The Alcohol Deprivation Effect: Marked Inhibition by Anticatalase Gene Administration into the Ventral Tegmental Area in Rats

Lutske Tampier*, María Elena Quintanilla*, Eduardo Karahanian, Mario Rivera-Meza, Mario Herrera-Marschitz, and Yedy Israel

Background: Animals that have chronically consumed alcohol and are subsequently deprived of it markedly increase their intake above basal levels when access to alcohol is reinstated. Such an effect, termed the alcohol deprivation effect (ADE), has been proposed to reflect (i) an obsessive–compulsive behavior, (ii) craving, or (iii) an increased reinforcing value of ethanol (EtOH). It has been reported that acetaldehyde, a highly reinforcing metabolite of EtOH, is generated in the brain by the action of catalase. Recent studies show that the administration of an anticatalase (shRNA)-encoding lentiviral vector into the brain ventral tegmental area (VTA) of naïve rats virtually abolishes (85 to 95%) their EtOH intake. It is hypothesized that the antireinforcing effect of the anticatalase vector will also inhibit the ADE.

Methods: Two-month-old Wistar-derived UChB alcohol drinker rats were offered free access to water and 10 and 20% EtOH for 67 days. Thereafter, the animals were deprived of EtOH for 15 days and were subsequently offered access to the EtOH solutions. At the start of the deprivation period, animals were microinjected a single dose of an anticatalase (or control) vector into the VTA. EtOH intake was measured on the first hour of EtOH re-exposure as well as on a 24-hour basis for 7 days.

Results: A marked ADE was observed when EtOH intake was measured on the first hour or 24 hours following EtOH re-exposure, compared to the corresponding controls. The administration of the anticatalase vector reduced ADE by 60 to 80% ($p < 0.001$) on the first hour and by 63 to 80% ($p < 0.001$) on the initial 24 hours of EtOH re-exposure (first and second ADE, respectively) without changing the total fluid intake, indicating a specific effect on EtOH drinking.

Conclusions: Ethanol intake associated with ADE—a binge-like drinking behavior—is markedly inhibited by the administration of an anticatalase vector into the VTA, which blocks the conversion of EtOH into acetaldehyde, strongly suggesting that the marked increased EtOH intake that follows an alcohol deprivation period is mediated by acetaldehyde and its reinforcing metabolite.

Key Words: Catalase, Alcohol Deprivation Effect, Acetaldehyde, Alcoholism, Reinforcement.

EARLY STUDIES BY Sinclair and Senter (1968, 1977) showed that chronic intake of ethanol (EtOH) by rats, followed by a period of alcohol deprivation and subsequent re-exposure to EtOH, leads animals to increase their EtOH intake above their basal predeprivation levels. This effect, termed the alcohol deprivation effect (ADE), can be defined as a temporary increase in the ratio of EtOH/total fluid intake and as an increase in voluntary intake of EtOH solutions over baseline drinking conditions when EtOH is reinstated after the period of alcohol deprivation (Rodd-Henricks et al., 2001; Spanagel and Hölter, 1999). An ADE can be observed after a short (1 to 3 days; Agabio et al., 2000; Sinclair and Li, 1989) or a long (up to 60 to 75 days) deprivation period (Sinclair et al., 1973; Spanagel and Hölter, 1999), but is not observed in nondeprived continuously alcohol-treated animals, suggesting that chronic exposure to EtOH alone is not sufficient to produce such a marked increase in EtOH intake (Spanagel and Hölter, 1999). Examination of the ADE phenomenon has revealed that at least 3 to 4 weeks of continuous alcohol-drinking experience are required before deprivation to elicit an ADE (Spanagel and Hölter, 1999). An ADE has been reproduced in rats (Füllgrabe et al., 2007; Heyser et al., 1997; Rodd-Henricks et al., 2001; Serra et al., 2003; Sinclair and Li, 1989; Spanagel and Hölter, 1999; Thielen et al., 2004; Vengeliene et al., 2013), mice (Tambour et al., 2008), and primates (Kornet et al., 1990). Studies of ADE have been reported in several rats lines/strains bred for their high voluntary alcohol intake, including the Indianapolis P, HAD-1

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and HAD-2 lines (Bell et al., 2008; Rodd et al., 2008a; Sinclair and Li, 1989), the Finnish AA line (Sinclair and Li, 1989); and the Sardinian alcohol preferring sP rats (Serra et al., 2003). A number of studies have also shown that repeated alcohol intake–deprivation–re-administration episodes increase the expression of ADE (see Rodd et al., 2008a).

