Long-term inhibition of ethanol intake by the administration of an aldehyde dehydrogenase-2 (ALDH2)-coding lentiviral vector into the ventral tegmental area of rats

Eduardo Karahanian[1]*, Mario Rivera-Meza[2]*, Lutske Tampier[2]*, María Elena Quintanilla[2], Mario Herrera-Marschitz[2] & Yedy Israel[2]

Center for Biomedical Research, Universidad Autónoma de Chile, Chile[1] and Molecular and Clinical Pharmacology Program, ICBM, Faculty of Medicine, University of Chile, Chile[2]

ABSTRACT

Previous studies suggest that acetaldehyde generated from ethanol in the brain is reinforcing. The present studies tested the feasibility of achieving a long-term reduction of chronic and post-deprivation binge ethanol drinking by a single administration into the brain ventral tegmental area (VTA) of a lentiviral vector that codes for aldehyde dehydrogenase-2 (ALDH2), which degrades acetaldehyde. The ALDH2 gene coding vector or a control lentiviral vector were microinjected into the VTA of rats bred for their alcohol preference. In the chronic alcohol administration model, naïve animals administered the control vector and subsequently offered 10% ethanol and water ingested 8–9 g ethanol/kg body weight/day. The single administration of the ALDH2-coding vector prior to allowing ethanol availability reduced ethanol drinking by 85–90% ($P < 0.001$) for the 45 days tested. In the post-deprivation binge-drinking model, animals that had previously consumed ethanol chronically for 81 days were administered the lentiviral vector and were thereafter deprived of ethanol for three 7-day periods, each interrupted by a single 60-minute ethanol re-access after the last day of each deprivation period. Upon ethanol re-access, control vector-treated animals consumed intoxicating 'binge' amounts of ethanol, reaching intakes of 2.7 g ethanol/kg body weight in 60 minutes. The administration of the ALDH2-coding vector reduced re-access binge drinking by 75–80% ($P < 0.001$). This study shows that endowing the ventral tegmental with an increased ability to degrade acetaldehyde greatly reduces chronic alcohol consumption and post-deprivation binge drinking for prolonged periods and supports the hypothesis that brain-generated acetaldehyde promotes alcohol drinking.

Keywords   Acetaldehyde, ADE, chronic ethanol intake, lentiviral vector, VTA.

Correspondence to: Yedy Israel, Molecular and Clinical Pharmacology Program, Laboratory of Genetics of Alcoholism, Institute of Biomedical Sciences, University of Chile, Independencia 1027, Independencia, Santiago RM 8380453, Chile. E-mail: yisrael@uchile.cl

INTRODUCTION

Conditions present at different stages in the path to human alcoholism include chronic drinking and, after detoxification, the inability to abstain once access to alcohol is re-initiated, leading to binge drinking. The present study aims at reproducing and preventing these conditions in Wistar-derived rats selectively bred to prefer drinking ethanol-containing solutions over water.

The possible involvement of the ethanol metabolite acetaldehyde as a mediator of the reinforcing effects of ethanol was proposed over 40 years ago, and the 'acetaldehyde hypothesis' has been strengthened since then (reviewed by Correa *et al.* 2012; Deehan, Brodie & Rodd 2013). Studies in the late 1970s (Brown, Amit & Rockman 1979) showed that naïve rats maintained in operant chambers self-administer acetaldehyde solutions into the cerebral ventricles. However, the concentrations

*These authors contributed equally to this work.

attained in the cerebrospinal fluid were on the order of 5×10^{-3} M, which are two to three orders of magnitude higher than those present in the arterial blood of animals. Blood levels of acetaldehyde attained after the administration of low doses of ethanol (1 g ethanol/kg) do not normally exceed 20×10^{-6} M (Quintanilla *et al.* 2007; Rivera-Meza *et al.* 2010). At the latter concentrations, acetaldehyde does not cross the blood–brain barrier (Peterson & Tabakoff 1979; Stowell *et al.* 1980) because acetaldehyde is cleared by the high-affinity aldehyde dehydrogenase-2 (ALDH2) present in the endothelial (tight-junction) cells that constitute the blood–brain barrier; thus, it is highly improbable that acetaldehyde generated from ethanol metabolism in the liver could reach the brain. Only after the exogenous administration of systemic acetaldehyde, when acetaldehyde concentrations exceed 100×10^{-6} M, is this metabolite found in the central nervous system (Tabakoff, Anderson & Ritzmann 1976).

Cohen, Sinet & Heikkila (1980) asked if ethanol could be oxidized by catalase in the brain. Their *in vivo* studies showed that the administration of ethanol prevented the inhibition of brain catalase by the drug 3-aminotriazole, which indicated that ethanol binds to the same oxidized intermediate of catalase ('compound I') that binds 3-aminotriazole. These studies suggested that the brain could also oxidize ethanol to acetaldehyde. It is noted that while alcohol dehydrogenase is not expressed in the brain, several *in vitro* studies have indicated that acetaldehyde is generated from ethanol by the action of brain catalase (Tampier & Mardones 1979; Aragon, Rogan & Amit 1992; Zimatkin *et al.* 2006) and, to a minor extent, by CYP2E1 (Zimatkin *et al.* 2006). Catalase generates some 70% of brain acetaldehyde, whereas CYP2E1 generates only 15–20% (Zimatkin *et al.* 2006).

It has been shown that the inhibition of catalase by the acute administration of 3-aminotriazole (Aragon & Amit 1992; Tampier, Quintanilla & Mardones 1995) or the administration of D-penicillamine, which condenses to acetaldehyde, reduces voluntary consumption of ethanol and binge drinking in animals (Font, Aragon & Miquel 2006; Orrico *et al.* 2013). The effect of these drugs is short-lived, thus requiring the current or daily administration of these agents to sustain the effects on chronic alcohol-related behaviors.

