), as indicated in previous experiments, induced an increase in time spent in the postconditioning phase ($n = 29$, 1159.5 ± 54.9 seconds) with respect to its pre-conditioning phase (621.2 ± 56.7 sec-

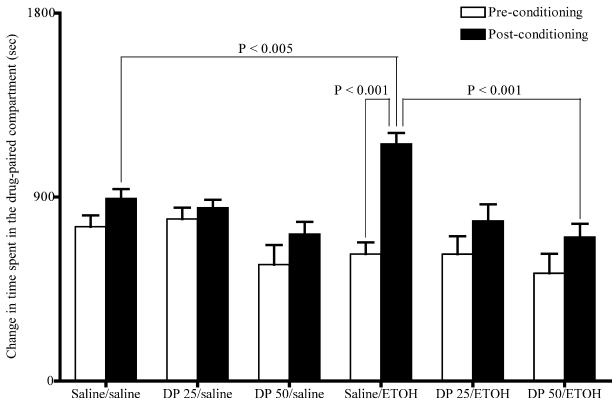


**Fig. 7.** Effect of DP-pretreatment (25 and 50 mg/kg, i.p.) on EtOH-induced cpp (1 g/kg, i.g.). Data are shown as time in seconds (±SEM; $n$ = 14–29). Significant differences between time spent during postconditioning phase compared to preconditioning phase or that of saline/saline or EtOH group are indicated (two-way ANOVA followed by Newman–Keuls, post hoc test).

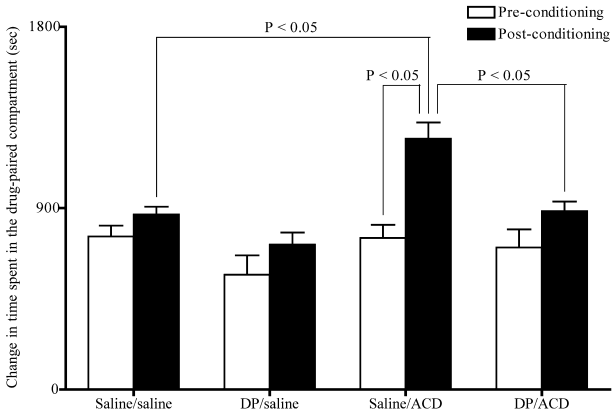


**Fig. 8.** Effect of D-penicillamine-pretreatment (50 mg/kg, i.p.) on ACD-induced cpp (20 mg/kg, i.g.). Data are shown as time in seconds (±SEM; $n$ = 6-14). Significant differences between time spent during postconditioning phase compared to preconditioning or postconditioning phase of saline/-saline or saline/ACD group are indicated (two-way ANOVA followed by Newman–Keuls, post hoc test).

onds; $p < 0.001$) and with respect to postconditioning phase of saline/saline group ($n = 33$, 892.9 ± 45.6 seconds; $p < 0.005$). When rats were pretreated with DP at the lower dose ($n = 14$, 25 mg/kg) showed a slight reduction in EtOH-induced cpp. In contrast, pretreatment with 50 mg/kg dose of DP fully decreased the effect of EtOH-induced cpp ($n = 14$, $p < 0.001$). In fact, the animals pretreated with DP spent less time in the compartment paired with DP/EtOH (25 mg/kg: 782.6 ± 82.0 seconds; 50 mg/kg: 704.9 ± 63.9 seconds) with respect to the group treated with saline/EtOH-induced cpp ($n = 29$, 1159.5 ± 54.9 seconds).