The biological bases underlying ADE have not been established. Hypotheses on the biological basis of ADE have ranged from alterations in taste-related inhibition to neuronal adaptation to the reinforcing and/or aversive properties of alcohol (Rodd et al., 2003a; Rodd-Henricks et al., 2001). It has been proposed that the ADE effect is akin to an obsessive–compulsive disorder (Vengeliene et al., 2009) or to craving (Heyser et al., 1997; Robinson and Berridge, 1993; Sinclair and Li, 1989), conditions often reported for alcoholics, implying the existence of biobehavioral changes *leading* the addict toward drug consumption. Rodd and colleagues (2003a), Oster and colleagues (2006), and Vengeliene and colleagues (2009) have proposed that the motivational and reinforcing effects of EtOH are increased in the ADE condition. The term relapse or relapse-like drinking behavior has also been used to refer to ADE (Sinclair and Li, 1989; Spanagel and Hölter, 1999; Tambour et al., 2008), a term that does not imply a mechanism for the postdeprivation increase in EtOH intake.

A number of studies have proposed that brain acetaldehyde, the first metabolite of EtOH, is a reinforcing and motivational molecule (Amit and Smith, 1985; Aragon and Amit, 1992; Brown et al., 1979; Tampier et al., 1995). Rodd and colleagues (2003b, 2005a) demonstrated that rats selectively bred as alcohol drinkers (strain P of Indianapolis) self-administer both EtOH and acetaldehyde into the brain ventral tegmental area (VTA). Acetaldehyde showed reinforcing effects at concentrations (6×10^{-6} M) that were 1,000 lower than those required for EtOH (17×10^{-3} M) self-administration in this area.

While liver-generated systemic acetaldehyde is known to generate an aversive reaction (see Eriksson, 2001), a major question in this field is whether systemic acetaldehyde (normally not exceeding 20×10^{-6} M in arterial blood after EtOH intake) can cross the blood–brain barrier. Studies indicate that as the capillaries of the blood–brain barrier have tight junctions (rather than open pores), acetaldehyde must first enter the endothelial cells of the barrier, which clear acetaldehyde. Thus, under normal conditions of EtOH metabolism, systemic acetaldehyde does not cross the blood–brain barrier (Lindros and Hillbom, 1979; Petersen and Tabakoff, 1979; Stowell et al., 1980). Only when systemic concentrations exceed 100 μM, following the exogenous administration of acetaldehyde, does this molecule enter the central nervous system (Tabakoff et al., 1976).

While alcohol dehydrogenase is not expressed in the brain (see Deitrich, 2011; Zimatkin et al., 2006), acetaldehyde can be generated in this organ by the action of catalase on EtOH and to a minor extent by CYP2E1, both enzymes present in the brain (Aragon et al., 1992; Deitrich, 2011; Tampier and Mardones, 1979; Zimatkin et al., 2006). In vitro studies indicate that catalase generates 60 to 70% of brain acetaldehyde while CYP2E1 some 15 to 20% (Zimatkin et al., 2006). The question remains as to whether enough acetaldehyde is generated in the brain to develop rewarding and reinforcing effects when EtOH is consumed orally.

Recently, a specific gene-blocking technique allowed inhibiting brain catalase synthesis. Karahanian and colleagues (2011) developed lentiviral vectors that coded for an shRNA designed to inhibit the synthesis of catalase. Lentiviral vectors permanently integrate into the cell genome. The single stereotaxic administration of an anticatalase-lentiviral vector into the VTA, which reduced catalase levels by 70 to 80% (Quintanilla et al., 2012), virtually abolished the voluntary EtOH consumption (up to 95%) by drinker UChB rats for the 40 to 60 days studied (Karahanian et al., 2011; Quintanilla et al., 2012). The lentiviral anticatalase shRNA administration also abolished the increases in dopamine release in nucleus accumbens induced by the acute administration of EtOH. Overall, the reinforcing effects of EtOH appear to be mediated by acetaldehyde generated in the brain by the action of catalase. We therefore hypothesize that an intact catalase is also required to generate the ADE condition.

Depending on the strain and EtOH administration schedule, chronically EtOH fed rats can metabolize up to 11 to 14 g EtOH/d (Khanna et al., 1982). Thus, in paradigms measuring ADE-induced consumption for 24 hours or for consecutive days, the percent increases in EtOH intake can be greater in animals that have consumed lower amounts of EtOH prior to the induction of ADE. In this study, we have investigated the effect on ADE of an anticatalase vector both on the first hour and following several 24-hour periods after a deprivation period in animals bred for their high EtOH consumption.

MATERIALS AND METHODS

Animals

The studies were conducted on female rats of the post-80th UChB generation of the Wistar-derived alcohol-drinking line (Mardones and Segovia-Riquelme, 1983; Quintanilla et al., 2006). Two-month-old female rats housed individually were offered free access to fluid from 3 tubes: water, and 10% (v/v) and 20% (v/v) EtOH for 67 days. Solid chow was available ad libitum. Rats were maintained on a 12-hour normal light/dark cycle (lights off at 1900 hours). Animals were weighed weekly. All procedures used in this study were in compliance with the Animal Experimentation Committee of the Faculty of Medicine and Chilean Ethical rules for animal experimentation.