A different experimental approach has greatly contributed to strengthening the acetaldehyde hypothesis. Rodd and co-workers (see Rodd *et al.* 2005; Deehan *et al.* 2013) demonstrated that rats selectively bred as alcohol drinkers (strain P of Indianapolis) placed in operant chambers self-administer both ethanol and acetaldehyde into the brain ventral tegmental area (VTA). Most importantly, the reinforcing concentrations of acetaldehyde $[(6–12) \times 10^{-6}$ M] are three orders of magnitude lower than those required for ethanol (17×10^{-3} M) (Oster *et al.* 2006).

Karahanian *et al.* (2011) and Quintanilla *et al.* (2012) aimed at inhibiting ethanol intake by permanently reducing VTA catalase *synthesis* by a genetic approach, thus adding to the view that endogenous acetaldehyde generation is required to exert the reinforcing effects of orally consumed ethanol. This was accomplished in UChB alcohol-drinker rats by single administration into the VTA of a lentiviral vector that coded for a short hairpin RNA (shRNA), which inhibited the synthesis of VTA catalase by 70%. Genes carried by lentiviral vectors permanently integrate into the cell genome. The single stereotaxic administration of the anti-catalase shRNA-coding lentiviral vector into the VTA greatly reduced (70–95%) the voluntary ethanol consumption for the 40–60 days studied (Karahanian *et al.* 2011; Quintanilla *et al.* 2012). There was also a full inhibition of the alcohol-dependent release of dopamine in the nucleus accumbens (Karahanian *et al.* 2011). While in the brain, enzymes other than catalase are mainly involved in the degradation of hydrogen peroxide (Halliwell 2006), a reduction of catalase activity might affect the levels of this molecule, considered an intracellular signal transduction molecule (Gill & Lavine 2013).

In the present study, we used an alternative approach aimed at increasing the VTA ability to *degrade* acetaldehyde, leading to a long-term reduction of ethanol voluntary intake and binge drinking; namely by transducing into the VTA a single dose of a lentiviral vector that codes for ALDH2. The affinity of ALDH2 is high ($K_m < 0.2 \times 10^{-6}$ M; Klyosov 1996), thus allowing maximal activity at the acetaldehyde concentrations $[(6–12) \times 10^{-6}$ M] that animals self-administer into the VTA (Oster *et al.* 2006).

In their studies using rats, Sinclair and co-workers showed that chronic ethanol intake followed by a period of alcohol deprivation and a subsequent ethanol re-access period led animals to markedly increase their ethanol intake above their pre-deprivation levels (Sinclair & Senter 1968). This increase in ethanol intake above basal levels has been termed the alcohol deprivation effect (ADE) (Spanagel & Hölter 1999; Rodd-Henricks *et al.* 2001). ADE is seen either after a short (1–3 days) (Sinclair & Li 1989; Agabio *et al.* 2000) or a long (up to 60–75 days) deprivation period (Sinclair, Walker & Jordan 1973; Spanagel & Hölter 1999), but prolonged exposure to ethanol alone without a period of deprivation did not produce such a marked increase in ethanol intake (Spanagel & Hölter 1999). Some 3–4 weeks of continuous alcohol drinking are required before deprivation to elicit an ADE (Spanagel & Hölter 1999). It is noteworthy that a number of studies have shown that repeated cycles of alcohol intake-deprivation re-access lead to additional

increases in ethanol intake (Oster *et al.* 2006; Rodd *et al.* 2009). Another characteristic of ADE is that animals allowed re-access to two or three concentrations of ethanol choose to consume most of the ethanol from solutions that are more concentrated than those consumed during their basal chronic intake (see Rodd *et al.* 2009). Such a behavior, referred to as 'relapse-like drinking' by some authors (Vengeliene, Noori & Spanagel 2013), allows the animals to attain a marked intoxication in a short re-access time.

An ADE has been reproduced by several groups (Sinclair & Li 1989; Heyser, Schulteis & Koob 1997; Spanagel & Hölter 1999; Rodd-Henricks *et al.* 2001; Serra *et al.* 2003), including experiments with the Indianapolis P; HAD-1 and HAD-2 rat strains (Sinclair & Li 1989; Bell *et al.* 2008; Rodd *et al.* 2009); the Finnish AA rat line (Sinclair & Li 1989); the Sardinian alcohol-preferring sP rats (Serra *et al.* 2003); and the Chilean UChB line (Tampier *et al.* 2013).

The biological bases underlying ADE have not been established, although it is conceivable that after chronic free access to ethanol, the deprivation period may lead to sensitization, as reported for other drugs (Morgan, Smith & Roberts 2005). In line with this view, in the ADE condition, animals work to a greater extent (to a higher break-point in progressive ratio schedules) to obtain ethanol (Rodd *et al.* 2003; Oster *et al.* 2006; Vengeliene *et al.* 2009). Vengeliene *et al.* (2005) showed that binge drinking is blunted after the intraperitoneal administration of ethanol. What is not clear is whether the same molecular agent that promoted chronic drinking is also involved in ADE intake. Two recent short-term studies suggest that acetaldehyde may be required as the infusion of D-penicillamine intra VTA administration or of an anti-catalase gene vector inhibited ADE (Orrico *et al.* 2013; Tampier *et al.* 2013). The present study aimed at inducing a long-term increase in the ability of the VTA to *degrade* acetaldehyde at the time of ADE expression. The effect of the ALDH2-coding vector on post-alcohol deprivation (ADE) binge drinking was determined during a 60-minute re-access to ethanol on each of three 7-day ethanol deprivation and re-access cycles.