*Effect of* D-*Penicillamine-Pretreatment on ACD-Induced cpp*. The effect of DP-pretreatment (50 mg/kg, i.p.) on ACD-induced cpp (20 mg/kg, i.g.) is shown in Fig. 8. This DP dose was chosen because it was found to be the minimal dose that fully prevented EtOH-induced cpp. Two-way ANOVA of ACD-induced cpp values in response to DP-pretreatment revealed a significant effect of group [$F(3,25) = 4.94$, $p < 0.01$], of conditioning group [$F(1,25) = 13.77$, $p < 0.001$] and group × conditioning group interaction [$F(3,25) = 1.79$, $p < 0.05$]. The saline/saline group in comparison with DP/saline group (as reported above), spent equal time both in preconditioning and postconditioning session, indicating an absence of cpp effects. Rats treated with saline/ACD showed an increase of time spent during the postconditioning phase (1245.0 ± 80.9 seconds; $n = 8$) with respect to that of saline/saline group (867.8 ± 40.2 seconds; $n = 7$; $p < 0.05$) and to its pre-conditioning phase (752.0 ± 65.4 seconds; $p < 0.05$). Further, as shown in Fig. 8, animals pretreated with DP before i.g. ACD did not show preference for the DP/ACD-paired compartment (885.4 ± 48.1 seconds; $n = 7$) with respect to its preconditioning phase (704.4 ± 90.3 seconds) and with respect to the postconditioning phase of saline/saline group (867.8 ± 40.2 seconds). Indeed, pretreatment with DP abolished the action of cpp induced by ACD, because the animals spent significantly less time in the compartment paired with DP/ACD with respect to the group conditioned with saline/ACD-induced cpp (1245.0 ± 80.9 seconds; $n = 8$; $p < 0.05$).

*Effect of* D-*Penicillamine-Pretreatment on Morphine-Induced cpp*. Two-way ANOVA of DP on morphine-induced cpp values yielded significant effects of group [$F(3,40) = 6.50$, $p < 0.005$], of conditioning group [$F(1,40) = 37.97$, $p < 0.0000005$] and group × conditioning group interaction [$F(4,40) = 8.47$, $p < 0.0005$]. Pretreatment with DP (50 mg/kg, i.p.; $n = 9$), 30 minutes before morphine, did not interfere with morphine-induced cpp. In fact, animals showed preference for the DP/morphine-paired compartment (1132 ± 59.8 seconds; $n = 8$) with respect to saline/saline group (as reported above, 788.7 ± 38.3 seconds; $p < 0.005$; $n = 15$) and with respect to its pre-conditioning phase (631.6 ± 97.4 seconds; $p < 0.005$; $n = 8$). Indeed, rats conditioned with DP/morphine spent equal time during the postconditioning phase (1132 ± 59.8 seconds) with respect to the group paired with saline/morphine (as reported above, 1210.6 ± 49.1 seconds; $n = 10$), (Fig. 6, panel *B*).

*Blood EtOH and ACD Levels*. In order to characterize blood EtOH and ACD levels, produced by an i.g. treatment with 1 g/kg dose of EtOH, an additional group of rats ($n = 18$) were used. Blood samples were taken at 30, 60, and 120 minutes after each treatment. Mean blood EtOH and ACD levels at each time point are listed in Table 1. Two-way ANOVA (group × time) yielded significant effects of group [$F(7,33) = 33.48$, $p < 0.001$]. As can be seen, blood EtOH concentrations were positively related to i.g. EtOH (1 g/kg, $n = 6$) where 4-MP (45 mg/kg, $n = 6$), significantly increased it (30 min: $p < 0.05$, 60 minutes $p < 0.05$, 120 minutes $p < 0.05$, respectively). Moreover, blood ACD

levels were increased after the same EtOH treatment achieved significant concentration at all time point. When rats ($n = 6$) were pretreated with a single dose of 4-MP showed a significant reduction in blood-ACD concentrations with respect to EtOH-treated group (30 min: $p < 0.01$, 60 minutes $p < 0.005$, 120 minutes $p < 0.005$, respectively).

