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Developmental lead exposure induces opposite effects on ethanol intake and locomotion in response to central vs. systemic cyanamide administration

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ABSTRACT

Lead (Pb) is a developmental neurotoxicant that elicits differential responses to drugs of abuse. Particularly, ethanol consumption has been demonstrated to be increased as a consequence of environmental Pb exposure, with catalase (CAT) and brain acetaldehyde (ACD, the first metabolite of ethanol) playing a role. The present study sought to interfere with ethanol metabolism by inhibiting ALDH2 (mitochondrial aldehyde dehydrogenase) activity in both liver and brain from control and Pb-exposed rats as a strategy to accumulate ACD, a substance that plays a major role in the drug's reinforcing and/or aversive effects.

To evaluate the impact on a 2-h chronic voluntary ethanol intake test, developmentally Pb-exposed and control rats were administered with cyanamide (CY, an ALDH inhibitor) either systemically or intracerebroventricularly (i.c.v.) on the last 4 sessions of the experiment. Furthermore, on the last session and after locomotor activity was assessed, all animals were sacrificed to obtain brain and liver samples for ALDH2 and CAT activity determination.

Systemic CY administration reduced the elevated ethanol intake already reported in the Pb-exposed animals (but not in the controls) accompanied by liver (but not brain) ALDH2 inactivation. On the other hand, a 0.3 mg i.c.v. CY administration enhanced both ethanol intake and locomotor activity accompanied by brain ALDH2 inactivation in control animals, while an increase in ethanol consumption was also observed in the Pb-exposed group, although in the absence of brain ALDH2 blockade. No changes were observed in CAT activity as a consequence of CY administration.

These results support the participation of liver and brain ACD in ethanol intake and locomotor activity, responses that are modulated by developmental Pb exposure.

1. Introduction

Developmental exposure to low doses of the non-essential metal lead (Pb) induces subtle neurobehavioral consequences that are noticeable later in life, including an enhanced vulnerability to drug addiction (Virgolini & Cancela, 2014). Interestingly, adult Pb-exposed animals evidenced attenuation in the pharmacological effects of ethanol (narcosis, lever press for the drug and pain responses) as well as higher ethanol consumption (Nation, Baker, Fantasia, Ruscher, & Clark, 1987; Nation, Dugger, Dwyer, Bratton, & Grover, 1991; Nation, Grover, & Bratton, 1991; Nation, Baker, Taylor, & Clark, 1986). Furthermore, in adolescent low-level developmentally Pb-exposed animals, we have reported a higher reactivity to the anxiolytic, motivational and hypnotic responses to the drug compared to non-exposed controls (Virgolini, Cancela, & Fulginiti, 1999). In addition, using a similar exposure scheme, we have recently demonstrated that Pb-exposed animals evidenced an enhanced ethanol intake and subsequent ethanol-induced locomotion, ascribing a critical role to brain ethanol metabolism in these responses. In effect, we showed that CAT pharmacological

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activation (3 nitropropionic acid) or inhibition (1,2,4 aminotriazole) resulted in parallel behavioral and biochemical changes in ethanol intake and CAT activity, respectively, in the Pb-exposed animals. We thus concluded that CAT-mediated ethanol oxidation (and brain ACD accumulation) is a putative mechanism involved in the heightened ethanol motivational effects observed in these animals (Mattalloni, De Giovanni, Molina, Cancela, & Virgolini, 2013).

As is well known, the enzyme alcohol dehydrogenase (ADH) presents low activity in the brain, by which catalase (CAT) and to a lesser extent cytochrome CYP2E1 catalyze central ethanol oxidation to acetaldehyde (ACD), whereas aldehyde dehydrogenase (ALDH) favors ACD oxidation to acetate, a step that is followed by acetyl CoA and CO_2 formation (Zimatkin, Pronko, Vasiliou, Gonzalez, & Deitrich, 2006). ALDH1A1 and ALDH2 are involved in ethanol-derived ACD oxidation to acetic acid, sharing a 68% amino acid similarity despite cytosolic ALDH1A1 having less affinity for ACD (Km 50–180 μM) than mitochondrial ALDH2 (Km < 1 μM) (Marchitti, Brocker, Stagos, & Vasiliou, 2008). Interestingly, a reported point mutation in ALDH2 (a glutamic acid substitution by a lysine in position 487 determining the ALDH2*2 variant) is responsible for the genetic susceptibility that leads to the "flushing syndrome" observed in East Asians as a consequence of systemic ACD accumulation (Higuchi, Matsushita, Murayama, Takagi, & Hayashida, 1995).