Overall, the study shows a marked long-term inhibitory effect on chronic alcohol intake of a single dose of an ALDH2-coding lentiviral vector when administered to naïve rats that are subsequently exposed to ethanol and on binge drinking in rats deprived of ethanol after chronic consumption and subsequently allowed re-access to ethanol.

MATERIALS AND METHODS

Animal experimental procedures were approved by the Ethics Committee for Experiments with Laboratory Animals, Faculty of Medicine, University of Chile (Protocol CBA#0507, FMUCH) and by the Chilean Council for Science and Technology Research, endorsing the principles of laboratory animal care (NIH; No. 86-23). Every effort was made to minimize the numbers and any suffering of animals.

Generation of lentiviral vectors

The lentiviral vector coding for rat ALDH2 was constructed cloning the rat ALDH2 cDNA sequence (Jeng & Weiner 1991) in pCDH1 (System Biosciences, Mountain View, CA, USA) under the control of the cytomegalovirus promoter. The control virus was generated from the same vector but it did code for ALDH2. The viruses were packed, purified and titrated as previously described (Karahanian *et al.* 2011).

Generation of ALDH2 in vitro by ALDH2-coding lentiviral vector

HEK-293 (ATCC #CRL-1573) cells (that do not express ALDH2) in 6-well plates were infected at 50% confluency with Lenti-ALDH2 or Lenti-Control (at a multiplicity of infection of 1 virus/cell) ($n = 3$). Three days after infection, ALDH2 enzymatic activity in cell lysates was determined spectrophotometrically by NADH generation in the presence of 0.8×10^{-3} M NAD$^+$ and 14×10^{-6} M propionaldehyde, according to Karahanian, Ocaranza & Israel (2005).

Intracerebral administration of lentiviral vectors

The studies were performed in Wistar-derived UChB rats bred for over 85 generations to ingest ethanol solutions in preference to water (Quintanilla *et al.* 2006). In these studies, 3- to 4-month-old female rats (having nearly stabilized their body weight) were used. Sixteen naïve female rats and 10 female rats that had prior access to 10% ethanol and water for 81 days were anesthetized with a mixture of air and isoflurane and placed in a stereotaxic frame for intracerebral administration of the viral vector into the left VTA (B-5.6, L-0.5, V-7.4; from Paxinos & Watson 1986). Further, 1 μl of a solution containing the control lentiviral vector (8×10^4 viral particles was microinjected into eight naïve and five ethanol-drinking rats) and 1 μl of ALDH2-coding viral vector (8×10^4 viral particles was also microinjected into eight naïve and five ethanol-drinking rats). The lentiviral vectors were injected in approximately 5 minutes, which should allow distribution into the whole VTA. It should be noted that, in line with the work of Rodd *et al.* (2005), in the present study, the VTA infusion was more posterior than in

previous studies (B-5.2) by Karahanian *et al.* (2011), but still in a total volume of 1 µl, likely spreading to most of the VTA-injected side.

Experiment 1

Effect of an ALDH2-coding vector injected into the VTA of alcohol-naïve rats on voluntary ethanol intake

After the intracerebral lentiviral (ALDH2, $n = 8$) or control (empty virus, $n = 8$) vector injection, the animals were returned to their individual home cage, and 4 days later, they were offered 24-hour continuous access to an ethanol solution (10% v/v) and water for 45 days. Both 10% ethanol and water consumption were recorded daily. Ethanol consumption was expressed as g ethanol/kg body weight/day.

Experiment 2

Effect on voluntary ethanol intake of an ALDH2-coding vector injected into the VTA of chronically alcohol-consuming rats

Ten rats that had prior 24-hour access to 10% ethanol and water for 81 days were divided into two groups with closely similar alcohol consumption. Five rats with final intakes of 8.8 ± 1.0 g ethanol/kg/day were injected with the ALDH2-coding viral vector into the VTA and five rats with intakes of 9.1 ± 0.8 g ethanol/kg/day were injected with the control viral vector. Animals were returned to individual home cage and continued under the 24-hour continuous access to ethanol solution (10% v/v) and water for 11 days. Ethanol consumption continued to be determined on a daily basis and expressed as gram ethanol/kilogram body weight/day.

Experiment 3

Effect on alcohol intake of an ALDH2-coding vector injected into the VTA rats that had chronically consumed ethanol and were subsequently exposed to ethanol deprivation and thereafter allowed ethanol re-access

Rats that had consumed 10% ethanol chronically for 81 days were intracerebrally injected with ALDH2- or control-lentiviral vectors ($n = 5$ rats/group) and intake was further determined for 11 days. The animals were then deprived of ethanol for 7 days and were subsequently allowed re-access to 10 and 20% ethanol solutions and water for only 60 minutes. The ethanol deprivation and subsequent re-access cycles were repeated three times. On each of the cycles, the 1-hour ethanol intake was determined as gram ethanol of each of the 10 and 20% solutions consumed/kilogram body weight. The experimental design follows studies that have shown that binge drinking occurs mainly in the first hour

of ethanol re-exposure (Rodd-Henricks *et al.* 2001; Bell *et al.* 2006; Tampier *et al.* 2013) and have demonstrated that repeated ethanol deprivation and re-access cycles result in significant increases in ethanol binge drinking (Oster *et al.* 2006; Rodd *et al.* 2009). On each cycle, after the 7-day ethanol deprivation period, ethanol re-access started at 1:00 PM on a regular 7:00 AM lights on to 7:00 PM lights off circadian cycle (thus not 'drinking-in-the-dark').

Statistical analyses

Data are expressed as means ± SEM. Statistical differences were analyzed by Student's *t*-test, or by one-way or two-way ANOVA using Bonferroni's as *post hoc test*. A level of $P < 0.05$ was considered statistically significant.

