

RESEARCH ARTICLE

# The acute anti-craving effect of acamprosate in alcohol-preferring rats is associated with modulation of the mesolimbic dopamine system

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## Abstract

*Acamprosate (Campral<sup>®</sup>) is a drug used clinically for the treatment of alcoholism. In order to examine further the time-course and mechanism of action of acamprosate, the effect of acute and repeated acamprosate administration was examined on (i) operant ethanol self-administration and (ii) voluntary home cage ethanol consumption by alcohol-preferring Fawn-Hooded, iP and Alko Alcohol (AA) rats. Acutely, acamprosate was shown to cause a significant decrease in operant ethanol self-administration by Fawn-Hooded and alcohol-preferring iP rats in part by decreasing the motivational relevance of a specific ethanol cue; however, repeated injection of acamprosate led to tolerance to this effect. Voluntary alcohol consumption in the home cage in Fawn-Hooded and AA rats was also reduced by an acute acamprosate injection; however, again tolerance developed to repeated injections. In a separate experiment, the effect of acamprosate on markers of the dopaminergic system was examined. Interestingly, acute acamprosate was also shown to cause increased dopamine transporter density and decreased dopamine D<sub>2</sub>-like receptor density within the nucleus accumbens but not in the caudate-putamen, suggesting a link between the decreased motivational salience of the ethanol cue and altered dopaminergic signalling within the nucleus accumbens. With repeated injections of acamprosate, markers of the dopaminergic system returned to steady state levels with a similar temporal profile to the development of tolerance in the behavioural studies. Along with previous studies, our findings indicate that acamprosate modulates the mesolimbic dopaminergic system and may thereby decrease ethanol reinforcement processes; however, these effects undergo tolerance in alcohol-preferring rats and may in part explain the fact why some subjects are non-responders to chronic acamprosate treatment.*

## Introduction

Acamprosate (Campral<sup>®</sup>, Lipha) is a drug used clinically for the treatment of alcoholism. Several clinical studies (Lhuintre *et al.*, 1985; Paille *et al.*, 1995; Sass *et al.*, 1996; Whitworth *et al.*, 1996; Pelc *et al.*, 1997; Kiefer *et al.*, 2003) and recent meta-analyses (Kranzler and Van Kirk, 2001; Mann, 2004) have indicated significantly higher continuous abstinence rates, decreased relapse rates and higher treatment retention rates compared with placebo. The mechanism of action of acamprosate remains somewhat unclear; however, an interaction with glutamatergic/excitatory amino acid (EAA) neurotransmission has been suggested (Spanagel and Ziegl-

gansberger, 1997; Naassila *et al.*, 1998a). Thus, acamprosate can attenuate the increased glutamate release within the nucleus accumbens in ethanol-withdrawn rats (Dahchour *et al.*, 1998), a phenomenon which has been linked to the hyperexcitability seen in withdrawal (Spanagel *et al.*, 1996a). Furthermore, a link was demonstrated recently between a hyper-glutamatergic state, enhanced alcohol intake and the action of acamprosate (Spanagel *et al.*, 2005). Thus, Per2<sup>Brdm1</sup> mutant mice (mice deficient for the Per2 clock gene), which exhibit decreased glutamate transport activity and subsequent elevated levels of extracellular glutamate within the nucleus accumbens, also show enhanced con-

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sumption of ethanol compared with wild-type mice. Per<sup>2</sup><sup>Brdm1</sup> mice respond well to acamprosate in terms of a reduction of extracellular glutamate levels within the nucleus accumbens to wild-type levels, as well as a decrease in ethanol consumption to the level of wild-type mice (Spanagel *et al.*, 2005). The effect of acamprosate on this hyper-glutamatergic state might be brought about via several overlapping mechanisms: acamprosate binds to mGlu5 receptors (Harris *et al.*, 2002), antagonizes Ca<sup>2+</sup> influx (Allgaier *et al.*, 2000) and interacts with NMDA receptors in a complex fashion (Naassila *et al.*, 1998a; Rammes *et al.*, 2001). This polymodal mechanism of action of acamprosate may explain the conflicting data obtained previously in electrophysiology studies (Zeise *et al.*, 1993; Madamba *et al.*, 1996; Berton *et al.*, 1998; Popp and Lovinger, 2000). However, at a behavioural level the mechanism of action of acamprosate is still unclear.

Acamprosate has been hypothesized to prevent relapse by preventing conditioned withdrawal, induced by environmental stimuli secondarily conditioned by ethanol (Littleton, 1995). Some studies have indicated that acamprosate can indeed prevent the conditioned behavioural responses to environmental stimuli associated previously with ethanol (Cole *et al.*, 2000; Quertemont *et al.*, 2002) as well as the reinstatement of alcohol-seeking behaviour by stimuli conditioned secondarily to ethanol (Bachteler *et al.*, 2005). At a neurochemical level, conditioned stimuli have been shown to cause the release of dopamine within the ventral striatum/nucleus accumbens; this appears to involve glutamate-induced activation of dopaminergic terminals within the nucleus accumbens (Spanagel and Weiss, 1999; Joseph *et al.*, 2003). Of particular interest, therefore, is the recent demonstration that acamprosate can prevent ethanol- and glutamate-induced (whether direct or indirect) release of dopamine within the nucleus accumbens (Olive *et al.*, 2002; Cano-Cebrian *et al.*, 2003a, b). In light of these studies, we have examined the effect of acute and repeated acamprosate injections on markers of dopaminergic neurotransmission within the dorsal striatum (caudate-putamen) and ventral striatum (nucleus accumbens). We have also examined the effect of acamprosate on operant ethanol self-administration and voluntary home cage ethanol consumption by multiple strains of alcohol-preferring rats, in order to determine whether the effect of acamprosate on markers of the dopaminergic system paralleled the effect of acamprosate on these behavioural measures.

## Methods

All experiments were performed in accordance with the Prevention of Cruelty to Animals Act, 1986, Australia under the guidelines of the Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia or in accordance with the German Law on the Protection of Animals, subsequent to approval by the Committee on Animal Care and Use of the relevant local governmental body.