It is noteworthy that ACD, ethanol's first metabolite, has opposite effects in the organism. In addition to the well-known aversive consequences of its accumulation in the periphery, centrally-formed ACD has positive reinforcing properties (Correa et al., 2012; Israel, Quintanilla, Karahanian, Rivera-Meza, & Herrera-Marschitz, 2015; Quertemont, Tambour, & Tirelli, 2005). In effect, ACD can be self-administered both orally (Peana, Muggironi, & Diana, 2010) and into VTA (Rodd-Henricks et al., 2002). Moreover, ADH inhibition prevents the ability of ethanol (but not ACD) to increase spontaneous firing activity of dopamine (DA) neurons in the VTA (Foddai, Dosia, Spiga, & Diana, 2004) and ERK phosphorylation in the nucleus accumbens (NAc) shell (Vinci et al., 2010), as well as ethanol-induced acquisition of conditioned place preference (Peana et al., 2008). Furthermore, ethanol favors DA release in the NAc (McBride, Le, & Noronha, 2002; Quertemont & Didone, 2006), an effect that was prevented by CAT inhibition (Diana et al., 2008; Melis, Enrico, Peana, & Diana, 2007). On the other hand, ACD's aversive effects are the basis of the first pharmacological approaches (including drugs such as disulfiram and cyanamide -CY) that prevent alcohol consumption by peripheral ACD accumulation resulting from ALDH inhibition, a strategy that is associated with symptoms that discourage the individual from further consumption (Koppaka et al., 2012). Cyanamide (approved by the European Medicine Agency -EMA- as calcium carbimide: Temposil®) potently inhibits liver ALDH, but is less effective against the brain enzyme, raising questions on whether the drug or its metabolites are able to cross the blood-brain barrier. This drug exerts a preferential ALDH2 inhibition (Crabb, Matsumoto, Chang, & You, 2004) that peaks 1–2 h after drug administration, with 80% restoration of the activity occurring within 24 h, a feature that has limited its clinical use because of the short duration compared to disulfiram (Deitrich, Troxell, & Worth, 1976). The first studies in animal models showed that CY depressed ethanol intake (Sinclair, Lindros, & Terho, 1980) and locomotion, a role ascribed to brain ALDH inhibition, as systemic ACD accumulation was prevented by the concurrent administration of CY plus 4-methyl pyrazole (4-MP, an ADH inhibitor) (Spivak, Aragon, & Amit, 1987). Similarly, ethanol-induced locomotor activity was partially suppressed by peripheral CY administration, while 4-MP reversed (Escarabajal & Aragon, 2002; Tambour, Closon, Tirelli, & Quertemont, 2007), and aminotriazole (AT, a CAT inhibitor) potentiated this inhibition (Sanchis-Segura, Miquel, Correa, & Aragon, 1999). Furthermore, when ethanol was administered into the VTA, it was reported that CY enhanced ethanol-induced locomotion (Martí-Prats et al., 2013). Interestingly, in the single report in which CY has been administered both intracerebroventricularly and systemically in rats that had never consumed ethanol, the lower doses employed enhanced subsequent ethanol intake, regardless of the administration route, a result that the authors ascribed to the endogenously-generated ACD in both, brain and periphery. Intriguingly, the 1.0 mg CY i.c.v. dose "consistently suppressed alcohol intake and the 0.5 mg dose produced mixed effects on the self-selection of alcohol" (Critcher & Myers, 1987).