RESULTS

Cells (HEK-293) transduced with the ALDH2-coding vector showed an ALDH2 activity (10.3 ± 0.76 nmol NADH/min/mg protein; $n = 3$) two orders of magnitude greater than that of cells transfected with the control vector (0.12 ± 0.09 nmol NADH/min/mg protein $n = 3$) ($P < 0.02$; Student's *t*-test).

Figure 1 shows that when administered to naïve animals, the single injection of an ALDH2-coding lentiviral vector into the VTA greatly reduced (85–90%) ethanol intake for a 45-day period [$F_{(1,43)} = 2730$, $P < 0.001$; two-way ANOVA]. Animal weights were not significantly different between the two groups [data not shown; $F_{(1,43)} = 3.59$, N.S.]. Similarly, total fluid intake was not different in the two groups (control vector: 108 ± 1.2 ml fluid/kg/day; 104 ± 1.4 ml fluid/kg/day for ALDH2-coding vector group). Water intake (from the water tubes) was markedly lower in animals that received the control lentiviral vector (15 ± 1.5 ml/kg/day) than in animals treated with the ALDH2-coding lentiviral vector (92 ± 1.5 ml/kg/day) ($P < 0.001$; Student's *t*-test).

Figure 2 shows that the ALDH2-coding vector that had greatly inhibited ethanol intake in naïve animals was inactive in reducing ethanol intake in rats that had consumed ethanol on a 24-hour basis for 81 days. In these animals, ethanol intake was monitored for 11 days following the ALDH2-coding (or control) viral vector injection (N.S.; two-way ANOVA). Possible reasons for this difference with the findings observed in naïve animals are discussed (*vide infra*).

Figure 3a shows that a 7-day ethanol deprivation of animals that had consumed ethanol chronically (92 days) results in binge-like drinking when ethanol re-access was allowed for only 1 hour. Data show that repeated deprivation and re-access cycles led to further increases in 60-minute ethanol intake [one-way ANOVA,
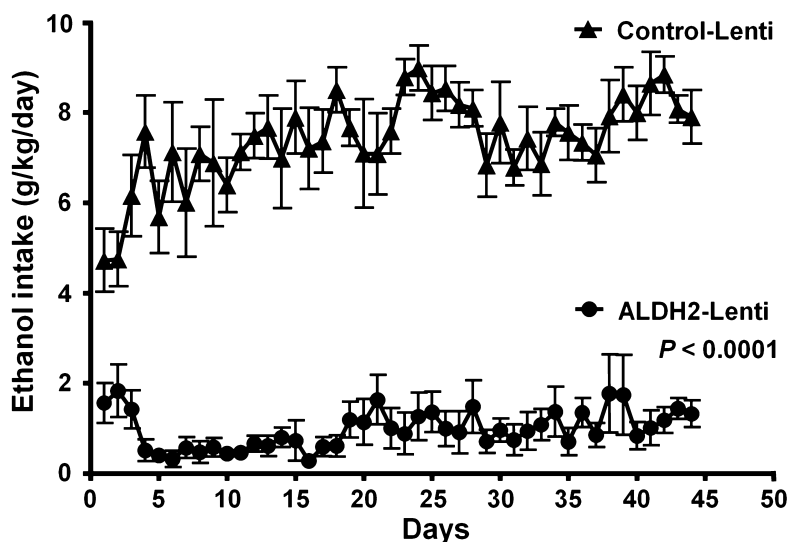
Figure 1 A single aldehyde dehydrogenase-2 (ALDH2)-lentiviral vector injection into the ventral tegmental area (VTA) reduces long-term alcohol intake in rats. Rats ($n = 8$ rats/group) markedly reduced their alcohol intake when an ALDH2 lentiviral vector was injected into the VTA prior to ethanol exposure. A two-way ANOVA analysis revealed a significant inhibition on ethanol intake (85–90%) by the administration of the ALDH2-lentiviral vector versus control vector ($P < 0.001$)
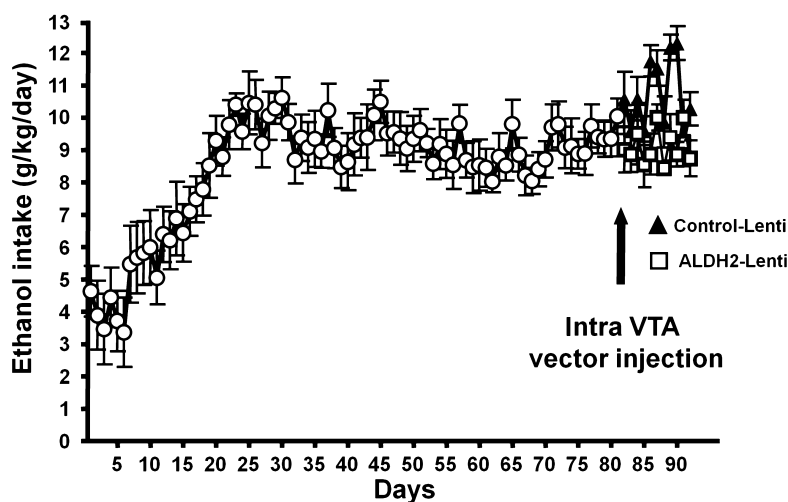

Figure 2 Aldehyde dehydrogenase-2 (ALDH2)-lentiviral vector injection did not reduce alcohol intake in rats that had chronically ingested ethanol. Arrow indicates the time of administration of either control-lentiviral vector (-▲-) or ALDH2-lentiviral vector (-□-). The number of animals was 8 rats/group. Neither the ALDH2-coding vector nor the control vector modified the voluntary ethanol intake of the animals for the 11 days studied (N.S.)