Generation of Lentiviral Vectors

The lentiviral vector expressing a rat anticatalase shRNA and the control virus containing no shRNA sequences were prepared and stored at −80°C until stereotaxically injected into the VTA as described by Karahanian and colleagues (2011). Rats were anesthetized and placed in a stereotaxic frame for intracerebral

administration of 1 μl of the solution containing the anticatalase shRNA lentiviral vector (8×10^4 virus/μl; body weight 190 ± 5.67 g, $n = 7$) or 1 μl the corresponding control viral vector (8×10^4 virus/μl; body weight 186 ± 7.12, $n = 7$) into the left VTA (B-5.2, L-0.8, V-7.2, according to Paxinos and Watson, 1986). The 1 μl of lentiviral vectors was injected in 2.5 minutes, which should allow distribution into the whole VTA.

Experimental Procedures

Animal Treatments. No Viral Vector Administration—Rats received 24-hour free-choice concurrent access to 10 and 20% EtOH solutions and water for 67 consecutive days. Fluid consumption was recorded daily. Baseline data shown corresponds to the average EtOH intake on the basis of 24 hours ($n = 7$ rats) or when access was restricted for only 1 h/d ($n = 7$ rats) during 7 days immediately prior alcohol deprivation on day 67. This study was conducted to assess the generation of ADE in animals, which did not receive a viral vector injection. This 1-hour group was later discarded from deprivation cycles as EtOH was only available for 1 hour in the last 7 days following 60 days of 24-hour intake.

Viral Vector Administration and EtOH Deprivation—On the 68th day, rats that had received 67 consecutive days of free-choice EtOH consumption were divided into 2 groups matched for similar 24-hour alcohol consumption and preference. The first group ($n = 7$) received an intracerebral administration of the control lentiviral vector and was immediately deprived for 15 consecutive days of both the 10 and 20% EtOH solution, while water was the sole fluid available. The second group ($n = 7$ rats per group) was injected into the VTA the anticatalase-Lenti-shRNA. As for the viral control group, these rats were returned to their home cage and deprived for 15 days of both 10 and 20% EtOH solutions.

EtOH Re-Exposure—Following the 15 days of EtOH deprivation, re-exposure to free-choice intake of 10 and 20% EtOH and water started at 1 PM and lasted for 7 days. Alcohol intake was recorded in all groups on the first hour of re-exposure and 24 hours after alcohol re-exposure each day. Thereafter, groups of rats injected into the VTA with anticatalase-Lenti and control-Lenti received a second period of 15 days of EtOH deprivation and further 7 days of EtOH drinking. Results are expressed as g of EtOH consumed/kg body weight on the first hour of re-exposure and on a daily basis (24-hour intakes). Water intake was also determined on a daily basis. Percentage EtOH preference (ml intake of 10 or 20% EtOH/ml total fluid intake × 100) was also calculated for lentiviral-treated rats and control rats after chronic EtOH intake (baseline), EtOH deprivation, and EtOH re-exposure for the first 24 hours.

Statistical Analyses

Data are expressed as the means ± SEM for each condition. Statistical differences were analyzed by the Student's *t*-test or a 2-way analysis of variance (ANOVA) (baseline vs. cycle of re-exposure) and treatment (control-Lenti vs. anticatalase-Lenti) followed by the post hoc Bonferroni *t*-test.

RESULTS

Figures 1 and 2 show that UChB rats displayed a clear ADE following 67 days of EtOH intake (10 and 20%) and EtOH re-exposure after the 15 days of deprivation. Figure 1 shows that EtOH intake during the first hour of EtOH re-exposure increased by 53% ($p < 0.001$) over the baseline, mainly due to an increase consumption of the 20% EtOH solution, in line with previous ADE studies with other rat strains/lines (Bell et al., 2008; Rodd-Henricks et al., 2001; Spanagel et al., 1996). Figure 2 shows the effect of deprivation on daily EtOH consumption prior to and following re-exposure. An ADE effect was only observed during the first 24 hours. On a 24-hour consumption basis, EtOH intake increased by 47% ($p < 0.001$) over baseline, again mainly due to 20% EtOH intake, reaching a total daily intake of the order of 12 g/kg body weight, near to the metabolic capacity of chronically alcohol fed rats.