Thus, on the basis of the demonstrated implication of CAT activity (and brain ACD formation) in the facilitator effect of developmental Pb exposure on ethanol consumption, the present study attempted to unravel the role of ALDH2 in the modulation of ACD accumulation in these animals. To that end, we assessed the interplay between central and peripheral ACD accumulation, given that CY, when systemically administered, would predominantly inhibit liver rather than brain ALDH (Deitrich et al., 1976), while central CY administration would impact directly on brain ACD accumulation. Therefore, to evaluate the two perspectives on ethanol intake and subsequent locomotor activity, CY was administered both centrally and systemically. Moreover, ALDH2 and peroxisomal CAT activities in the liver, whole brain and relevant brain areas were assessed to evidence CY-induced differential enzymatic inactivation in the ethanol intake and stimulant properties of the drug in control and Pb-exposed animals.

2. Material and methods

2.1. Animals

Adult Wistar female rats (250–300 g), bred and raised at the Facultad de Ciencias Químicas vivarium, were mated in a 2/1 index. Pregnant females were housed two per cage and exposed to 220 ppm Pb (0.4 g/l Pb acetate Mallinckrodt, J.T. Baker; Argentina) or filtered tap water (which contains less than 5.0 μg/l Pb) until the pups were weaned at postnatal day 25 (PND 25), when Pb exposure was interrupted. Animals were maintained at 22 °C under a 12 h light/dark cycle, with free access to food (Batistella, Córdoba, Argentina) and water, or the Pb solution. All procedures were handled in accordance with the NIH Guide for the Care and Use of Laboratory Animals, as approved by the Animal Care and Use Committee of the Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Argentina. The number of animals used for each experiment is indicated in the corresponding figures. Studies began in male pups at PND 35, which is considered the periadolescence period in rats, a time of particular vulnerability to drug addiction (Smith, 2003). Only one pup from each litter was used for each experimental condition, as suggested by Maurissen (2010), except for the ethanol intake tests in which two litter-mates were housed in one cage and considered a single experimental subject. This ensured that no isolation-related stress would interfere with voluntary ethanol intake, which may be a confounding factor particularly in juvenile rats, as reviewed in Anacker and Ryabinin (2010).

2.2. Ethanol intake and group conformation

Thirty-day-old pup males were housed two per cage with 2 h/day access (between 9.00 a.m. and 1.00 p.m.) to four tubes, two containing water and the other two increasing concentrations of ethanol according to the following scheme (v/v): days 1–4: 2%; days 5–8: 4%; days 9–12: 6%; days 13–16: 8%, and 10% from day 17

to the end of the experiments. The rationale for providing two instead of only one tube for either water or ethanol was intended to avoid competition for the solutions between the two animals that shared the home cage. On the last 4 days of the test (which in all cases was preceded by 4 days of a baseline 10% ethanol intake, regardless of the drug's route of administration), the animals were injected with: a) saline (*SAL group*); b) cyanamide (25 mg/kg i.p., 30 min before the ethanol free-choice test: *CY i.p. group*); c) vehicle (animals implanted with a cannula in the lateral ventricle and injected with cerebrospinal fluid - see below: *VEH group*), and d) cyanamide (animals implanted with a cannula in the lateral ventricle and i.c.v. injected with CY at a dose of 0.1, 0.2 or 0.3 mg dissolved in vehicle immediately before the ethanol free-choice test: *CY i.c.v. group*). It should be noted that the last intake session was scheduled to last only 1 h in order to evidence maximum ethanol consumption and resultant locomotion or enzymatic activity, given that limited alcohol-access schedules imply greater ethanol intake during the early portion of the drinking session (Becker, 2013). The CY doses were selected on the basis of behavioral data published in rats as well as on pilot studies (data not shown for the i.p. dose and presented as part of the supplementary data for the i.c.v. CY doses). Daily intake was registered and expressed as g ethanol consumed/kg body weight, and as the percentage of ethanol with respect to water. Two additional groups were included as controls: 35 day-old and 63 day-old animals that had not consumed ethanol, labeled as 35d non-ethanol and 63d non-ethanol rats, respectively.