$F_{(2,14)} = 14.88$, $P < 0.001$]. In control vector-treated animals, intake was 2.0 g ethanol/60 minutes/kg after the first deprivation re-access cycle, increasing to 2.7 g ethanol/60 minutes/kg on the third deprivation and re-access cycle (Bonferroni's test, $P < 0.001$). The ALDH2-coding lentiviral viral vector administration significantly inhibited ethanol intake during the three re-access cycles compared to the Lenti-Control vector group. A two-way ANOVA (Treatment × Deprivation cycles) of total ethanol intake data (Fig. 3a) showed a significant overall inhibitory effect of ALDH2 lentiviral vector treatment [$F_{(1,29)} = 65.46$, $P < 0.001$]. A *post hoc* Bonferroni's test showed that rats treated with Lenti-ALDH2 vector displayed a statistically significant greater inhibitory effect on total ethanol intake compared to the Lenti-Control group as the number of deprivation and re-access cycles increased (first re-access: $P < 0.05$; second re-access: $P < 0.01$; third re-access: $P < 0.001$). On the third deprivation and re-access cycle, the ALDH2-

coding vector reduced alcohol intake of animals by 75–80%. Figure 3b shows the intake (means ± SEM) of 20% ethanol solution during the three post-deprivation re-access periods. ALDH2 treatment also resulted in a marked inhibition of ethanol intake [two-way ANOVA, $F_{(1,29)} = 76.86$, $P < 0.001$]. A *post hoc* Bonferroni's test showed that rats treated with Lenti-ALDH2 vector displayed a statistically significant greater effect on 20% ethanol intake compared to the Lenti-Control group on each of the three deprivation re-access cycles (first re-access: $P < 0.05$; second re-access: $P < 0.001$; third re-access: $P < 0.001$). Figure 3c shows the intake (means ± SEM) of the 10% ethanol solution during the three post-deprivation and re-access cycles. ALDH2 treatment resulted in a significant inhibition of ethanol intake [two way-ANOVA, $F_{(1,29)} = 8.18$, $P < 0.001$]. Additionally, Fig. 3b and c shows that ethanol deprivation and re-access cycles led animals to prefer the more concentrated ethanol solution available [two-way ANOVA for the
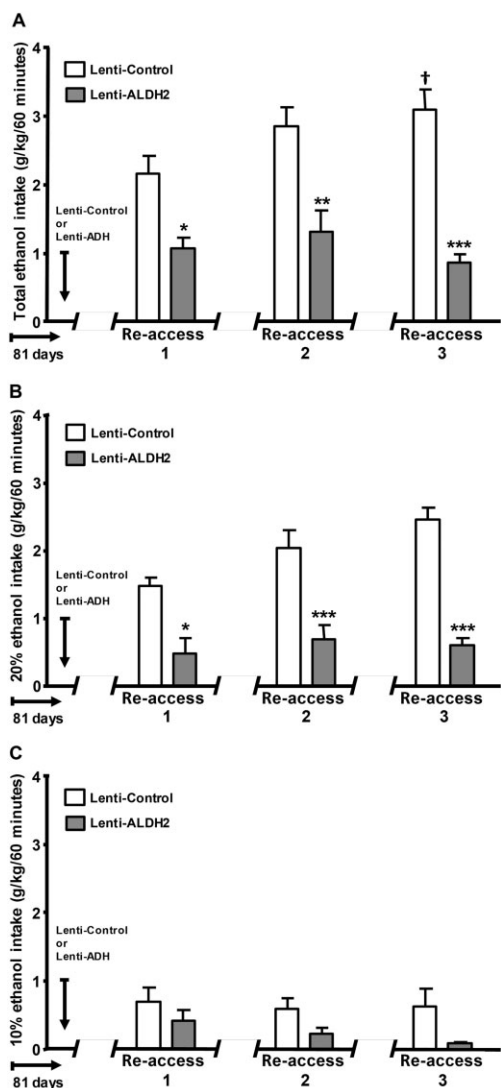
Figure 3 The injection of an aldehyde dehydrogenase-2 (ALDH2)-lentiviral vector inhibits ethanol intake on re-access after repeated access-deprivation cycles. Animals that had voluntarily consumed 10% ethanol for 81 days were administered an ALDH2-coding lentiviral vector or a control-lentiviral vector into the ventral tegmental area (VTA) (*n* = 5/group). On day 91, animals were deprived for 7 days followed by 1 hour of access to 10 and 20% ethanol and water. These deprivation and ethanol re-access cycles were repeated three times. Ethanol consumption on re-access was expressed as g ethanol/kg/60 minutes. (a) Values represent the mean ± SEM of total ethanol consumption (from both the 10 and 20% ethanol solutions) on the three deprivation (-//-) and re-access cycles. Effect of ALDH2 treatment: *P* < 0.001. (b) Values represent the mean ± SEM of 20% ethanol solution consumption during the three deprivation (-//-) and re-access cycles. Effect of ALDH2 treatment: *P* < 0.001. (c) Values represent the mean ± SEM of 10% ethanol solution consumption during the three re-access cycles. Effect of ALDH2 treatment: *P* < 0.001. Overall (a–c), the effect of ALDH2 treatment was increased following each deprivation cycle: *****P* < 0.05, ******P* < 0.01, *******P* < 0.001 versus Lenti-Control group. The symbol † indicates a greater total ethanol intake (*P* < 0.001, by Bonferroni's test) versus the first Lenti-Control re-access

Table 1 Water intake of control-lentiviral vector and ALDH2-lentiviral vector treated rats subjected to three deprivation and ethanol re-access cycles.