## DISCUSSION

The present results suggest that EtOH-derived ACD participates in mediating motivational effects of EtOH ingested, as indexed by cpp method. In fact, the most interesting finding was that 4-MP, a peripheral competitive inhibitor of ADH and DP, a selective ACD-sequestrating agent reduced i.g. EtOH-induced cpp. Moreover, DP, but not 4-MP, prevented i.g. ACD-induced cpp. In addition, both pretreatments did not interfere with morphine-induced cpp indicating that these functional antagonists specifically modulate the motivational properties of EtOH and ACD. Thus, the ability of 4-MP and DP to decrease EtOH-induced cpp could be mediated by a reduction of ACD levels formed after peripheral and central EtOH metabolism. The lack of effect of 4-MP on ACD-induced cpp, tends to rule out the effect of high blood levels of EtOH produced (see also Waller et al., 1982) by blockade of its metabolism with 4-MP, further supporting the notion that ACD could play a key role in the affective/motivational properties of EtOH ingested as well as in its abuse liability.

Alcohol dehydrogenase represents the main peripheral metabolic pathway by which EtOH, contained in alcoholic beverages, is converted into ACD upon ingestion and it is normally found in gastric and hepatic human tissue (Baraona et al., 1991). Importantly, in this study, we show that pretreatment with 4-MP, reduced i.g. EtOH-induced cpp in a dose-dependent manner, a finding suggestive that ACD metabolically derived from EtOH could be responsible for this effect. Since the brain does not possess physiologically active ADH, the effect of 4-MP is restricted to the periphery, where it prevents ACD formation (Escarabajal and Aragon, 2002a,b). In contrast, brain ACD production is only slightly affected by the administration of 4-MP, because EtOH is metabolized by alternative pathways within the brain. Quertemont et al. (2005) reported that 4-MP prevents the effects of cyanamide, an ALDH inhibitor, on EtOH-induced behaviors; concluding that these effects are mediated by a peripheral accumulation of ACD. A possible explanation for the ability of 4-MP to block EtOH-induced cpp would be that 4-MP per se might have motivational properties. However, treatment with 4-MP (22.5, 45, and 67.5 mg/kg) did not produce neither rewarding nor aversive effects since when paired with saline, it failed to affect place conditioning. Thus, the observation that 4-MP failed to produce cpp or cpa per se, but reduced EtOH-induced place preference, suggests that EtOH primary action on cpp can be attributed to EtOH-derived ACD. Moreover, the present study revealed that 4-MP did not affect the positive effects of i.g. ACD in rats. In fact, on the test day, the animals pretreated with 4-MP during the conditioning session with ACD, showed the same level of preference with respect to the group treated with saline/ACD. These observations further suggest that the effect of 4-MP could be mediated by a reduction of ACD levels formed after peripheral EtOH metabolism and support the above interpretation. On the other hand, it seems unlikely that the increase of EtOH-circulating levels after 4-MP administration can produce cpp; as blockade of ADH increases blood EtOH levels roughly by 5 to 6 times (Waller et al., 1982) and these high concentrations of EtOH are known not to induce cpp effect (Bozarth, 1990; Van der Kooy et al., 1983). On the other hand, from 2 g/kg of EtOH (not induced cpp) derived blood levels of ACD should correspond to ACD dose (40 mg/kg) not inducing cpp. Coherently, our present study revealed that 4-MP did not affect the lack of cpp effect of EtOH administered at the lower dose (0.5 mg/kg, i.g.) thereby further supporting the notion that motivational properties of EtOH could be due to its first metabolite, ACD. Indeed, EtOH accumulation (0.5 g/kg, i.g.) consequent to 4-MP pretreatment did not induce cpp effect. Nevertheless, the observation that 4-MP neither affects ACD nor morphine-induced cpp further suggests that the ability of 4-MP to block EtOH-induced cpp could be mediated by a reduction in ACD levels formed after peripheral EtOH metabolism. We did not rule out that 4-MP produced a shift to the right in cpp EtOH dose–response but data with DP well supported the idea of ACD role in EtOH rewardings properties. Indeed, the lack of EtOH-induced cpp could be ascribed to high blood EtOH concentration, and consequent behavioral effects. However, this possibility is not supported by present findings and recent experiments showing that EtOH and ACD-induced cpp was precluded by DP administration (Font et al., 2006a) that, while preventing ACD effects by virtue of its sequestering properties (Nagasawa et al., 1978), does not increase blood EtOH levels. In addition, ICV administration of DP, but not its peripheral administration, selectively prevents spontaneous EtOH intake (Font et al., 2006b) whereas its peripheral actions produced changes in the ingestive and flavor properties of sucrose and EtOH, thereby suggesting a crucial role for central EtOH-derived ACD into the motivational properties of EtOH self-administration.