### Animals

Male Fawn-Hooded (FH) rats were obtained from Central Animal Services, Monash University, or from the breeding colony at the Howard Florey Institute, University of Melbourne, at 3 weeks of age. Parental stock had been obtained

previously from the University of North Carolina, School of Medicine, Chapel Hill, USA. Alcohol-preferring iP rats were obtained from the breeding colony at the Howard Florey Institute, University of Melbourne. Parental stock had previously been obtained from Professor T. K. Li (Indiana University, Indianapolis, USA). Alcohol-preferring AA (Alko Alcohol line, National Public Health Institute, Helsinki, Finland) rats were generously supplied by Petri Hyytia. Rats were housed in a 12-hour light/dark cycle with free access to standard rat chow and tap water.

### Operant ethanol self-administration

To examine oral self-administration of ethanol by FH rats ( $n = 12$ ;  $357 \pm 20$  g at 4 months), operant chambers supplied by Med Associates (St Albans, Vermont, USA) were employed. Each chamber was housed individually in sound-attenuation cubicles, featuring a fan to provide airflow and mask external noise, and the chambers were connected to a computer running Med-PC IV software (Med Associates) to record activity. Within the chambers, a house light provided soft illumination during operant sessions. On either side of the operant chambers a retractable lever (exserted during operant sessions) was placed centrally below a stimulus light and adjacent to a fluid receptacle. Each receptacle was fed by a solenoid-controlled liquid dispenser with a 20 ml reservoir. Access by the rat to either receptacle was recorded by interruption of an infrared photobeam.

Training of the FH rats consisted of two initial overnight sessions in the chambers with one receptacle loaded with water and the other with 5% v/v ethanol sweetened with 12% w/v sucrose. Standard rat chow was available *ad libitum* on the floors of the operant chambers during these overnight sessions. An initial fixed response ratio of 1 was used; each lever response was reinforced with the delivery of 100  $\mu$ l of either water or ethanol. All rats learnt to respond for ethanol and water during this first overnight session. In the second overnight session, a fixed ratio of 2 (FR2) was used and the location of the ethanol and water reservoirs was reversed.

Following the overnight sessions, daily training sessions continued using a session length of 40 minutes. A 10% v/v ethanol solution/12% sucrose was used initially. The ethanol and water response levers were alternated from left to right between sessions to avoid place-preference, with a small drop of the current solution (either water or ethanol/sucrose) left in each receptacle to indicate the current orientation of the solutions. As overall preference to ethanol became apparent, a sucrose fade procedure was used (Samson *et al.*, 1988) such that the rats gradually received less and less sucrose until it was completely absent from the 10% ethanol solution; the fixed ratio requirement was also increased to 3 (FR3). For each session, total ethanol and water responses and the number of times the ethanol and water receptacles were accessed (photobeam breaks) were recorded. Ethanol and water reinforcer delivery times and beam break times were recorded, such that the latency of activities could be determined. The difference in fluid in the ethanol reservoir between the beginning and end of the session was also recorded to ensure correct calibration of the delivery system.

Drug administration commenced once responding for ethanol was stable across sessions for most rats ( $< 10\%$

variation across sessions). Three rats were excluded: one due to illness during the initial drug-administration week, and the remaining two due to high variability (> 10% across sessions). The remaining rats ( $n=9$ ) were divided into two groups such that the drinking behaviour of the two groups was not significantly different. Drug administration weeks were structured so that Mondays, Thursdays and Fridays were no-injection days, vehicle (saline) was injected on Tuesdays intraperitoneally (i.p.) and drugs administered on Wednesdays (i.p.). Group 1 was administered 100 mg/kg of acamprosate i.p. in week 1, and 200 mg/kg in week 2. Group 2 received 200 mg/kg and 100 mg/kg i.p. doses of acamprosate, respectively, in weeks 1 and 2. Acamprosate was administered 1 hour prior to placing the rats into the operant chambers.

A separate group of 12 alcohol-preferring iP rats ( $478 \pm 6$  g at the commencement of experimental procedures) were trained to self-administer oral ethanol under operant conditions using the same protocol as for the FH rats. However, as the iP rats appeared to display a deprivation-induced increase in responding for ethanol on the first test day of each week (Monday), both Mondays and Tuesdays were no-injection days; vehicle (saline) was injected on Wednesdays (i.p.) and acamprosate was injected on Thursdays. Friday was again a no-injection day. On two consecutive weeks, all 12 rats received acamprosate (100 mg/kg i.p.) 1 hour prior to placing the rats into the operant chambers. For each session, total ethanol and water responses and the number of times the ethanol and water receptacles were accessed (photobeam breaks) were recorded.

#### Continual access ethanol consumption

To examine the effects of acamprosate on voluntary home cage consumption of ethanol by alcohol-preferring rats, two independent experiments in two different laboratories were conducted. In the first experiment FH rats ( $n=12$ ;  $338 \pm 9$  g at 3 months) were given a choice of 5% ethanol or tap water. After acquiring an ethanol preference, rats were allowed a further 14 days of baseline drinking. The last 3 days of consumption data were analysed before the rats were assigned to either vehicle- or acamprosate-treatment groups. Rats were treated with a daily injection of acamprosate (200 mg/kg i.p.,  $n=6$ ) or vehicle (saline, 1 ml/kg,  $n=6$ ) for 5 days. In comparison to the operant studies a higher dose of acamprosate has been chosen for these experiments, as it has been shown in previous studies that a 200 mg/kg dose of acamprosate is most effective on 24 hours of measurements (e.g. Spanagel *et al.*, 1996a; Rimondini *et al.*, 2002). Body weight, water intake and 5% ethanol intake were measured daily, from which total fluid consumption (ml/day), ethanol consumption per kg body weight (g/kg/day) and preference for ethanol solution (% ml ethanol solution/ml daily fluid intake) were calculated, both before and during drug administration.