	Water intake (ml/kg/day)		
	First re-access	*Second re-access*	*Third re-access*
Control-lentiviral vector (*n* = 5)	104 ± 3	95 ± 7	97 ± 4
ALDH2-coding lentiviral vector (*n* = 5)	108 ± 7	91 ± 6	96 ± 9

Data (means ± SEM) correspond to the 24-hour intake of water (from the water tube) on the day of ethanol re-access. N.S. = ANOVA did not show significant differences.

Lenti-Control group, effect of alcohol concentration: $F_{(1,29)} = 63.97$, $P < 0.001$]; ethanol intake from the 20% ethanol solution was two to three times larger than that from 10% ethanol (Bonferroni's test: first re-access: $P < 0.05$; second re-access: $P < 0.001$; third re-access: $P < 0.001$).

Table 1 shows that water intake was not affected by the ethanol deprivation and re-access cycles or by the viral vectors [two-way ANOVA (Treatment × Deprivation cycle); treatment effect: $F_{(1,29)} = 0.007$, N.S.; deprivation effect: $F_{(2,29)} = 2.319$, N.S.].

DISCUSSION

The data presented earlier show that in naïve animals the administration of an ALDH2-coding lentiviral vector into the VTA markedly reduced (80–95%) ethanol intake. The study supports the hypothesis that ethanol reinforcement is mediated by acetaldehyde generated endogenously in the VTA. Previous studies (Karahanian *et al.* 2011; Quintanilla *et al.* 2012) showed that inhibition of catalase synthesis, which reduced the generation of acetaldehyde, is also effective in markedly reducing chronic ethanol intake in alcohol-preferring rats. Thus, either increasing the metabolism of acetaldehyde or reducing its generation in the VTA leads to a marked reduction of ethanol intake. In both cases, total fluid intake was unchanged, but water was replaced by ethanol solution.

Genetic manipulation of ALDH2 activity is likely more specific than approaches reported previously. An additional advantage of lentiviral delivery is the fact that since the genes are incorporated into the genome, the duration of their effect is prolonged, thus not requiring multiple intracranial administrations. The present study shows that ALDH2 gene transduction did not alter body weight or total fluid intake. In lentiviral-ALDH2-treated animals, water intake (from the water tube) was higher than in the

lentiviral-control animals, as the ALDH2-treated animals greatly reduced their water intake from the 10% ethanol solution. Similar effects on ethanol and water intake were observed in animals treated with a shRNA anti-catalase lentiviral-coding vector (Karahanian *et al.* 2011).

An effect that requires further studies is the observation that unlike the strong inhibitory effect (80–95%) on ethanol intake generated by the administration of the ALDH2-coding vector to naïve animals, animals that had consumed ethanol chronically for 2–3 months did not reduce their ethanol intake upon the administration of the ALDH2-coding vector. Cues such as odor are associated with increases in alcohol self-administration in habitual drinkers (Perkins *et al.* 2003). In hazardous drinkers, ethanol odor *per se* increases the activity in the nucleus accumbens, potentially enhancing the desire to drink (Bragulat *et al.* 2008). In mice exposed to a reinstatement paradigm similar to ADE, alcohol cues in an alcohol context increased alcohol self-administration (Tsiang & Janak 2006). Thus, odor may constitute a cue that perpetuates ethanol drinking when the primary molecular mechanisms that lead to chronic drinking are blunted by the administration of the ALDH2-coding vector. Alternatively, after chronic ethanol intake, perpetuation of alcohol self-administration may not be dependent on VTA acetaldehyde or may reside in brain areas other than the VTA (see Chaudhri *et al.* 2013). Studies by Engleman *et al.* (2009) suggest that at intoxicating levels of ethanol, the nucleus accumbens may also be involved in ethanol reinforcement. In the opiate field, it has been described that after chronic opiate treatment of animals, reward no longer occurs via the VTA dopaminergic control (Laviolette & Van der-Kooy 2004; Vargas-Perez, Ting-A-Kee & van der Kooy 2009). Additionally, the possibility that in rats that have consumed ethanol chronically a system that degrades VTA acetaldehyde may be activated cannot be discarded.

A marked inhibitory effect (75–80%; $P < 0.001$) on ethanol intake of the ALDH2-coding vector was however observed in animals exposed to chronic ethanol intake, followed by ethanol deprivation and subsequent alcohol re-access. Further, binge drinking was increased on each ethanol deprivation cycle, whereas the inhibitory effect on ethanol intake generated by ALDH2 overexpression was enhanced after each deprivation period, being significantly greater after the third deprivation cycle. Data in this study suggest that acetaldehyde is required for the binge drinking induced by the ADE.

The binge-like alcohol intake resulting from the ADE reached 2.7 g ethanol/kg in 60 minutes. The higher ethanol intake following repeated ethanol deprivation cycles might be due to a repeated reversal of tolerance to the putatively hedonic effect of ethanol, thus leading to sensitization to the rewarding effects of ethanol. In line with the development of sensitization in the ADE condition, Rodd *et al.* (2003), Oster *et al.* (2006) and Vengeliene *et al.* (2009) showed that animals performed more work (bar-pressed to a higher break-point) to obtain ethanol than they did before the chronic intake and deprivation cycles, thus suggesting that deprivation increases the hedonistic effect of ethanol. Previous studies suggest that physical dependence does not occur in this animal model (Quintanilla *et al.* 2012); thus, it is unlikely that negative reinforcement would play a major role in the increases in ethanol intake.

Overall, the present study shows that a single injection into the VTA of a lentiviral vector that codes for the high-affinity alcohol dehydrogenase-2 markedly reduces chronic ethanol drinking in naïve rats and also greatly reduces the binge-like ethanol consumption that is seen in animals that have consumed alcohol chronically, are alcohol deprived and are subsequently allowed ethanol re-access. Data also strongly suggest that brain-generated acetaldehyde mediates these two effects, suggesting clinically relevant therapeutic avenues.