The selection of doses for gavage administrations of EtOH and ACD was based on i.p. doses, reported by other authors (Bozarth, 1990; Quertemont and De Witte, 2001), considering that an i.g. treatment would be subjected to substantial first pass metabolism and that the rise in blood concentration could be more slow than after i.p. doses. Therefore, a comparison of doses between the two routes of administration can only be taken as indicative.

The present results showed that EtOH-induced positive motivational properties, as indexed by a slightly biased place conditioning paradigm. In fact, administration of EtOH, at the dose of 1 g/kg i.g., produced a significant cpp while failing to affect spontaneous preference when conditioning was performed at 0.5 and 2 g/kg, similarly to that reported by others

using a similar dose range and method but employing different routes of administration (Biala and Kotlińska, 1999; Bozarth, 1990). Previous studies, on the effects of EtOH on cpp in rats have generated conflicting results, reporting cpa (Fidler et al., 2004) or, at low doses, no effect (Bozarth, 1990). Our cpp results are in agreement with Roma and Riley (2005) and Cunningham et al. (2003) which reported that place conditioning was apparent only when EtOH was paired with the initially less preferred cue, and not when paired with the preferred cue (Cunningham et al., 2003). Our findings contrast with the results of the study by Quertemont and De Witte (2001) reporting the lack of EtOH-induced cpp; however, an insufficient number of conditioning pairings (eight in the study by Quertemont and De Witte, 2001) might give reason for these differences since in agreement with Bozarth (1990), Bieńkowski et al. (1996) and Biala and Kotlińska (1999) we could obtain EtOH-induced cpp following eight conditioning pairings. In this regard, the observation that EtOH can induce cpp, cpa or have no motivational effects, suggest that its motivational properties may result from a complex interaction among a number of variables including genetics, route of administration (Fidler et al., 2004), experimental design (biased vs. unbiased), number and time of conditioning trials and doses of EtOH (Bozarth, 1990; Carr et al., 1989).

Similarly to EtOH, ACD administered orally by gavage, displayed a similar bell-shaped dose–response curve on cpp effect at doses well in the range of those previously reported by Quertemont and De Witte (2001) in mice. ACD has been reported to be a more potent reinforcer than EtOH; in agreement with Rodd-Henricks et al. (2002), it seems relevant to observe that in this study, ACD-induced cpp could be obtained after four pairings of the dose of 20 mg⁄kg with the less preferred compartment whereas EtOH required eight pairings with 1 g⁄kg. Moreover, after a single i.g. dose of EtOH-induced cpp (1000 mg⁄kg) blood EtOH levels are about 0.132 mg⁄ml, well in the range of those determined in mice by Cunningham et al. (2002). Furthermore, after the same EtOH dose, ACD-blood levels are about 0.0034 mg⁄ml, corresponding at a range dose of ACD similar to the one inducing cpp (20 mg⁄kg i.g.). Thus i.g 1 g⁄kg dose of EtOH achieved similar blood ACD levels than those produced by ACD treatment (20 mg⁄kg, i.g.).