In the second experiment, alcohol-preferring AA rats ( $n=16$ ;  $388 \pm 14$  g at 3 months) were given access to tap water, a 5% and 20% ethanol solution (v/v) for 28 days. The last 3 days of consumption data were analysed before the rats were assigned to either vehicle- or acamprosate-treatment groups. Rats were treated with acamprosate (200 mg/kg i.p.,

twice daily,  $n=8$ ) or vehicle (saline, 3 ml/kg, twice daily,  $n=8$ ) for a total of five injections. Water and ethanol intake were measured daily, and each rat's body weight was recorded 24 hours before the first injection and 12 hours after the last injection. From these data, total fluid consumption (ml/day), ethanol consumption per kg body weight (g/kg/day) and preference for ethanol solutions (5% and 20% solutions; % ml ethanol solution/ml daily fluid intake) were calculated, both before and during drug administration.

#### Neurochemistry

Twenty FH rats ( $299 \pm 15$  g at 4 months) were divided into four groups. Two groups were treated daily with acamprosate (200 mg/kg i.p.) and killed by decapitation following 1 or 3 days of treatment, 1 hour after the last treatment, i.e. the same acute pre-treatment time as used for the operant responding protocol. The third group was injected daily with saline vehicle and finally killed by decapitation 1 hour after the last injection. The fourth group was drug-naïve. After decapitation all brains were removed rapidly and frozen over liquid nitrogen. Coronally cut sections (14  $\mu$ m) were mounted onto gelatin/chrom alum-coated slides for autoradiography, and poly-L-lysine-coated slides for *in-situ* hybridization histochemistry. Brain sections were collected encompassing the caudate-putamen and nucleus accumbens (from Bregma 2.2 mm). The slides were stored at  $-80^\circ\text{C}$  and thawed to room temperature when required for use.

#### Autoradiography

In general, for each brain region analysed per ligand, four slide-mounted sections from the region of interest from each animal (typically 100 sections) were used to determine specific binding, with an additional 10 sections chosen randomly from individual animals to determine non-specific binding. Tissue levels of the dopamine transporter were determined using a previously published protocol (McGregor *et al.*, 2003). Sections were pre-incubated in buffer (0.1 M  $\text{NaH}_2\text{PO}_4/0.1$  M sucrose, pH 7.4) for 30 minutes at room temperature. Sections were then incubated in buffer containing 50 pM [ $^{125}\text{I}$ ]RTI-55 (2200 Ci/mmol; Perkin Elmer, USA) and 100 nM fluoxetine (60 minutes at room temperature). GBR12935 (10  $\mu\text{M}$ ) was used to determine non-specific binding. Sections were then washed in ice-cold buffer (1  $\times$  1 min, 2  $\times$  20 minutes), rinsed in ice-cold  $\text{dH}_2\text{O}$  and desiccated overnight. Slides were then apposed to Kodak X-omat AR film in the presence of standard [ $^{14}\text{C}$ ]microscales (American Radiolabelled Chemicals, St Louis, USA) for 7 hours.

Tissue levels of the dopamine  $\text{D}_1$  receptor were determined using a standard protocol (Djouma and Lawrence, 2002). Sections were incubated in buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM  $\text{CaCl}_2$ , 1 mM  $\text{MgCl}_2$  for 30 minutes at room temperature) with 50 nM ketanserin (to occlude binding to  $5\text{HT}_{2A}$  receptors) and 50 pM [ $^{125}\text{I}$ ]SCH23982 (2200 Ci/mmol; Perkin Elmer, USA). SKF77434 (10  $\mu\text{M}$ ) was used to determine non-specific binding. Sections were washed in ice-cold buffer (2  $\times$  5 minutes) and rinsed in ice-cold  $\text{dH}_2\text{O}$ . Following overnight desiccation, slides were apposed to film for 23 hours.

Tissue levels of the dopamine D<sub>2</sub>-like receptor were determined using a standard protocol (Lawrence *et al.*, 1995; Djouma and Lawrence, 2002). [<sup>125</sup>I]NCQ298 was freshly prepared from the des-iodo derivative NCQ634 using [<sup>125</sup>I]Na (2200 Ci/mmol; Amersham International, UK) via the chloramine-T iodination technique (Lawrence *et al.*, 1995). [<sup>125</sup>I]NCQ298 was purified from the iodination reaction mixture by paper chromatography as described previously (Lawrence *et al.*, 1995). Sections were pre-incubated in buffer (170 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 0.001% w/v ascorbic acid) for 30 minutes. Sections were then incubated in buffer for 1 hour with 0.3 nM [<sup>125</sup>I]NCQ298. Non-specific binding was determined using 10 μM raclopride. Sections were washed in ice-cold buffer (4 × 2 minutes) and rinsed in ice-cold dH<sub>2</sub>O (2 × 30 seconds). Following overnight desiccation, slides were apposed to film for 3 hours for striatal sections and 23 hours for mesencephalon sections.

Films were developed with Kodak D-19 developer and analysed using the SCION imaging system for densitometry, by comparing the optical densities resulting from the radioactive ligands with that of corresponding standard microscales under constant illumination. Data are expressed as dpm/mm<sup>2</sup>.

#### Data analysis

Statistical analysis was carried out using SPSS 12.0.1. A significance level of  $p=0.05$  was used throughout. Operant ethanol self-administration and home cage ethanol consumption were analysed using repeated-measures analysis of variance (RM-ANOVA). The effect of acamprosate on neurochemical parameters were analysed using one-way ANOVA. Bonferroni corrections for multiple comparisons were used in all *post-hoc* tests.