Subsequent studies were conducted to evaluate the effect of anticatalase (or control) vector administration into the VTA on ADE (Figs 3 and 4). Figure 3 shows the average of 1 h/d EtOH intake during the 7-day baseline period prior to deprivation. During the baseline period, rats consumed nearly identical amounts of 10 and 20% EtOH (1.09 ± 0.06 g/kg; Fig. 3*A*,*B*). When EtOH was deprived for 15 days, rats that had received the anticatalase-lentiviral vector into the VTA showed a marked reduction in EtOH intake compared with the viral control. Noteworthy, the viral control injection did not affect the generation of an ADE. After re-exposure, total EtOH consumption was increased in the virus control group, to 1.52 ± 0.10 g/kg/h

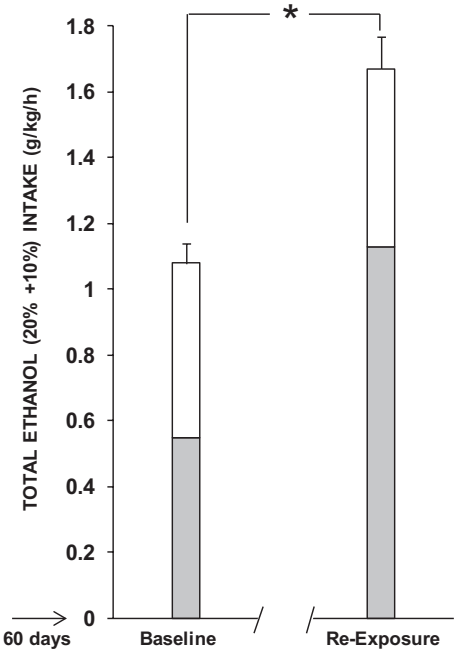

Fig. 1. Increase in ethanol (EtOH) intake during the first hour of re-exposure to alcohol solutions after 67 days of 24-hour free-choice of 10 and 20% EtOH and further EtOH deprivation for 15 days. The -/ /- symbol in the *x*-axis represents the 15-day deprivation period. Baseline consumption corresponds to 1-hour intake of during the final 7 days of rats previously kept on a 24-hour access. The total height of each bar represents the sum of EtOH intake (g EtOH/kg/1 h from the 10% solution) (empty bars) plus that from the 20% solution (gray bars). Significant difference between EtOH intake displayed during re-exposure and that of the mean of 7 baseline days: *$p < 0.001$. Difference due to a significant increase in consumption of the 20% EtOH solution ($p < 0.005$) Student's *t*-test ($n = 7$ animals per group).

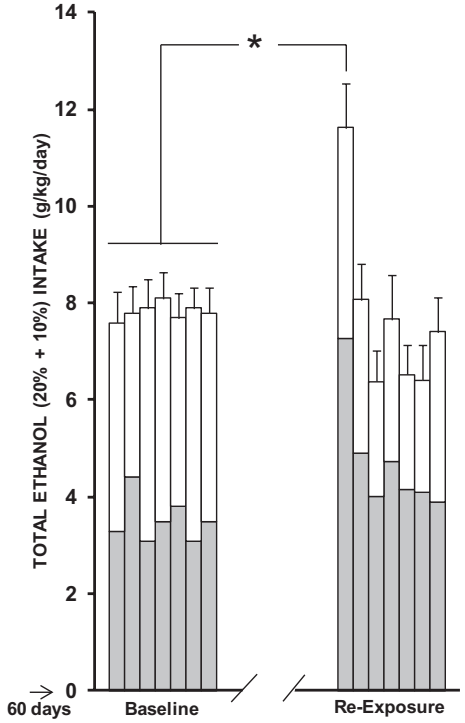

Fig. 2. Increase in 24-hour ethanol (EtOH) intake following re-exposure to alcohol solutions after 67 days of free-choice 10 and 20% EtOH availability and EtOH deprivation for 15 days. The total height of each bar represents the sum of EtOH intake (g EtOH/kg/24 h) from the 10% solution (empty bars) plus that from the 20% solution (gray bars). The -/ /- symbol in the *x*-axis represents the 15-day deprivation period. Baseline data correspond to the average of EtOH intake on the basis of 24 hours, for 7 days immediately prior to alcohol deprivation ($n = 7$/group). Significant difference between EtOH intake displayed during re-exposure and that of the mean of 7 baseline days: $*p < 0.001$. Difference due to a significant increase in consumption of the 20% EtOH solution ($p < 0.001$). Student's *t*-test.