The effect of ACD administration (20 mg⁄kg, i.g.) on cpp paradigm adds further support to the hypothesis that high blood ACD levels from EtOH metabolism can saturate blood–brain barrier (BBB) and therefore cross into the brain, potentially adding to local ACD produced from EtOH via the catalase system (Quertemont et al., 2005). Moreover, several studies demonstrated that systemic ACD injections (20 mg⁄kg higher doses) saturated both liver and endothelial ALDH (Hoover and Brien, 1981; Ward et al., 1997). Under these circumstances, ACD can cross BBB and exert some actions in the brain. On the other hand, the notion that ACD can cross the BBB is supported by previous literature which showed that peripherally administered EtOH yields significant ACD levels in the brain (Isse et al. 2005) and peripherally administered ACD is found in relevant concentrations in the CNS (Ward et al., 1997; Quintanilla and Tampier 2003; Quintanilla et al. 2002; Quertemont and Tambour 2004; Heap et al. 1995).

In line with the above results, the amino acid, DP, at doses not producing any effect by itself, decreased the effect of i.g. EtOH and ACD-induced cpp. Moreover, morphine-induced cpp in rats was not affected by DP pretreatment, indicating that DP specifically modulates the motivational properties of EtOH and ACD. We selected these doses of DP (25 and 50 mg⁄kg) based on previous findings (Font et al., 2005, 2006a,b) demonstrating an interaction between DP on i.p. treatment of EtOH or ACD. The mechanism by which DP reduced EtOH and ACD-induced cpp could be envisaged in previous findings indicating that DP can condense, *in vivo,* with EtOH-derived ACD and form adducts. The condensation product of ACD is the cyclic amino acid, 2,5,5-trimethylthiazolidine-4-carboxylic acid, that shows enough stability to be excreted in urine (Cohen et al., 2000; Font et al., 2006a; Nagasawa et al., 1975, 1987). Further, Nagasawa et al. (1977, 1978, 1980) demonstrated that i.p. administration of DP to rats, at different intervals before an injection of EtOH resulted in a significant and sustained lowering of blood ACD levels, with an average reduction of 70% (Yusof et al., 2000), without increasing blood EtOH levels, whereas it is detected in the brain 30 minutes after administration of 100 mg⁄kg of DP (Yusof et al., 2000).

The results of the present experiments are in line with previous observations which reported that ACD inactivation with DP, prevents behavioral activation produced by i.p. ACD and EtOH in mice (Font et al., 2005), abolishes voluntary EtOH consumption in unselected rats (Font et al., 2006b) and reduces the i.p. EtOH-induced cpp in mice (Font et al., 2006a). Although these behavioral paradigms provide a sound and reliable way to test drug affective properties, the lack of a related neurochemical evidence does not allow to precisely ascribe to ACD inactivation by DP the effects observed, especially considering the properties of DP as nitric oxide donor (Feelisch, 1998) and their possible consequences on EtOH effects (Spanagel et al., 2002). However, ACD self-administration into the VTA of alcohol-preferring rats (Rodd-Henricks et al., 2002), together with electrophysiological findings (Foddai et al., 2004) indicating a dose-related, ACD-induced, increase of neuronal activity in VTA (presumably dopamine-containing) neurons, accompanied by a 4-MP-induced reduction of EtOH-induced increments (Foddai et al., 2004), makes the mesolimbic dopamine system a good candidate to elucidate the neurochemical mechanism underlying EtOH-derived ACD-induced motivational properties described in the present study. In line with this possibility, we recently found that EtOH-derived ACD augments dopamine outflow in the nucleus accumbens of freely behaving rats (Enrico et al., 2006).

Considering these data as a whole, it is tempting to speculate a different role for ACD naturally contained in wine and other alcoholic beverages (Liu and Pilone, 2000), as being much

more than merely a volatile flavor compound (Genovese et al., 2005). In fact, ACD ingested together with alcoholic drinks may reach the CNS, actively participating to the rewarding and motivational effects of EtOH.