## Results

#### Operant ethanol self-administration

During the first week of administration to FH rats, acamprosate caused a significant decrease in responding for ethanol ( $F_{2,14}=5.630$ ,  $p=0.016$ ; Fig. 1a), a significant decrease in the number of times the ethanol receptacle was accessed ( $F_{2,14}=6.417$ ,  $p=0.011$ ; Fig. 1b) and a significant increase in latency until the first ethanol-reinforced response ( $F_{2,14}=4.484$ ,  $p=0.031$ : vehicle,  $48 \pm 15$  seconds; acamprosate,  $323 \pm 134$  seconds); however, there was no effect of acamprosate on the latency until the ethanol receptacle was initially accessed ( $F_{2,14}=1.777$ ,  $p=0.20$ : vehicle,  $25 \pm 8$  seconds; acamprosate,  $20 \pm 6$  seconds). Acamprosate had no significant effect on responses for water ( $F_{2,14}=2.912$ ,  $p=0.088$ ; data not shown), the number of times the water receptacle was accessed ( $F_{2,14}=2.271$ ,  $p=0.140$ ) or the latency until the first water-reinforced response ( $F_{2,14}=0.065$ ,  $p=0.937$ : vehicle,  $314 \pm 156$  seconds; acamprosate,  $211 \pm 131$  seconds). Interestingly, the dose of acamprosate administered (100 or 200 mg/kg) was not a significant factor in any measure analysed: ethanol ( $F_{1,7}=0.001$ ,  $p=0.989$ ) and water ( $F_{1,7}=0.237$ ,  $p=0.641$ ) responses; number of times the ethanol ( $F_{1,7}=0.079$ ,  $p=0.787$ ) or water ( $F_{1,7}=0.504$ ,  $p=0.501$ ) receptacle was

accessed; or latency to the first ethanol-reinforced ( $F_{1,7}=3.902$ ,  $p=0.089$ ) or water-reinforced ( $F_{1,7}=1.515$ ,  $p=0.258$ ) response.

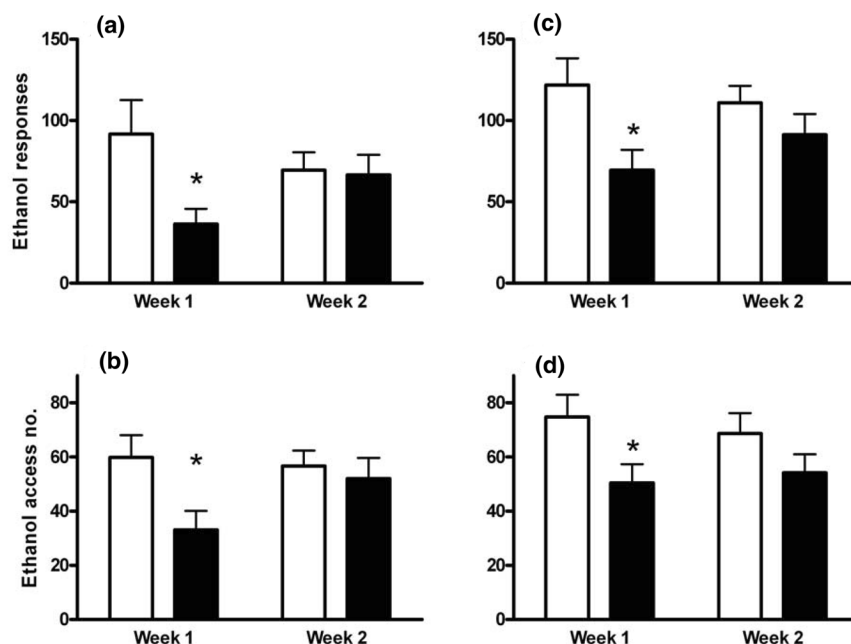
In contrast with the first week of acamprosate administration, the second dose of acamprosate that the rats received (whether 100 mg/kg or 200 mg/kg) had no significant effect on any measure analysed: ethanol ( $F_{2,14}=0.135$ ,  $p=0.875$ ; Fig. 1a) and water ( $F_{2,14}=0.761$ ,  $p=0.486$ ; data not shown) responses, number of times the ethanol ( $F_{2,14}=0.391$ ,  $p=0.684$ ; Fig. 1b) or water ( $F_{2,14}=0.141$ ,  $p=0.684$ ) receptacle was accessed; or latency until the first ethanol-reinforced ( $F_{2,14}=0.712$ ,  $p=0.508$ ) or water-reinforced ( $F_{2,14}=0.138$ ,  $p=0.872$ ) response.

In alcohol-preferring iP rats, treatment with acamprosate (100 mg/kg) led to a significant decrease in responding for ethanol ( $F_{2,22}=5.330$ ,  $p=0.013$ ; Fig. 1c) and a significant decrease in the number of times the ethanol receptacle was accessed ( $F_{2,22}=4.572$ ,  $p=0.022$ ; Fig. 1d) in the first week of treatment. However, acamprosate (100 mg/kg) had no significant effect on operant ethanol self-administration in the second week of administration: ethanol ( $F_{2,22}=0.735$ ,  $p=0.491$ ; Fig. 1c) and water ( $F_{2,22}=2.456$ ,  $p=0.109$ ) responses and the number of times the ethanol ( $F_{2,22}=1.680$ ,  $p=0.210$ ; Fig. 1d) or water ( $F_{2,14}=0.141$ ,  $p=0.684$ ) receptacle was accessed.

#### Continual-access ethanol consumption

Prior to drug administration, no significant difference was observed between FH rats selected for vehicle administration and rats selected for acamprosate administration in terms of ethanol consumption (g/kg/day;  $F_{1,10}=3.944$ ,  $p=0.075$ ; Fig. 2a), ethanol preference ( $F_{1,10}=0.294$ ,  $p=0.6$ ; Fig. 2b), water consumption ( $F_{1,10}=0.463$ ,  $p=0.512$ ; data not shown), and total fluid intake ( $F_{1,10}=0.004$ ,  $p=0.951$ ; Fig. 2c). Compared with vehicle-treated rats, treatment with acamprosate (200 mg/kg/day) caused a significant decrease in ethanol consumption (g/kg/day;  $F_{1,10}=4.969$ ,  $p=0.050$ ; Fig. 2a) and ethanol preference ( $F_{1,10}=7.091$ ,  $p=0.024$ ; Fig. 2b). While there was no significant effect of treatment day on ethanol preference (factor day,  $F_{4,40}=1.598$ ,  $p=0.184$ ) the effect of acamprosate on ethanol consumption diminished over time, such that there was a significant effect of treatment day ( $F_{4,40}=2.704$ ,  $p=0.001$ ) and a significant interaction between day and treatment ( $F_{4,40}=2.107$ ,  $p=0.001$ ). A significant decrease in total fluid intake ( $F_{1,10}=23.188$ ,  $p=0.001$ , Fig. 2c) was entirely attributable to the effect of acamprosate on ethanol consumption.