2.3. Locomotor activity

The testing apparatus consisted of eight rectangular cages (30.5 × 19.5 × 46.5 cm) equipped with two parallel infrared photocell beams located 3 cm above the floor. Interruption of either beam resulted in a photocell count. Immediately after the last voluntary ethanol intake session (exceptionally of 1 h in length), subsets of animals from all groups were placed individually in each cage with motor activity counts monitored at 10-min intervals during 60 min under white light in a quiet room. They were thereafter sacrificed to collect samples for blood, liver, and brain CAT activity, and liver and brain ALDH2 activity determinations, as described below. It should be noted that all animals were habituated to the locomotor cages on the day before the experiment by free exploration of the apparatus during 60 min (data not shown).

2.4. Surgery

On day 21 of the above-described ethanol intake protocol, and once animals had been offered 10% ethanol solution for 4 days, rats were anesthetized with Ketamine and Xilazine (in a 2/3 ratio) and mounted into a Stoelting stereotaxic instrument with the incisor bar at −3.3 cm above the interaural line. Afterwards, a cannula (14 mm, 22 gauge stainless steel) was implanted unilaterally in the lateral ventricle (AP -0.9, ML -1.5, DV -3.6) according to the coordinates from Paxinos and Watson (2009). It should be noted that in all cases the placement was visually checked with a magnifier right after decapitation and before the tissue was dissected for enzymatic determination. Nevertheless, in a separate group of animals the cannula placement was checked in slices that were stained with Cresyl Violet (data not shown). The cannula was secured in place with two stainless steel screws tapped into the skull and dental cement. On the day of surgery, ethanol consumption was interrupted to allow the animals to drink water *ad libitum;* they were also s.c. injected with neocilin (penicillin/streptomycin), a wide-spectrum antibiotic to prevent infections. The day after, the water was removed, to resume the 2-h free-choice ethanol/water test the following day for another four days; the brain CY administration protocol was then applied as explained below.

2.5. Microinjection procedure

Once assured that ethanol intake was restored to pre-surgery levels during 4 days, a subset of animals were i.c.v. microinfused with either vehicle or CY for an additional 4 days as follows: immediately prior to each daily free-choice test session, the obturators were removed from the guide cannulae and replaced by an injection needle (30-gauge stainless steel) that extended 2.5 mm below the tip of the guide cannula into the lateral ventricle. Unilateral infusions (0.1, 0.2 or 0.3 mg CY) were performed daily at a rate of 1 μl/min (total volume: 2.5 μl) delivered by a pump (Harvard Apparatus model #22, Holliston, MA). Sixty seconds later, the injector was removed and the rat re-introduced into the home cage for the ethanol intake test.

2.6. Liver, and brain ALDH2 activity determinations

ALDH2 activity was measured in all the groups defined in the previous sections (except in the 0.2 and 0.1 mg CY i.c.v. non-ethanol animals) according to Gill, Amit, and Smith (1996), and Escrig, Pardo, Aragon, and Correa (2012) with slight modifications. For the systemic CY administration studies, the enzymatic activity was determined in liver and brain (either whole brain or tissue, which included the cerebellum, frontal cortex, hippocampus, striatum, hypothalamus, and nucleus accumbens) whereas, for central CY administration, ALDH2 activity was determined in the whole brain and the regions mentioned above. It should be noted that, due to substrate affinity and CY preferential inhibition (Crabb et al., 2004), ALDH2 rather than ALDH1 was assessed; all determinations were performed in mitochondrial homogenates obtained by a differential centrifugation method. Results are expressed as nmol NADH/min/mg protein ($\epsilon_{NADH\ 340\ nm} = 6.31\ mmol^{-1}\ mm^{-1}$).