Acknowledgements

Authors Contributions

YI, LT and MEQ conceived and designed the experiments; EK, MR-M, MEQ and MH-R performed the experiments: MEQ, MR-M and YI performed data analysis; YI, LT and MEQ wrote the paper. All authors critically reviewed content and approved final version for publication.

References

Agabio R, Carais MAM, Lobina C, Pani M, Reai R, Vacca G, Gessa GL, Colombo G (2000) Development of short-lasting alcohol deprivation effect in Sardinian alcohol-preferring rat. Alcohol 21:59–62.

Aragon CM, Amit Z (1992) The effect of 3-amino-1,2,4-triazole on voluntary ethanol consumption: evidence for brain catalase involvement in the mechanism of action. Neuropharmacology 31:709–712.

Aragon CM, Rogan F, Amit Z (1992) Ethanol metabolism in rat brain homogenates by a catalase-H_2O_2 system. Biochem Pharmacol 44:93–98.

Bell RL, Rodd ZA, Lumeng A, Murphy JM, McBride WJ (2006) The alcohol-preferring P rat and animal models of excessive alcohol drinking. Addict Biol 11:270–288.

Bell RL, Rodd ZA, Schultz JA, Peper CL, Lumeng L, Murphy JM, McBride WJ (2008) Effects of short deprivations and

re-exposure intervals on the ethanol drinking behavior of selectively bred high alcohol-consuming rats. Alcohol 42:407–416.

Bragulat V, Dzemidzic M, Talavage T, Davidson D, O'Connor SJ, Kareken DA (2008) Alcohol sensitizes cerebral responses to odors of alcoholic drinks: an fMRI study. Alcohol Clin Exp Res 32:1124–1134.

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

Chaudhri N, Woods CA, Sahuque LL, Gill YM, Janak PH (2013) Unilateral inactivation of the basolateral amygdala attenuates context-induced renewal of Pavlovian-conditioned alcohol-seeking. Eur J Neurosci 38:2751–2761.

Cohen G, Sinet PM, Heikkila R (1980) Ethanol oxidation by rat brain *in vivo*. Alcohol Clin Exp Res 4:366–370.

Correa M, Salamone JD, Segovia KN, Pardo M, Longoni R, Spina L, Peana AT, Vinci S, Acquas E (2012) Piecing together the puzzle of acetaldehyde as a neuroactive agent. Neurosci Biobehav Rev 36:404–430.

Deehan GA Jr., Brodie MS, Rodd ZA (2013) What is in that drink: the biological actions of ethanol, acetaldehyde, and salsolinol. Curr Top Behav Neurosci 13:163–184.

Engleman EA, Ding Z-M, Oster SM, Toalston JE, Bell RL, Murphy JM, McBride WJ, Rodd ZA (2009) Ethanol is self-administered into the nucleus accumbens shell but not core: evidence of genetic sensitivity. Alcohol Clin Exp Res 33:2162–2171.

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

Gill T, Lavine A (2013) Mitochondria-derived hydrogen peroxide selectively enhances T cell receptor-initiated signal transduction. J Biol Chem 288:26246–26255.

Halliwell B (2006) Oxidative stress and neurodegeneration: where are we now? J Neurochem 97:1634–1658.

Heyser JC, Schulteis G, Koob GF (1997) Increased ethanol self-administration after a period of imposed ethanol deprivation in rats trained in a limited access paradigm. Alcohol Clin Exp Res 21:784–791.

Jeng JJ, Weiner H (1991) Purification and characterization of catalytically active precursor of rat liver mitochondrial aldehyde dehydrogenase expressed in *Escherichia coli*. Arch Biochem Biophys 289:214–222.

Karahanian E, Ocaranza P, Israel Y (2005) Aldehyde dehydrogenase (ALDH2) activity in hepatoma cells is reduced by an adenoviral vector coding for an ALDH2 antisense mRNA. Alcohol Clin Exp Res 29:1384–1389.

Karahanian E, Quintanilla ME, Tampier L, Rivera-Meza M, Bustamante D, Gonzalez Lira V, Morales P, Herrera-Marschitz M, Israel Y (2011) Ethanol as a prodrug: brain metabolism of ethanol mediates its reinforcing effects. Alcohol Clin Exp Res 35:606–612.

Klyosov AA (1996) Kinetics and specificity of human liver aldehyde dehydrogenases toward aliphatic, aromatic, and fused polycyclic aldehydes. Biochemistry 35:4457–4467.

Laviolette SR, Van der-Kooy D (2004) GABAa receptors signal bidirectional reward transmission from the ventral tegmental area to the tegmental pedunculopontine nucleus as a function of opiate state. Eur J Neurosci 20:2179–2187.

Morgan D, Smith MA, Roberts DCS (2005) Binge self-administration and deprivation produces sensitization to the reinforcing effects of cocaine in rats. Psychopharmacology (Berl) 178:309–316.

Orrico A, Hipolito L, Sanchez- Catalan MJ, Marti-Prats L, Zornoza T, Granero L, Polache A (2013) Efficacy of D-penicillamine, a sequestering acetaldehyde agent, in the prevention of alcohol relapse drinking in rats. Psychopharmacology (Berl) 228:563–575.

Oster SM, Toalston JE, Kuc KA, Pommer TJ, Murphy JM, Lumeng L, Bell RL, McBride WJ, Rodd ZA (2006) Effect of multiple alcohol deprivations on operant ethanol self-administration by high-ethanol drinking replicate rat lines. Alcohol 38:155–164.

Paxinos G, Watson C (1986) The Rat Brain in Stereotaxic Coordinates. New York: Academic Press.