## CONCLUSIONS

In summary, we propose that the ability of 4-MP and DP to reduce i.g. EtOH-induced cpp could be mediated by a reduction in ACD levels formed after EtOH metabolism. Understandably, further research in this direction is needed, for a better view of the neurochemical substrates mediating the expression of the conditioned motivational properties of EtOH, nevertheless the present results challenge significantly the assumption that EtOH per se mediates its own rewarding properties and provide further support to the hypothesis that motivational actions of EtOH ingestion could be mediated by its first metabolite, ACD. It seems plausible to suggest that modulation of EtOH-derived ACD, either by reducing its production and/or by using sequestrating agents, may exert a profound influence on the euphoriant effects and discriminative stimulus of EtOH, thereby decreasing the motivational effects associated with EtOH intake. The present observations may also bear important theoretical consequences on the therapeutic side of alcoholism. Indeed, these results suggest that pharmacological blockade of EtOH metabolism would deprive it of its rewarding properties and, quite possibly, discourage individuals from drinking.

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## REFERENCES

Amit Z (1977) Brain and liver aldehyde dehydrogenase: relations to ethanol consumption in wistar rats. Neuropharmacology 16:781–784.

Baraona E, Yokoyama A, Ishii H, Hernandez-Munoz R, Takagi T, Tsuchiya M, Lieber CS (1991) Lack of alcohol dehydrogenase isoenzyme activities in the stomach of Japanese subjects. Life Sci 49:1929–1934.

Biala G, Kotlińska J (1999) Blockade of the acquisition of ethanol-induced contitioned place preference by N-methyl-D-Aspartate receptor antagonist. Alcohol Alcohol 28(Suppl. 2):345–351.

Bieńkowski P, Kuca P, Piasecki J, Kostowski W (1996) Low doses of ethanol induce conditioned place preference in rats after repeated exposure to ethanol or saline injection. Alcohol Alcohol 32:547–553.

Bozarth MA (1990) Evidence for the rewarding effects of ethanol using the conditioned place preference method. Pharmacol Biochem Behav 35:485–487.

Brown ZW, Amit Z, Rockman GE (1979) Intraventricular self-administration of acetaldehyde, but not ethanol, in naïve laboratory rats. Psychopharmacology (Berl) 64:271–276.

Brown ZW, Amit Z, Smith BR (1980) Intraventricular self-administration of acetaldehyde and voluntary consumption of ethanol in rats. Behav Neural Biol 28:150–155.

Brown ZW, Amit Z, Smith BR, Sutherland EA, Selvaggi N (1983) Alcohol-induced euphoria enhanced by disulfiram and calcium carbimide. Alcohol Clin Exp Res 7:276–278.

Carr GD, Fibiger HC, Phillips AG (1989) Conditioned place preference as a measure of drug reward, in The Neuropharmacological Basis of Rewar (Liebman J, Cooper S eds), pp 264–319. Oxford Science Publication, Clarendon Press, Oxford.

Chevens LCF (1953) Antabuse addiction. Br J Med 1:1450–1451.

Cohen JF, Elberling JA, DeMaster E, Lin RC, Nagasawa HT (2000) N-Terminal dipeptides of D-(-)-penicillamine as sequestration agents for acetaldehyde. J Med Chem 43:1029–1033.

Cunningham CL, Clemans GM, Fidler TL (2002) Injection timing determines whether intragastric ethanol produces conditioned place preference or aversion in mice. Pharmacol Biochem Behav 72:659–668.

Cunningham CL, Ferree NK, Howard MA (2003) Apparatus bias and place conditioning with ethanol in mice. Psychopharmacology 170:409–422.

Enrico P, Peana AT, Sirca D, Mereu M, Demurtas L, Columbano N, Diana M (2006) . Are the hedonistic properties of oral ethanol mediated by acetaldehyde? In vivo microdialysis study in the rat. Program No. 193.17/NN762006 Neuroscience Meeting Planner. Society for Neuroscience, 2006. Online, Atlanta, GA.