2.7. Blood, liver, and brain CAT activity determinations

CAT activity was measured in all the groups defined in the previous sections (except in the 0.2 and 0.1 mg CY i.c.v. non-ethanol animals) by Aebi's method (Aebi, 1984). Thus, to determine the erythrocyte CAT activity, heparinized whole blood was collected by cardiac puncture from ether-anesthetized rats. Samples were first used to assess Hb levels by Drabkin's method (Drabkin & Austin, 1935); subsequently they were centrifuged at 5739 *g*, red cells washed, hemolyzed, and diluted. Brain CAT activity was measured either in whole brain or in the areas mentioned above. Although rats were not perfused, no blood contamination was found in brain tissue, as determined by nondetectable hemoglobin (Hb) content in all brain regions assayed (data not shown). Blood and brain resultant CAT activity was expressed as μmol H_2O_2 decomposed/min/g hemoglobin or μmol H_2O_2 decomposed/min/mg protein, as applicable ($\epsilon_{H2O2\ 240\ nm} = 0.0394\ mmol^{-1}cm^{-1}$).

2.8. Drugs

Ethanol solutions were prepared fresh daily from a stock of 96% redistilled ethanol (Porta Hnos., Córdoba, Argentina), dissolved in filtered water to achieve the different concentrations administered during the free-choice paradigm (2–10% ethanol v/v).

Cyanamide (Sigma-Aldrich, Argentina) preparation: for systemic injections CY was dissolved in 0.9% saline and injected at dose of 25 mg/kg i.p., 30 min before the free-choice test, during the last 4 days of the experiment. For i.c.v. injections, a solution

containing 0.1, 0.2 or 0.3 mg/ml CY was prepared aseptically, dissolved in standard Krebs solution saturated with 95% O_2 and 5% CO_2 and administered immediately before the last 4 free-choice sessions of the experiment.

3. Statistical analysis

To facilitate statistical analysis, daily ethanol intake and ethanol preference data was collapsed into the four days that belong to the same ethanol concentration. Thus, the average for the 2%, 4%, 6%, 8% and 10% ethanol concentrations (test days 1–24) was analyzed by a two-way repeated measures ANOVA, contrasting the group (C *vs.* Pb) against the time/ethanol concentration as the repeated variable. Once chronic CY pretreatment was in effect, data was analyzed by a two-way ANOVA by comparisons between group (C *vs.* Pb) and drug (SAL *vs.* CY i.p. or VEH *vs.* CY i.c.v., as applicable). Locomotor activity counts were analyzed in separate analyses according to ethanol consumption (ethanol or non-ethanol) in 10 min blocks by a three-way repeated measures ANOVA contrasting group, drug, and time as the repeated factor. Total accumulated counts were analyzed by a two-way ANOVA (group x drug). Blood and tissue CAT and tissue ALDH2 activity were analyzed by a two-way ANOVA: group x drug according to the drug dose and ethanol consumption, with each brain region compared in a separated two-way ANOVA analysis. In all cases, when a significant interaction was found, a Tukey's test was performed as a *post hoc* analysis, with resulting *p* values indicated in the corresponding figure's legends.

4. Results

4.1. Systemic CY

Ethanol intake: Fig. 1A depicts voluntary ethanol intake and Fig. 1B ethanol preference in basal conditions (test days 1–24), and after systemic CY administration (25 mg/kg i.p.; days 25–28) across time/ethanol concentrations. As previously reported, perinatal Pb exposure increased baseline voluntary ethanol intake, an effect that emerged early as a trend, reaching statistical significance at both the 8% and the 10% ethanol concentrations (group: $F[1240] = 48.92$, $p < 0.001$, time: $F[5240] = 29.60$, $p < 0.001$, and group × time interaction: $F[5, 240] = 4.46$, $p < 0.001$). Interestingly, CY administration reduced ethanol consumption selectively in the Pb-exposed rats, showing a significant group: $F[1,46] = 38.63$, $p < 0.001$, drug: $F[1,46] = 22.32$, $p < 0.001$, and group x drug effect: $F[1,46] = 21.95$, $p < 0.001$. Similar results were obtained for baseline ethanol preference (with group: $F[1240] = 32.86$, $p < 0.001$ and time: $F[5240] = 43.90$, $p < 0.001$ significant effects). Moreover, after CY administration, statistical significance was found in all variables: group: $F[1,46] = 28.18$, $p < 0.001$, drug: $F[1,46] = 9.99$, $p < 0.01$, and the interaction between both: $F[1,46] = 16.58$, $p < 0.001$. No differences in body weight were observed among the groups (data not shown).