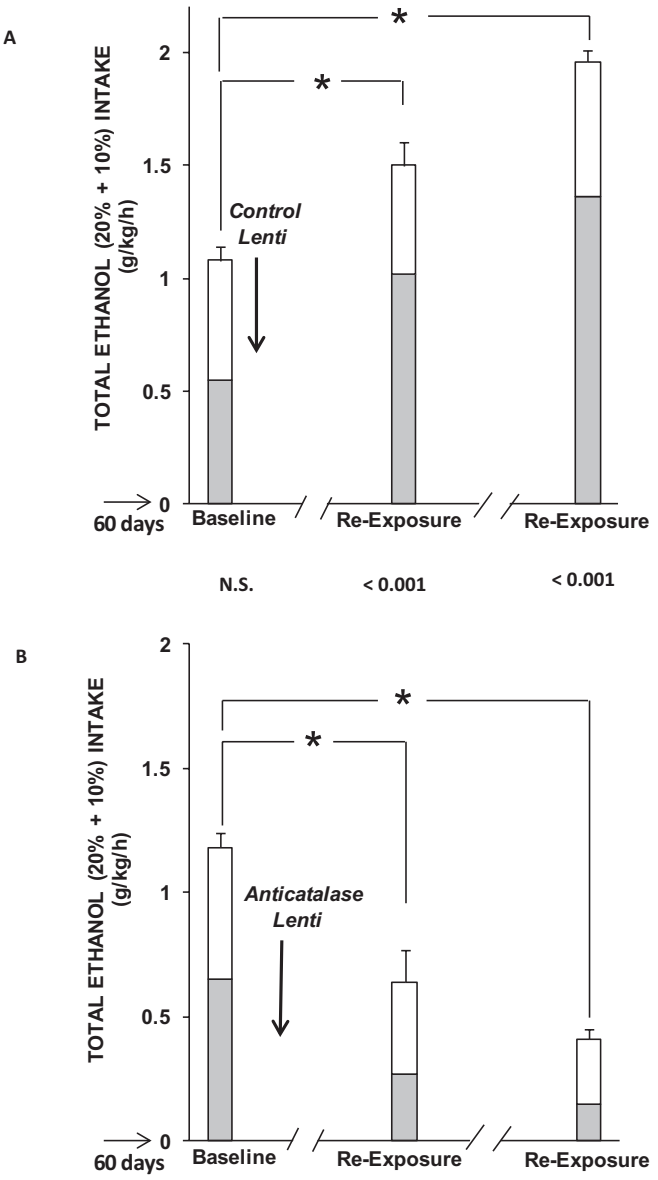

Fig. 3. A single intra-ventral tegmental area injection of an anticatalase-lentiviral vector inhibits first-hour ethanol (EtOH) intake after the first and second EtOH deprivation periods of 15 days. The total height of each bar represents the sum of EtOH intake (g EtOH/kg/1 h) of the 10% solution (empty bars) plus that of the 20% solution (gray bars). The -/ /- symbol in the *x*-axis represents the 15-day deprivation period. (**A**) Control viral vector. (**B**) Anticatalase viral vector. Arrows indicate the administration of either control lentiviral vector ($n = 7$) or anticatalase-lentiviral vector ($n = 7$) prior to the 15 days of deprivation. Baseline data correspond to the average of EtOH intake restricted to only 1 hour a day, for 7 days immediately prior to alcohol deprivation. The first and second re-exposure consumptions were significantly different from baseline: $*p < 0.001$. (Student's *t*-test). Additionally, a 2-way ANOVA (baseline vs. re-exposure) and treatment (control vector vs. anticatalase vector) show a significant effect of treatment, $F(1, 36) = 197.3$, $p < 0.0001$, and a significant treatment group × deprivation cycles interaction, $F(2, 36) = 59.84$, $p < 0.0001$. A post hoc Bonferroni *t*-test shows a highly significant difference between both treated groups on alcohol deprivation effect expression ($p < 0.001$) (*p*-values shown between **A** and **B**).

of EtOH ($p < 0.001$) during the first hour, mainly due to a 20% EtOH increase (Fig. 3*A*). Thereafter, EtOH was continuously available for a 6-day period before deprivation. Following a second 15-day deprivation period, total EtOH intake was again increased (ADE) during the first hour, mainly due to 20% EtOH intake (1.91 ± 0.02 g/kg/h, $p < 0.001$ vs. baseline and $p < 0.02$ vs. the intake following the first ADE). These highly intoxicating (see Lê and Israel, 1994) 1-hour oral consumption levels are clearly akin to a "binge-like" drinking behavior. However, as shown in Fig. 3*B*, after the administration of anticatalase-Lenti vector into the VTA, no ADE was observed following the first 15-day deprivation. Moreover, EtOH intake in animals treated with anticatalase vector was decreased by 40% versus baseline ($p < 0.001$) and 60% versus control vector. The inhibitory effect of the anticatalase vector was even more remarkable following the second 15-day deprivation period, being 67% below baseline ($p < 0.001$) and 80% ($p < 0.001$) lower than intake on the second control ADE intake (ANOVAs in Fig. 3).

When total EtOH consumption was recorded 24 h/d, a marked ADE was observed on the first day of EtOH re-exposure in control-Lenti rats (Fig. 4*A*), reaching a total EtOH intake of 12 to 14 g/kg/24 h. A full obliteration of the 24-hour ADE EtOH intake was observed in the anticatalase

group (Fig. 4*B*). The remarkable inhibitory effect on EtOH intake (60%; $p < 0.001$ vs. baseline values) of the single dose of the anticatalase vector remained after the second deprivation period (75%; $p < 0.001$ vs. baseline levels). Statistical analyses also show a highly significant inhibition on ADE expression (63 to 80%) when the anticatalase vector is compared against the control vector ($p < 0.001$) (ANOVAs in Fig. 4).