Escarabajal MD, Aragon CM (2002a) The effect of cianamide and 4-methylpyrazole on the ethanol-induced locomotor activity in mice. Pharmacol Biochem Behav 72:389–395.

Escarabajal MD, Aragon CM (2002b) Concurrent administration of diethyldithiocarbamate and 4-methylpyrazole enhances ethanol-induced locomotor activity: the role of brain ALDH. Psychopharmacology 160:339–343.

Feelisch M (1998) The use of nitric oxide donors in pharmacological studies. Naunyn Schmiedebergs Arch Pharmacol 358:113–122.

Fidler TL, Bakner L, Cunningham CL (2004) Conditioned place aversion induced by intragastric administration of ethanol in rats. Pharmacol Biochem Behav 77:731–743.

Foddai M, Dosia G, Spiga S, Diana M (2004) Acetaldehyde increases dopaminergic neuronal activity in the VTA. Neuropsychopharmacology 29:530–536.

Font L, Aragon CM, Miquel M (2006a) Ethanol-induced conditioned place preference, but not aversion, is blocked by treatment with D-penicillamine, an inactivation agent for acetaldehyde. Psychopharmacology 184:56–64.

Font L, Aragon CM, Miquel M (2006b) Voluntary ethanol consumption decreases after the inactivation of central acetaldehyde by d-penicillamine. Behav Brain Res 171:78–86.

Font L, Miquel M, Aragon CM (2005) Prevention of ethanol-induced behavioral stimulation by D-penicillamine: a sequestration agent for acetaldehyde. Alcohol Clin Exp Res 29:1156–1164.

Genovese A, Dimaggio R, Lisanti MT, Piombino P, Moio L (2005) Aroma composition of red wines by different extraction methods and gas chromatography-sim/mass spectrometry analysis. Ann Chim 95:383–394.

Heap L, Ward RJ, Abiaka C, Dexter D, Lawlor M, Pratt O, Thomson A, Shaw K, Peters TJ (1995) The influence of brain acetaldehyde on oxidative status, dopamine metabolism and visual discrimination task. Biochem Pharmacol 50:263–270.

Hoover DJ, Brien JF (1981) Acetaldehyde concentration in rat blood and brain during the calcium carbimide-ethanol interaction. Can J Physiol Pharmacol 59:65–70.

Hunt WA (1996) Role of acetaldehyde in the actions of ethanol on the brain. Alcohol 13:147–151.

Isse T, Matsuno K, Oyama T, Kitagawa K, Kawamoto T (2005) Aldehyde dehydrogenase 2 gene targeting mouse lacking enzyme activity shows high acetaldehyde level in blood, brain, and liver after ethanol gavages. Alcohol Clin Exp Res 29:1959–1964.

Liu S, Pilone GJ (2000) An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implications. Int J Food Sci Technol 35:49–61.

Medicamenta (1991–1992). Vol.1 parte generale: Tecnica Farmaceutica, Diluizione dell'alcool, Tab. 18-19, Cooperativa Farmaceutica Milanese, Milan, Italy. pp 814–815.

Myers WD, Ng KT, Singer G (1984) Effects of naloxone and buprenorphine on intravenous acetaldehyde self-injection in rats. Physiol Behav 33:449–455.

Nagasawa HT, Elberling JA, DeMaster EG (1980) Structural requirements for the sequestration of metabolically generated acetaldehyde. J Med Chem 23:140–143.

Nagasawa HT, Elberling JA, Roberts J (1987) β-substituted cysteines as sequestering agents for ethanol-derived acetaldehyde in vivo. J Med Chem 30:1373–1378.