Prior to drug administration, no significant difference was observed between alcohol-preferring AA rats selected initially for vehicle administration and rats selected for acamprosate administration in terms of ethanol consumption (g/kg/day;  $F_{1,14}=0.010$ ,  $p=0.924$ ; Fig. 2d), overall preference for ethanol ( $F_{1,14}=0.013$ ,  $p=0.910$ ; Fig. 2e) and total fluid intake ( $F_{1,14}=0.289$ ,  $p=0.599$ ; Fig. 2f). Treatment with acamprosate led to a marked reduction in ethanol consumption on the first day of treatment (Fig. 2d), with a significant effect of treatment day ( $F_{1,14}=17.806$ ,  $p=0.001$ ) and a highly significant interaction between treatment and day ( $F_{1,14}=28.568$ ,  $p<0.001$ ). Treatment with acamprosate led to a small but significant decrease in preference for ethanol on



**Figure 1.** Effect of acamprosate on operant responding (a, c) for orally available ethanol and ethanol access frequency (b, d) by Fawn-Hooded (a, b) and iP (c, d) rats. Treatment with acamprosate (filled columns) caused a significant decrease in the responses for ethanol (a, c) and the number of times the ethanol receptacle was accessed (b, d) in the first week of treatment, but not the second week of treatment; \* $p < 0.05$  compared with saline-treated rats (open columns). Note that the data for 100 mg/kg versus 200 mg/kg acamprosate was combined for the figure as there was no significant dose effect.

the first day of treatment (factor treatment  $F_{1,14} = 5.019$ ,  $p = 0.042$ , factor day  $F_{1,14} = 48.194$ ,  $p < 0.001$ ; Fig. 2e), concurrent with a significant decrease in total fluid intake (treatment  $\times$  day interaction  $F_{1,14} = 17.040$ ,  $p = 0.001$ ; Fig. 2f).

#### Neurochemistry

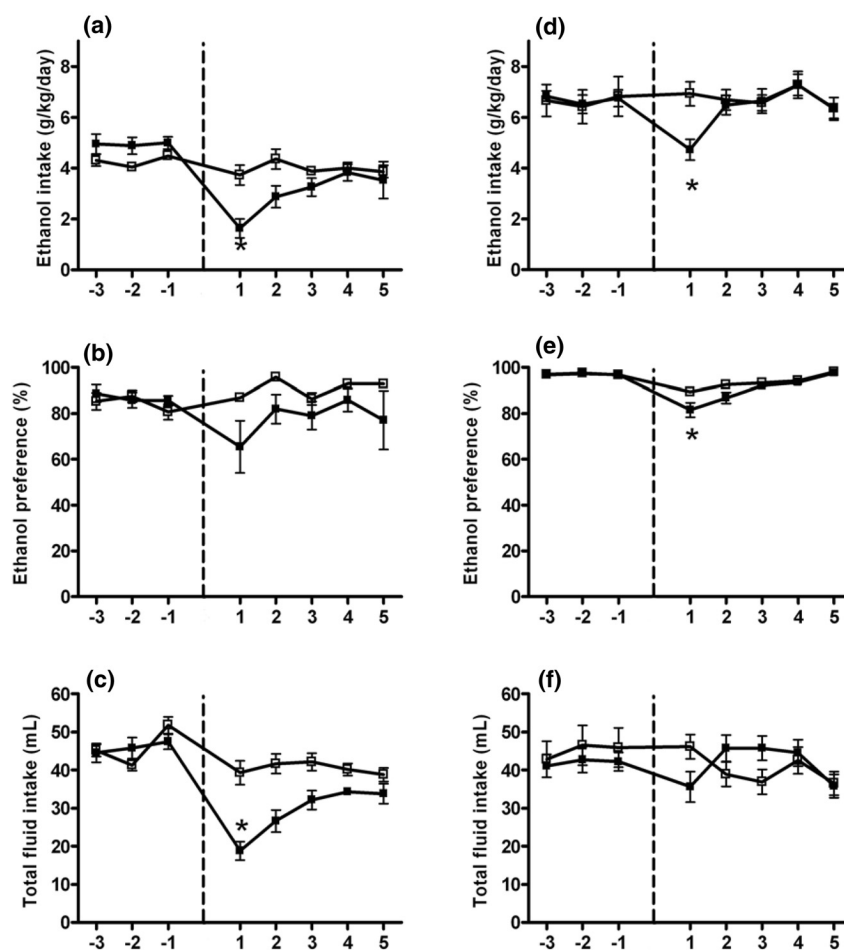
The effect of acute and chronic treatment with acamprosate on protein markers of dopamine neurotransmission is presented in Fig. 3. Under the conditions employed, [ $^{125}$ I]RTI-55 bound specifically to DAT and was sensitive to GBR12935 (Fig. 3a, d). Significant alterations in [ $^{125}$ I]RTI-55 binding were observed in response to acamprosate in the nucleus accumbens ( $F_{4,152} = 3.685$ ,  $p = 0.013$ ). *Post-hoc* analysis indicated small, but significant, increases in binding following acute (1 day) treatment with acamprosate in the nucleus accumbens (+8%; Fig. 3g) compared with vehicle-treated rats. No significant effect of acamprosate treatment was observed in the caudate-putamen ( $F_{4,152} = 1.361$ ,  $p = 0.257$ ; data not shown).