Table 1 shows water intake measured from the water tube only. Rats that did not receive any intracerebral treatment (no intracerebral vector) showed a water intake of 9.8 ± 3.1 ml/kg/d from the water tube measured at baseline, an amount that was not significantly changed (11.1 ± 3.3 ml/kg/d) after the 15-day EtOH deprivation period and the subsequent EtOH re-exposure. A similar result was observed in rats injected the empty Lenti-virus into the VTA. However, following the anticatalase vector, water intake *from the water tube* was significantly increased upon EtOH re-exposure (>6-fold and ~4-fold, by first and second ADE, respectively; compensating the water intake that was not consumed from the EtOH solution tubes). Table 2 shows that the anticatalase vector markedly reduced EtOH/water preference, indicating a specific effect of the anticatalase vector on EtOH drinking rather than on total fluid intake.

DISCUSSION

Rats of the Wistar-derived UChB alcohol drinker line markedly increase their EtOH consumption after a period of chronic EtOH intake followed by EtOH deprivation and subsequent EtOH re-exposure. The marked increase in EtOH consumption after the deprivation period also resulted from a shift in preference toward the solution with the higher EtOH concentration. Further, the increases in EtOH intake were observed primarily on the first hour after the EtOH deprivation cycle (see also Hölter et al., 1998; Rodd-Henricks et al., 2001; Spanagel and Hölter, 1999). This clearly intoxicating (Lê and Israel, 1994) oral EtOH intake in rats reaching 1.9 g EtOH/kg in 1 hour (exceeding 5 drinks/70 kg in humans) is akin to a "binge drinking" behavior. The anticatalase vector greatly inhibited (75 to 80%) the increases in EtOH consumption following deprivation, whether determined for a 60-minute period or a 24-hour EtOH intake period.

There are significant differences in the expression of an ADE among different rat drinker line/strains. It has been noted that preference for voluntary EtOH intake in these lines does not correlate with the development of ADE; marked differences in ADE development are seen in Indianapolis high drinker P, HAD-1 and HAD-2 lines, with HAD-2 rats being the strain that develops the greatest levels of ADE (Bell et al., 2008). Noteworthy, an ADE was also clearly shown in a rat strain specifically bred for their *low* EtOH consumption such as NP, LAD-1, and LAD-2 (Bell et al., 2004). However, for the latter, multiple exposure and deprivation cycles are needed. A marked ADE is also seen in