Nagasawa HT, Goon DJ, Constantino NV, Alexander CS (1975) Diversion of ethanol metabolism by sulfhydryl amino acids. D-penicillamine-directed excretion of 2,5,5-trimethyl-D-thiazolidine-4-carboxylic acid in the urine of rats after ethanol administration. Life Sci 17:707–713.

Nagasawa HT, Goon DJ, DeMaster EG (1978) 2,5,5-Trimethylthiazolidine-4-carboxylic acid, a D(-)-penicillamine-directed pseudometabolite of ethanol. Detoxication mechanism for acetaldehyde. J Med Chem 21:1274–1279.

Nagasawa HT, Goon DJ, DeMaster EG, Alexander CS (1977) Lowering of ethanol-derived circulating blood acetaldehyde in rats by D-penicillamine. Life Sci 20:187–193.

Quertemont E, De Witte P (2001) Conditioned stimulus preference after acetaldehyde but not ethanol injections. Pharmacol Biochem Behav 68:449–454.

Quertemont E, Tambour S (2004) Is ethanol a pro-drug? The role of acetaldehyde in the central effects of ethanol. Trends Pharmacol Sci 25:130–134.

Quertemont E, Tambour S., Tirelli E (2005) The role of acetaldehyde in the neurobehavioral effects of ethanol: A comprehensive review of animal studies. Prog Neurobiol 75:247–274.

Quintanilla ME, Callejas O, Tampier L (2002) Aversion to acetaldehyde: differences in low-alcohol-drinking (UChA) and high-alcohol-drinking (UChB) rats. Alcohol 26:69–74.

Quintanilla ME, Tampier L (2003) Brain mitochondrial aldehyde dehydrogenase: relation to acetaldehyde aversion in low-alcohol-drinking (UChA) and high-alcohol-drinking (UChB) rats. Addict Biol 8:387–397.

Rodd-Henricks ZA, Melendez RI, Zaffaroni A, Goldstein A, McBride WJ, Lu TK (2002) The reinforcing effects of acetaldehyde in the posterior ventral tegmental area of alcohol-preferring rats. Pharmacol Biochem Behav 72:55–64.

Roma PG, Riley AL (2005) Apparatus bias and the use of light and texture in place conditioning. Pharmacol Biochem Behav 82:163–169.

Smith BR, Amit Z, Splawinsky J (1984) Conditioned place preference induced by intraventricular infusions of acetaldehyde. Alcohol 1:193–195.

Spanagel R, Siegmund S, Cowen M, Schroff KC, Schumann G, Fiserova M, Sillaber I, Wellek S, Singer M, Putzke J (2002) The neuronal nitric oxide synthase gene is critically involved in neurobehavioral effects of alcohol. J Neurosci 22:8676–8683.

Stowell RM, Greenway AR, Batt RD (1977) Acetaldehyde formation during deproteinization of human blood samples containing ethanol. Biochem. Med. 18:392–401.

Suh JJ, Pettinati HM, Kampman KM, O'Brien CP (2006) The status of disulfiram: a half of a century later. J Clin Psychopharmacol 26:290–302.

Van der Kooy D, O'Shaunessy M, Mucha R, Kalant H (1983) Motional properties of ethanol in naive rats as studied by place contditioning. Pharmacol Biochem Behav 19:441–445.

Waller MB, McBride WJ, Lumeng L, Li TK (1982) Effects of intravenous ethanol and of 4-methylpyrazole on alcohol drinking in alcohol-preferring rats. Pharmacol Biochem Behav 17:763–768.

Ward RJ, Colantuoni C, Dahchour A, Quertemont E, De Witte P (1997) Acetaldehyde-induced changes in monoamine and amino acid extracellular microdialysate content of the nucleus accumbens. Neuropharmacology 36:225–232.

Yusof M, Neal R, Aykin N, Ercal N (2000) High performance liquid chromatography analysis of D-penicillamine by derivatization with N-(1-pyrenyl)maleimide (NPM). Biomed Chromatogr 14:535–540.