Ethanol-induced locomotor activity: Locomotor activity, assessed immediately after the free-choice test in response to SAL or CY administration, is plotted in Fig. 1C, while the corresponding non-ethanol data is presented in Fig. 1D. Replicating previous results, enhanced locomotor activity deriving from the ethanol ingested in the preceding intake session was observed selectively in the Pb-exposed animals. Interestingly, similar to the effect of the drug in the ethanol free-choice test, CY pretreatment was able to prevent this increase in the Pb-group, with no effect in the control animals. The ANOVA results for the 10-min bins analysis showed a group x drug × time interaction: $F[5330] = 2.53$, $p < 0.05$, and group: $F[1330] = 20.08$, $p < 0.001$, drug: $F[1330] = 37.03$, $p < 0.001$ and time: $F[5330] = 144.27$, $p < 0.001$ effects, as well as a group x drug: $F[1330] = 20.61$, $p < 0.001$ and a group x time: $F[5330] = 3.86$, $p < 0.01$ interaction. Total counts analysis revealed a group: $F[1,66] = 20.08$, $p < 0.001$ and drug $F[1,66] = 27.03$, $p < 0.001$ effect, and group × drug interaction $F[1,66] = 20.61$, $p < 0.001$. In contrast, in animals that had not consumed ethanol, the ANOVA for the 10-min bins revealed significant group x drug x time: $F[5250] = 2.77$, $p < 0.05$, and group x time: $F[5250] = 3.82$, $p < 0.01$ interactions, as well as time: $F[5250] = 53.52$, $p < 0.001$, and drug: $F[1250] = 9.43$, $p < 0.01$ effects. Total counts analysis showed only a drug effect at $F[1,50] = 9.42$, $p < 0.01$.

4.2. Intracerebral 0.3 mg CY

Ethanol intake: Fig. 2A and B represent ethanol intake and preference respectively, in Pb-exposed and control animals submitted to the voluntary ethanol scheme that were microinfused with CY (0.3 mg i.c.v.). Based on the higher manifestations of the effects, plotted data stands for the CY 0.3 mg dose, which was selected out of the three CY doses tested (results for the 0.1 and 0.2 mg are presented as supplementary data and in Fig. S1). As before, Pb-exposed rats voluntarily consumed more ethanol than their control counterparts (group: $F[1200] = 42.68$, $p < 0.001$ and time: $F[5200] = 22.19$, $p < 0.001$ effects, and a group × time interaction: $F[5200] = 2.92$, $p < 0.05$), an effect that was evident early in the experiment, but only reached statistical significance at the 8% ethanol concentration (see the corresponding figure legend for statistical details). Interestingly, CY administration resulted in a statistical difference in the group: $F[1,38] = 37.50$, $p < 0.001$ and drug: $F[1,38] = 56.72$, $p < 0.001$ variables, although a non-significant interaction among these was found, ascribed to the fact that both groups increased their ethanol intake. On the other hand, a significant interaction was found when ethanol preference was assessed in both basal conditions (group: $F[1200] = 50.14$, $p < 0.001$, time: $F[5200] = 31.23$, $p < 0.001$, and group x time: $F[5200] = 2.83$, $p < 0.05$) and after CY administration (group: $F[1,38] = 18.01$, $p < 0.001$, drug: $F[1,38] = 48.92$, $p < 0.001$ and group x drug: $F[1,38] = 29.05$, $p < 0.001$).

Ethanol-induced locomotor activity: As before, the Pb-exposed group showed increased locomotor activity in relation to controls. Interestingly, prior brain CY administration elicited a significant increase in locomotor activity counts in the control group (Fig. 2C), while no further increase in locomotion was evident in the Pb-exposed animals in spite of their trend towards heightened ethanol intake resulting from brain CY administration. Thus, in assessing the 10-min bins, group: $F[1310] = 4.45$, $p < 0.05$ and time: $F[5310] = 141.28$, $p < 0.001$ effects emerged as well as group x drug: $F[1310] = 5.59$, $p < 0.05$, time x group: $F[5310] = 5.30$, $p < 0.001$, time x drug: $F[5310] = 8.87$, $p < 0.001$, and group x drug x time: $F[5310] = 2.90$, $p < 0.05$ interactions. Regarding the total counts, there was a group effect: $F[1,62] = 4.45$, $p < 0.05$, and group × drug interaction $F[1,62] = 5.50$, $p < 0.05$. As expected, no significant differences were present in 10-min bins or total locomotor count animals that had not been submitted to the ethanol/water free-choice test (Fig. 2D).