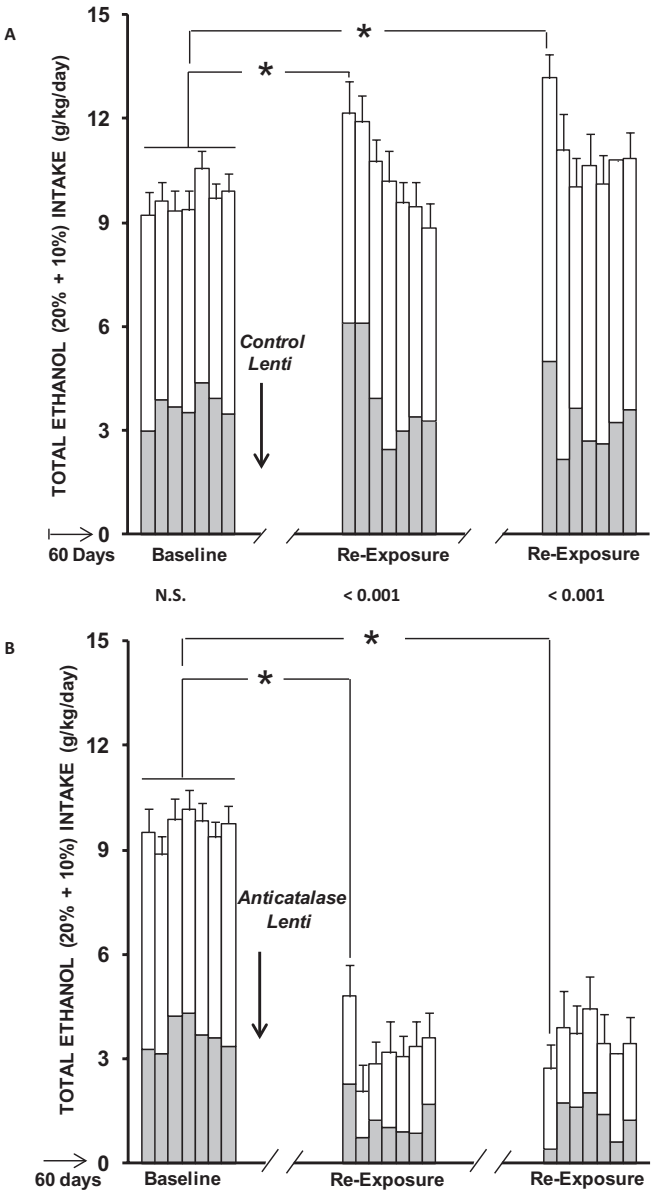

Fig. 4. A single intra-ventral tegmental area injection of an anticatalase-lentiviral vector inhibits the first 24-hour ethanol (EtOH) intake after the first and second deprivation periods of 15 days. The total height of each bar represents the sum of EtOH intake (g EtOH/kg/24 h of the 10% solution) (empty bars) plus that of the 20% solution (gray bars). (**A**) Control viral vector. (**B**) Anticatalase viral vector. The -/ /- symbol in the *x*-axis represents the 15-day deprivation period. Arrows indicate the administration of either control lentiviral vector ($n = 7$) or anticatalase-lentiviral vector ($n = 7$) immediately prior to the 15 days of deprivation. Baseline data correspond to the average of EtOH intake on the basis of 24 hours, for 7 days immediately prior to alcohol deprivation. Panels (**A**) and (**B**) show a significant increase between EtOH intake on the first day of re-exposure and the mean of the 7 baseline days of both the first and the second deprivation cycles: $*p < 0.001$ (Student's *t*-test). Noteworthy, EtOH intake after EtOH re-exposure dropped below baseline levels in the anticatalase animals. A 2-way ANOVA for deprivation cycles (baseline vs. re-exposure) and treatment (control vector vs. anticatalase vector) shows a significant effect of treatment, $F(1, 36) = 82.44$, $p < 0.0001$, and a significant treatment group × deprivation cycles interaction, $F(2, 36) = 25.80$, $p < 0.0001$. A post hoc Bonferroni *t*-test shows a highly significant difference between both groups on alcohol deprivation effect expression ($p < 0.001$) (*p*-values shown between **A** and **B**).

Table 1. Following EtOH Deprivation, the Anticatalase Vector Increases Water Intake from the Water-Only Tubes

Water intake			
Treatment	Baseline (ml/kg/24 h)	1st ADE (ml/kg/24 h)	2nd ADE (ml/kg/24 h)
Control (no vector)	9.8 ± 3.1	11.1 ± 3.3	n.d.
Control (empty) lentiviral vector	10.0 ± 1.4	10.7 ± 4.5	11.9 ± 5.0
Anticatalase-lentiviral vector	9.9 ± 1.4	60.7 ± 4.5**	37.3 ± 3.4**

n.d., not done; ADE, alcohol deprivation effect; EtOH, ethanol.

Mean ± SEM ($n = 7$, for each group); **$p < 0.001$, significant versus the corresponding baseline (Student *t*-test).

Water intake *from the water-only tube* is shown during baseline (average of 7 days predeprivation) and during the first day following deprivation and subsequent EtOH re-exposure (ADE). The increase in water intake compensates the reduction in water intake derived from the EtOH solutions (see Table 2).

Table 2. Anticatalase Vector Reduces EtOH Preference

Treatment	Baseline	1st ADE	2nd ADE
Control (no vector)			
10% EtOH preference (%)	58 ± 6.6	43 ± 5.1	–
20% EtOH preference (%)	22 ± 1.6	48 ± 5.2*	–
Total EtOH solutions preference (%)	80 ± 3.0	91 ± 2.2*	–
Control (empty) lentiviral vector			
10% EtOH preference (%)	67 ± 4.8	58 ± 6.3	69 ± 2.9
20% EtOH preference (%)	10 ± 1.2	34 ± 7.0*	22 ± 2.7*
Total EtOH solutions preference (%)	77 ± 3.0	92 ± 6.7*	91 ± 2.9*
Anticatalase-lentiviral vector			
10% EtOH preference (%)	66 ± 5.5	30 ± 4.7*	36 ± 7.2*
20% EtOH preference (%)	14 ± 2.2	13 ± 3.1	3 ± 1.4*
Total EtOH solutions preference (%)	81 ± 3.8	43 ± 3.9*	39 ± 4.3*

ADE, alcohol deprivation effect; EtOH, ethanol.

Means ± SEM ($n = 7$, for each group); *$p < 0.05$, significant versus the corresponding baseline (Student's *t*-test).