Neither acute nor repeated treatment with acamprosate led to significant alterations in [ $^{125}$ I]SCH23982 binding to D<sub>1</sub> receptors (Fig. 3b) in either the nucleus accumbens ( $F_{3,139} = 1.293$ ,  $p = 0.279$ ; Fig. 3e) or the caudate-putamen ( $F_{3,139} = 0.660$ ,  $p = 0.578$ ; data not shown). In contrast, treatment with acamprosate caused a significant alteration in [ $^{125}$ I]NCQ298 binding to D<sub>2</sub>-like receptors (Fig. 3c) in the nucleus accumbens ( $F_{4,148} = 7.455$ ,  $p < 0.001$ ; Fig. 3f). Acute acamprosate caused a significant decrease in [ $^{125}$ I]NCQ298 binding in the nucleus accumbens compared with all other days ( $p < 0.05$ ) and further, binding density was no different to control levels following 3 days of

acamprosate treatment. Although acamprosate appeared to cause a significant alteration in [ $^{125}$ I]NCQ298 binding in the caudate-putamen ( $F_{3,148} = 4.406$ ,  $p = 0.005$ ), *post-hoc* analysis indicated acute acamprosate caused a significant decrease in the caudate-putamen compared only with drug-naive rats but not vehicle-treated rats; further, drug-naive rats were significantly different to vehicle-treated rats (data not shown).

#### Discussion

In the present study, acamprosate was shown to cause a significant decrease in operant ethanol self-administration by FH and alcohol-preferring iP rats and an increased latency until the first ethanol-reinforced response. Interestingly, the effect of acamprosate was not dose-dependent in FH rats, suggesting that the effect of acamprosate was already maximal at 100 mg/kg. Measures of water responding were unaffected by acamprosate; however, the second dose of acamprosate that the rats (both iP and FH) received had no significant effect on ethanol self-administration, suggestive of the development of tolerance. That this profile was obtained both in FH rats and iP rats suggests that this effect was not specific to rat strain. As with operant ethanol self-administration, acamprosate caused a significant reduction in voluntary home cage ethanol consumption in a two-bottle choice test (ethanol versus water) by both FH and alcohol-preferring AA rats, again having no impact on water intake. However, the effect of acamprosate also rapidly diminished in this model, such that the effect was significant only on the first day of administration and ethanol consumption by acamprosate-treated rats matched that of saline-treated rats within a day or so (both strains of rat). Bi-daily treatment with acamprosate had no

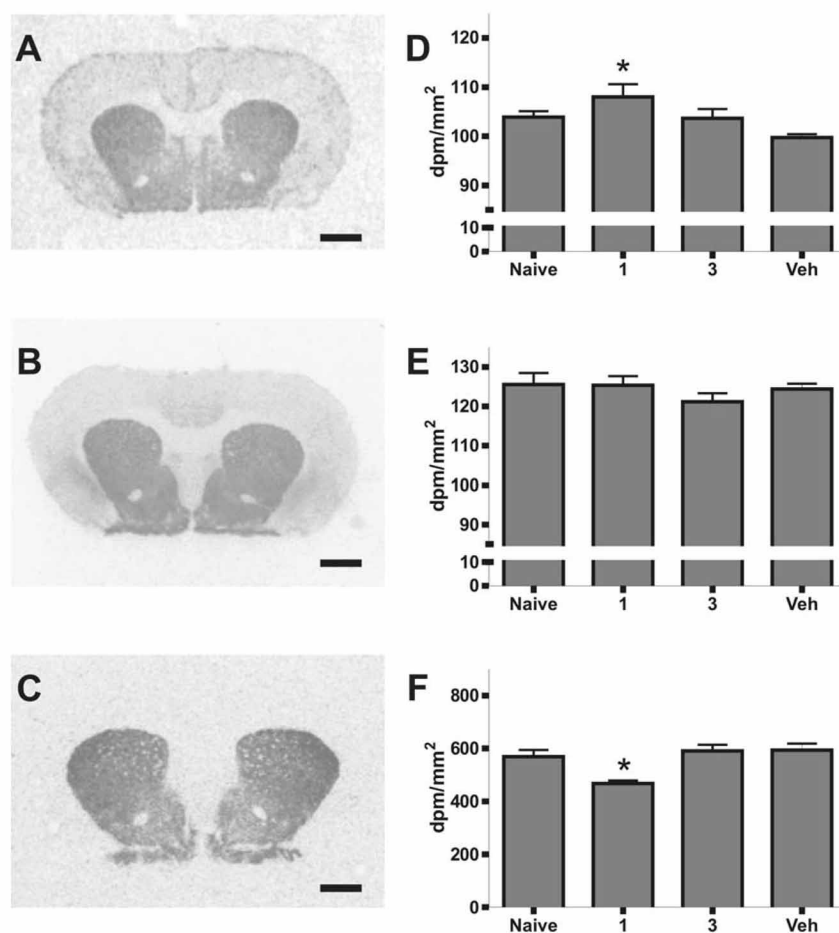


**Figure 2.** Ethanol consumption (g/kg/day; a, d), ethanol preference (b, e) and total fluid intake (c, f) by Fawn-Hooded rats ( $n = 6$  per group, a–c) and AA rats ( $n = 8$ ; d–f) prior to (days –3 until –1) and during (days 1–5) treatment with acamprosate (200 mg/kg/day i.p., closed symbols) or vehicle (open symbols). AA rats received twice-daily injections of the same dose (days 1–2). Acamprosate caused an overall significant decrease in ethanol intake, ethanol preference and total fluid intake by Fawn-Hooded and AA rats, relative to vehicle-treated controls; \* $p < 0.05$  compared with vehicle-treated rats.

impact on this time-course. Notably, acamprosate also caused significant alterations in FH rat brain on markers of the dopaminergic system, with protein changes exhibiting a temporal profile that essentially mirrored the behavioural data. Thus, an acute dose of acamprosate caused an increase in dopamine transporter (DAT) binding and a significant decrease in dopamine  $D_2$ -like binding in the nucleus accumbens. Interestingly, these effects occurred predominantly following an acute dose of acamprosate (1 hour post-injection) and re-normalized with repeated injections, reflecting the time-course of efficacy of acamprosate in the behavioural models used.