4.3. ALDH2 activity

Liver ALDH2 measured in 35 day-old rats (i.e. before the free-choice test scheme) showed no differences between both groups (C = 0.59 ± 0.10; Pb-exposed = 0.41 ± 0.10 nmol NADH/min/mg protein; n = 5 for each condition). Regarding the adult animals, baseline liver ALDH2 activity levels were comparable in the control and Pb-exposed groups, as the respective two-way ANOVA failed to evidence a significant group effect, either in animals that had consumed ethanol (Fig. 3A) or in their non-ethanol counterparts

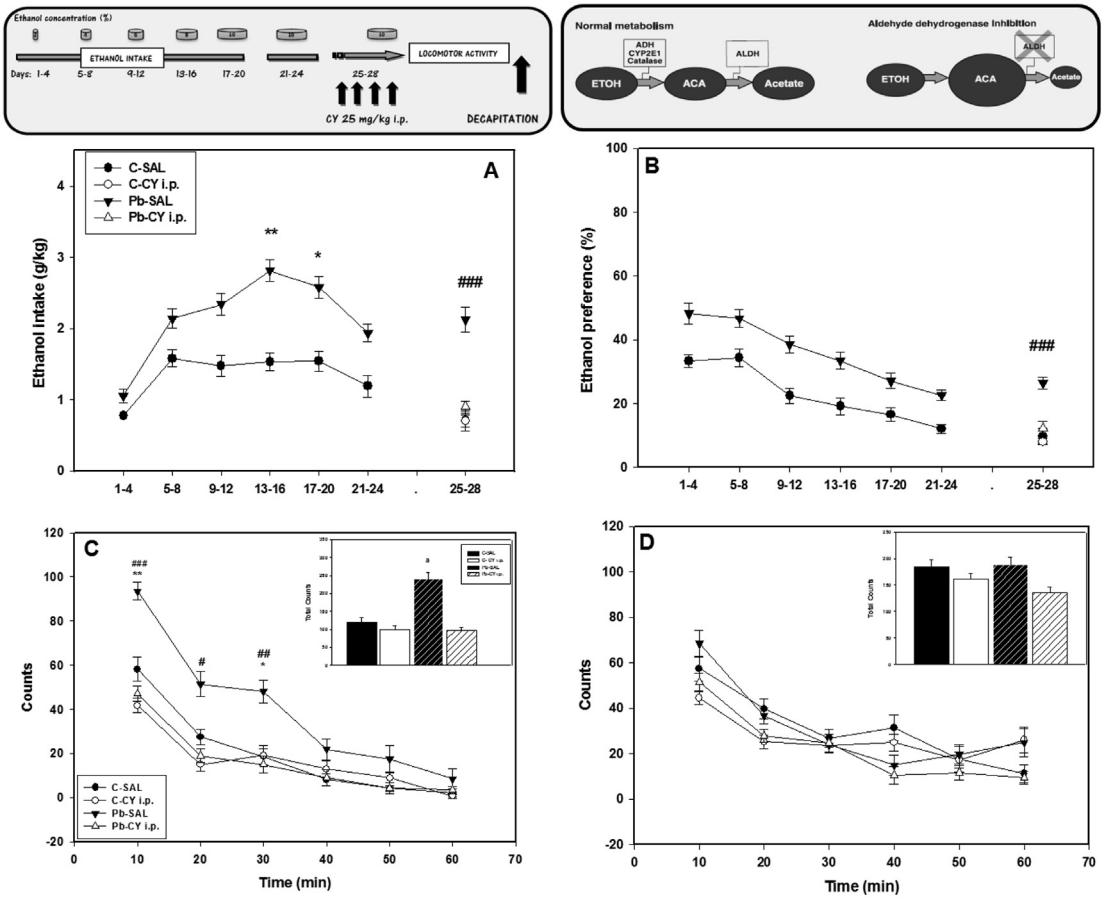

Fig. 1. Behavioral responses to systemic CY (25 mg/kg i.p.) administration. The upper left panel corresponds to the protocol employed in this study with increasing ethanol concentrations over time represented in cylinders, while the upper right panel depicts normal (left) and ALDH inhibited (right) ethanol metabolism *(adapted from* Quertemont & Didone, 2006*)*. C = control; Pb= Pb-exposed; SAL = saline; CY i.p. = systemic cyanamide. **A-B. Voluntary ethanol intake expressed as g ethanol/kg body weight (A) and percentage of preference (B)**. Data (mean ± SE) grouped in 4-day blocks along the horizontal axis correspond to ethanol intake in response to increasing ethanol concentrations (days 1–4: 2%; days 5–8: 4%; days 9–12: 6%; days 13–16: 8%, and days 17–24: 10%). *Baseline: *denotes significant differences compared to C at *p < 0.05 and **p < 0.01. CY administration: #indicates differences between the Pb-SAL and the Pb-CY groups at ###p < 0.001.* C-SAL = 13; C-CY i.p. = 10; Pb-SAL = 14; Pb-CY i.p. = 13 animals per group. The arrows indicate CY administration. **C-D. Locomotor activity assessed immediately after the ethanol/water free choice test in ethanol (C) and non-ethanol (D) animals**. **indicates differences between the C and the Pb groups injected with SAL at *p < 0.05; **p < 0.01. #denotes differences between the Pb-SAL and the Pb-CY groups at #p < 0.05; ##p < 0.01, and ###p < 0.001. Insert: the letter "a" indicates a significant difference of the Pb-SAL animals compared to all other groups at p < 0.001.* Total animals in the ethanol group: C-SAL = 17; C-CY i.p. = 16; Pb-SAL = 18; Pb-CY i.p. = 19; and in the non-ethanol group: C-SAL = 11; C-CY i.p. = 13; Pb-SAL = 12; Pb-CY i.p. = 18.