Percent EtOH preferences are shown for controls and lentiviral-treated rats following chronic EtOH intake (baseline) and following EtOH deprivation on the first 24 hours of subsequent EtOH re-exposure (first and second ADE). Total volume consumed (10% EtOH + 20% EtOH + water) corresponds to 100%.

low-consumer Wistar rats (Füllgrabe et al., 2007; Sinclair and Li, 1989; Spanagel et al., 1996; Vengeliene et al., 2005). Overall, repeated exposure to cycles of ADE increases the robustness of the ADE, as the volume of the higher EtOH solution consumed as well as extending the ADE to the second day and subsequent days postdeprivation (Rodd-Henricks et al., 2001). In the present studies, only 2 ADE cycles were imposed; which in the UChB line allowed a clear demonstration of a greater ADE effect on the second deprivation cycle.

Moreno and colleagues (1995) has demonstrated the existence of catalase in most brain areas including the VTA. When compared to other tissues, brain catalase levels are lower (Halliwell, 2006), which may be surprising because the brain displays the greatest utilization of oxygen per unit tissue weight, which leads to superoxide and hydrogen peroxide formation. However, it has become clear that in the brain, enzymes other than catalase are responsible for the elimination of hydrogen peroxide (Halliwell, 2006), namely most active glutathione peroxidases and peroxiredoxins (Rhee et al., 2005; Turrens, 2003). As indicated earlier (Karahanian et al., 2011), inhibition of catalase in the VTA (Quintanilla et al., 2012) does not change animal body weight, motility, or show stereotyped behaviors.

A note should be made with regard to the site and volume of the lentiviral vector injected in the present study. Rodd and colleagues (2005b) reported that rats self-administer EtOH in volumes of 100 nl into the posterior VTA (B −5.3 to −6.0). In the present studies, in which 1 μl was injected at B −5.2 (see also Karahanian et al., 2011 and Quintanilla et al., 2012), the 10-fold greater volume injected likely reached both the posterior and the anterior VTA. As the anterior VTA is inert with respect to EtOH self-administration, a smaller volume of the viral vector may conceivable be effective if injected only in the posterior VTA.

There have been studies in which whole brain catalase is inhibited by the systemic administration of 3-aminotriazole, in which animals that showed reductions in voluntary EtOH intake (Aragon et al., 1992; Rotzinger et al., 1994; Tampier et al., 1995). Rodd and colleagues (2005b) reported that in their studies 3-aminotriazole did not inhibit EtOH self-administration into the VTA. However, in these studies, brain EtOH was allowed to be co-infused by the animals *along* with 3-aminotriazole, while the presence of EtOH is known to block the inhibition of catalase by 3-aminotriazole (Cohen et al., 1980). Thus, it is uncertain whether catalase in VTA was indeed inhibited in these self-administration studies.

Rodd and colleagues (2003a), Oster and colleagues (2006), and Vengeliene and colleagues (2009) showed that animals experiencing ADE will work to a greater extent to obtain EtOH (achieving a higher break point in lever pressing on a progressive ratio schedule) than they do before the deprivation phase, thus suggesting that the deprivation increases the reinforcing or motivational effect of EtOH. Vengeliene and colleagues (2005) showed that the intraperitoneal administration of EtOH obliterates the ADE effect, suggesting that the cycle of EtOH deprivation influences the pharmacological effects of EtOH generated on the chronic EtOH consumption period. In line with the above studies, in the present studies, the anticatalase vector administration, which has been reported to block the reinforcing effects of EtOH (Karahanian et al., 2011), blocked the expression of ADE. The possibility that both cue-conditioning and a

reinforcing effect may act in concert to achieve the expression of ADE should also be considered. Work by Volkow and colleagues (2011) showed that addicts exposed to cues related to their drug of choice release dopamine in striatum (including nucleus accumbens), an effect that is also observed after the administration of virtually all addictive drug administered to a naïve individual (Wise, 2004). Thus, by activating dopamine release in nucleus accumbens, both cue-exposure (cognition) and the motivational/reinforcing effects might *potentiate* each other. There is increasing evidence that dopamine's role in reinforcement is more complex than just coding for reward per se (hedonic pleasure) and that stimuli that induce fast and large dopamine increases also trigger conditioned responses and elicit incentive motivation to procure them, acquiring the ability to increase dopamine in anticipation of the reward (see Owesson-White et al., 2009; Volkow et al., 2011).

It is noteworthy that in the present studies, the ADE effect was evident in animals kept on a regular 24-hour cycle (lights on from 7 AM to 7 PM) while the 1-hour EtOH intake of ADE was recorded from 1 to 2 PM, thus showing a significant ADE effect even at a time of minimal animal activity. Thus, the concomitant food intake that occurs in the first hours of a dark cycle is not required for the ADE effect; indicating that there is no need for an additional hedonistic stimulus as may be provided by food intake.

Overall, the present study shows that the increase in EtOH intake induced by chronic EtOH administration and subsequent EtOH deprivation (ADE) is markedly inhibited by a gene vector that blocks the synthesis of brain catalase, the major enzyme that metabolizes EtOH into acetaldehyde in this organ.

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