The effect of acamprosate on ethanol self-administration and consumption is in general agreement with previous pre-clinical (Boismare *et al.*, 1984; Le Magnen *et al.*, 1987b; Rassnick *et al.*, 1992; Spanagel *et al.*, 1996a; Hölter *et al.*, 1997; Heyser *et al.*, 1998) and clinical studies (Lhuintre *et al.*, 1990; Paille *et al.*, 1995; Sass *et al.*, 1996; Mann *et al.*, 2004). The effective dose in the present study (apparent maximal effect under operant conditions following a single dose, 100 mg/kg acamprosate) is slightly lower than in some

(Heyser *et al.*, 1998; Olive *et al.*, 2002) but not all (Rassnick *et al.*, 1992) studies of the acute effect of acamprosate; however, this may reflect the relatively long delay between injection of acamprosate and access to ethanol in the present study. In humans, acamprosate is much more slowly absorbed by the gastrointestinal tract than it is eliminated renally (Saivin *et al.*, 1998); thus the plasma  $t_{1/2}$  of acamprosate in healthy volunteer humans is 10 times longer when given as an oral solution than when injected intravenously (Saivin *et al.*, 1998). No absorption data are currently available for the rat; However, Spanagel *et al.* (2005) have demonstrated recently in mice that acamprosate caused a maximal reduction in extracellular glutamate overflow within the ventral striatum 80–120 minutes after an i.p. injection. Slow absorption of acamprosate subsequent to an intraperitoneal injection would certainly explain the ability of acamprosate to decrease ethanol consumption in the chronic (24-hour access) models used in the present study. Interestingly, combined treatment with naltrexone and acamprosate led to greater absorption of acamprosate in humans than when administered alone (Johnson *et al.*,



**Figure 3.** Effect of treatment with acamprosate (200 mg/kg; single daily injections for 1 and 3 days) on [<sup>125</sup>I]RTI-55 binding (a, d) to the dopamine transporter, [<sup>125</sup>I]SCH23982 binding (b, e) to the dopamine D<sub>1</sub> receptor and [<sup>125</sup>I]NCQ298 binding (c, f) to dopamine D<sub>2</sub>-like receptors within the rostral forebrain. (a–c) Representative autoradiograms; (d–f) binding data for the nucleus accumbens. Treatment with acamprosate caused a significant elevation in dopamine transporter binding within the nucleus accumbens on the first day of treatment, and a significant decrease in dopamine D<sub>2</sub>-like binding within the nucleus accumbens on the first day of treatment. These effects re-normalized to control levels within 3 days of repeated acamprosate treatment. \*Significantly different to vehicle-treated rats (Veh) ( $p < 0.05$ ). Scale bar = 150  $\mu$ m (a, b), 120  $\mu$ m (c).

2003) and a combination of naltrexone and acamprosate appeared to have additive effects clinically (Kiefer *et al.*, 2003).

Acamprosate has been hypothesized to be an anti-craving drug (Littleton, 1995; Spanagel and Zieglgansberger, 1997); specifically, one hypothesis is that the ability of acamprosate to overcome relapse may lie in its ability to prevent conditioned withdrawal, and thus craving, in response to environmental cues associated with alcohol consumption (Littleton, 1995). Acamprosate appears to have some effect on conditioned withdrawal (Cole *et al.*, 2000) and numerous studies have shown that acamprosate can diminish or prevent the behavioural and neurochemical consequences of ethanol withdrawal (Gewiss *et al.*, 1991; Putzke *et al.*, 1996; Spanagel *et al.*, 1996b; Dahchour *et al.*, 1998), the mechanisms of which are believed to be recruited in conditioned withdrawal (Littleton, 1995). Further, Bachteler *et al.* (2005) have demonstrated recently that acamprosate can prevent cue-induced reinstatement of alcohol-seeking behaviour. In clinical studies, continuous abstinence until first relapse was

greater in acamprosate-versus placebo-treated patients (Paille *et al.*, 1995; Sass *et al.*, 1996), indicating a significant pre-ingestive effect of acamprosate on ethanol consumption. However, although most pre-clinical studies have not specifically examined ethanol-seeking behaviour or appetitive function *per se*, in one study acamprosate was shown to have no effect on appetitive function for ethanol (Czachowski *et al.*, 2001) and only post-ingestive effects were observed. In the present study of operant ethanol self-administration, acamprosate caused a delay until the first ethanol-reinforced response; however, the rats had already accessed the ethanol receptacle (as indicated by the latency until the first beam break) without any significant delay. The effect of acamprosate on ethanol consumption does not appear to be due to taste aversion (Le Magnen *et al.*, 1987a). Therefore, in the present study, although the amount of ethanol available in the receptacle was too small, and the time-frame too limited, to have any pharmacological effect, the ability of the drop of ethanol in the receptacle to act as a cue or priming dose (Reid, 1996) appears to have been dampened. Interestingly, acam-

proprate was able to prevent the development of a conditioned place preference to ethanol (McGeehan and Olive, 2003) but not alter the discriminative stimulus properties of ethanol (Spanagel *et al.*, 1996c), suggesting that acamprosate does not alter the recognition of the cue but rather its relevance or salience. Therefore, it would appear that in the present study ethanol a specific cue for ethanol availability had diminished salience, yet the more general environmental cue of the operant chamber itself was unaltered. This would concur with a previous study demonstrating that following treatment with acamprosate, the ethanol-specific cue was unable to reinstate previously extinguished alcohol-seeking behaviour (Bachteler *et al.*, 2005).