Perkins KA, Ciccocioppo M, Jacobs L, Doyle T, Caggiula A (2003) The subjective and reinforcing effects of visual and olfactory stimuli in alcohol drinking. Exp Clin Psychopharmacol 11:269–275.

Peterson DR, Tabakoff B (1979) Characterization of brain acetaldehyde oxidizing systems in the mouse. Drug Alcohol Depend 4:137–144.

Quintanilla ME, Israel Y, Sapag A, Tampier L (2006) The UChA and UChB rat lines: metabolic and genetic differences influencing ethanol intake. Addict Biol 11:310–323.

Quintanilla ME, Tampier L, Karahanian E, Rivera-Meza M, Herrera-Marschitz M, Israel Y (2012) Reward and relapse: complete gene-induced dissociation in an animal model of alcohol dependence. Alcohol Clin Exp Res 36:517–522.

Quintanilla ME, Tampier L, Sapag A, Gerdtzen Z, Israel Y (2007) Sex differences, alcohol dehydrogenaser, acetaldehyde burst and aversion to ethanol in the rat: a systems perspective. Am J Physiol Endocrinol Metab 293:E531–E537.

Rivera-Meza M, Quintanilla ME, Tampier L, Mura CV, Sapag A, Israel Y (2010) Mechanism of protection against alcoholism by an alcohol dehydrogenase polymorphism: development of an animal model. FASEB J 24:266–274.

Rodd ZA, Bell RL, Kuc KA, Murphy JM, Lumeng L, Li T-K, McBride WJ (2003) Effect of repeated alcohol deprivations on operant ethanol self-administration by alcohol-preferring (P) rats. Neuropsychopharmacology 28:1614–1621.

Rodd ZA, Bell RL, Kuc KA, Murphy JM, Lumeng L, McBride WJ (2009) Effects of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of high-alcohol-drinking (HAD) rats. Addict Biol 14:152–164.

Rodd ZA, Bell RL, Zhang Y, Murphy JM, Goldstein A, Zaffaroni A, Li TK, McBride WJ (2005) Regional heterogeneity for the intracranial self-administration of ethanol and acetaldehyde within the ventral tegmental area of alcohol-preferring (P) rats: involvement of dopamine and serotonin. Neuropsychopharmacology 30:330–338.

Rodd-Henricks ZA, Bell RL, Kuc KA, Murphy JM, McBride WJ, Lumeng L, Li T-K (2001) Effect of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of alcohol-preferring rats. Alcohol Clin Exp Res 25:1140–1150.

Serra S, Brunetti G, Vacca G, Lobina C, Carai MAM, Gessa GL, Colombo G (2003) Stable preference for high ethanol concentrations after ethanol deprivation in Sardinian alcohol-preferring (sP) rats. Alcohol 29:101–108.

Sinclair JD, Li T-K (1989) Long and short alcohol deprivation effects on AA and P alcohol-preferring rats. Alcohol 6:505–509.

Sinclair JD, Senter RJ (1968) Development of an alcohol-deprivation effect in rats. Q J Stud Alcohol 29:863–867.

Sinclair JD, Walker S, Jordan W (1973) Behavioral and physiological changes associated with various durations of alcohol deprivation in rats. Q J Stud Alcohol 34:544–757.

Spanagel R, Hölter SM (1999) Long-term alcohol self-administration with repeated alcohol deprivation phases: an animal model of alcoholism? Alcohol Alcohol 34:231–243.

Stowell A, Hillbom M, Salaspuro M, Lindros KO (1980) Low acetaldehyde levels in blood, breath and cerebrospinal fluid of intoxicated humans as assayed by improved methods. Adv Exp Med Biol 132:635–645.

Tabakoff B, Anderson RA, Ritzmann RF (1976) Brain acetaldehyde after ethanol administration. Biochem Pharmacol 25:1305–1309.

Tampier L, Mardones J (1979) Catalase mediated oxidation of ethanol by rat brain homogenates. IRCS Med Sci 7:384.

Tampier L, Quintanilla ME, Mardones J (1995) Effects of aminotriazole on ethanol, water, and food intake and on brain catalase in UChA and UChB rats. Alcohol 12:341–344.

Tampier L, Quintanilla ME, Karahanian E, Rivera-Meza M, Herrera-Marschitz M, Israel Y (2013) The alcohol deprivation effect (ADE): marked inhibition by anticatalase gene administration into the ventral tegmental area in rats. Alcohol Clin Exp Res 37:1278–1285.

Tsiang MT, Janak PH (2006) Alcohol seeking in C75BL6 mice induced by conditions cues and contexts in the extinction-reinstatament model. Alcohol 38:81–88.

Vargas-Perez H, Ting-A-Kee R, van der Kooy D (2009) Different neural systems mediate morphine reward and its spontaneous withdrawal aversion. Eur J Neurosci 29:2029–2034.

Vengeliene V, Bahteler D, Danysz W, Spanagel R (2005) The role of the NMDA receptor in alcohol relapse: a pharmacological mapping study using the alcohol deprivation effect. Neuropharmacology 48:822–829.

Vengeliene V, Celerier E, Chaskiel L, Penzo F, Spanagel R (2009) Compulsive alcohol drinking in rodents. Addict Biol 14:384–396.

Vengeliene V, Noori HR, Spanagel R (2013) The use of a novel drinkometer system for assessing pharmacological treatment effects on ethanol consumption in rats. Alcohol Clin Exp Res 37:322–328.

Zimatkin SM, Pronko SP, Vasiliou V, Gonzalez FJ, Deitrich RA (2006) Enzymatic mechanisms of ethanol oxidation in the brain. Alcohol Clin Exp Res 30:1500–1505.