Analysis of the effect of acamprosate on ethanol consumption and ethanol-seeking behaviour was hampered by the apparent development of tolerance to the effect of acamprosate. That this effect occurred in different behavioural paradigms, in different laboratories and in all three strains of rat indicates this effect was neither strain-specific nor paradigm-specific. Such an effect does not appear to have been directly reported previously, although there are some indications in the literature that such an effect could occur. For example, chronic treatment with acamprosate was shown to prevent the enhancement of binding of [<sup>3</sup>H]dizocilpine to NMDA receptors in rat cortical membranes by low doses of acamprosate (al Qatari *et al.*, 1998) and a single dose of oral acamprosate appeared to lead to higher plasma levels of acamprosate than chronic treatment with acamprosate in healthy human volunteers (Saivin *et al.*, 1998). Thus, tolerance to acamprosate could involve neural mechanisms and/or alterations in absorption or elimination. However, previous studies have indicated a significant or longer-lasting effect of acamprosate on ethanol consumption in rats made physically dependent on ethanol, in contrast with consumption by ethanol non-dependent rats (Boismare *et al.*, 1984; Le Magnen *et al.*, 1987b; Rimondini *et al.*, 2002). Therefore, the clinical implications of the present data are unclear, but the development of tolerance clinically may explain in part why the effect size of acamprosate treatment is relatively moderate (Mann, 2005).

The significant decrease in dopamine D<sub>2</sub>-like binding in the nucleus accumbens, concurrent with an increase in pre-synaptic dopamine transporter binding within the same nucleus, subsequent to acute acamprosate administration, are suggestive of altered dopaminergic tone within the ventral striatum during the time frame within which the FH and iP rats gained access to ethanol via operant self-administration. The most likely mechanism of these effects is an interaction between acamprosate and the mGlu5 receptor (Harris *et al.*, 2002). A functional antagonism between mGlu5- and dopamine D<sub>2</sub>-mediated effects within the striatum (or striatal neurones) has been observed previously (Diaz-Cabiale *et al.*, 2002; Parelkar and Wang, 2003). Further, whereas CHPG-mediated activation of the mGlu5 receptor caused a significant inhibition of striatal dopamine transporter capacity and efficiency (Page *et al.*, 2001), we have demonstrated recently that inhibition of the mGlu5 receptor by the selective antagonist MTEP increased levels of the dopamine transporter within the ventral striatum (unpublished observations). Interestingly, dopamine transporter (DAT) knockout mice demonstrated enhanced

ethanol consumption (although this was sex- and concentration-dependent; Hall *et al.* 2003); conversely, dopamine D<sub>2</sub> receptor antagonists, whether given systemically (Naassila *et al.*, 1998b) or directly into the nucleus accumbens (Samson and Hodge, 1993), can decrease ethanol consumption by rats and mice.

Of themselves, the changes observed in the present study in markers of dopaminergic neurotransmission in response to treatment with acamprosate would be consistent with diminished dopamine-mediated responses in the nucleus accumbens (increased uptake, decreased dopamine D<sub>2</sub>-like receptor activation), although the level of dopamine in the nucleus accumbens at this time-point (1 hour post-injection) can only be surmised. Although high concentrations of acamprosate applied focally to the nucleus accumbens have been shown to cause a dose-dependent overflow of dopamine (as measured by microdialysis) within the nucleus accumbens (Cano-Cebrian *et al.*, 2003b), a low concentration was without effect on dopamine release (Cano-Cebrian *et al.*, 2003b) while preventing the NMDA-mediated release of dopamine within the nucleus accumbens (Cano-Cebrian *et al.*, 2003a, b). Further, at doses germane to the present study, acamprosate prevented the ethanol-induced dopamine release within the nucleus accumbens (Olive *et al.*, 2002). The exact involvement of dopamine in the reinforcing properties of ethanol (among other drugs of abuse) is unclear (Spanagel and Weiss, 1999; Joseph *et al.*, 2003); however, disruption of dopaminergic signalling has been shown to disrupt ethanol consumption and self-administration previously (e.g. Hodge *et al.*, 1997; Slawecki *et al.*, 1997). If acamprosate diminishes the relevance of the ethanol cue (or other, ethanol-associated cues) to produce a decrease in ethanol consumption, it is interesting that dopamine has been hypothesized to play a role in signalling the presence of, or controlling the attention paid to, drug-associated stimuli (Spanagel and Weiss, 1999; Joseph *et al.*, 2003). Further studies, for example using *in vivo* voltammetry or possibly microdialysis, are needed to examine whether acamprosate does prevent the release of dopamine in response to secondarily conditioned cues. The return of markers of the dopaminergic system to steady-state levels with repeated dosing with acamprosate is again indicative for the development of tolerance.

In summary, acamprosate was demonstrated to cause a significant and profound decrease in ethanol consumption and ethanol-seeking behaviour in a range of experimental paradigms and rat strains. Under operant conditions of responding for ethanol and water, acamprosate had no effect on water responding but appeared to decrease the motivational relevance of a specific ethanol cue. During the same period of time within which acamprosate was effective in decreasing ethanol self-administration, acamprosate was shown to cause alterations in markers of dopaminergic neurotransmission in the nucleus accumbens, suggesting a link between the decreased motivational salience of the ethanol cue, on one hand, and disruption of dopaminergic signalling within the nucleus accumbens, on the other hand. Tolerance developed to the effect of acamprosate on ethanol consumption and ethanol-seeking behaviour; the time-course of tolerance in the chronic ethanol consumption model paralleled the diminishing effect of acamprosate on markers

of the dopaminergic system. However, this apparent tolerance may be due to altered central nervous system responsiveness to acamprosate, or altered absorption or elimination of acamprosate. The development of tolerance to acamprosate in these pre-clinical models suggests the possibility that tolerance could develop in the clinical situation. Therefore, future studies should examine the effect of acamprosate on these various neurochemical markers, both in ethanol-non-dependent rats and in rats previously made dependent on ethanol, as well as to explore further the pharmacokinetics of acamprosate.

### Acknowledgements

This study was supported by a programme grant from the NHMRC, Australia (236805), of which AJL is a senior research fellow, and MSC a CJ Martin Fellow.

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