(Fig. 3B). However, as expected, systemic CY administration (25 mg/kg i.p.) abruptly reduced liver ALDH2 activity in both control and Pb-exposed animals, regardless of ethanol consumption (drug effect: $F[1,27] = 13.08$, $p < 0.01$ for the ethanol, and $F[1,17] = 24.46$, $p < 0.001$ for the non-ethanol animals). On the other hand, data revealed that, after ethanol intake, perinatal Pb-exposure alone reduced brain ALDH2 activity in comparison to controls (group effect for the animals that had consumed ethanol: $F[1,15] = 13.92$, $p < 0.01$), while this inhibition was absent in both groups when CY was systemically administered in animals with or without previous ethanol intake (Fig. 3C&D, respectively). Interestingly, brain ALDH2 activity in response to i.c.v. CY administration showed a dramatic inhibition only in the control animals that had consumed ethanol (group × drug interaction: $F[1,13] = 24.55$, $p < 0.001$; Fig. 3E) whereas no differences emerged in the non-ethanol group (Fig. 3F). This may be related to the fact that the Pb-exposed animals injected with vehicle showed lower ALDH2 activity than the controls only as a consequence of ethanol intake, replicating results of Fig. 3C. Regarding ALDH2 activity measured in brain regions, data from all the experimental conditions assessed in this study is depicted in Table 1. The absence of values in some regions is due to the fact that many brains were required to be pooled to generate detectable enzymatic levels (i.e. up to 10 brains for the NAc ALDH activity). The statistical analysis revealed that systemic CY administration elicited a significant effect of the drug parameter: $F[1,17] = 4.49$, $p < 0.05$ only in the cerebellum, although the *post hoc* Tukey's test showed no differences among the groups that had consumed ethanol. In the case of their non-ethanol counterparts, it was also the cerebellum which showed significant differences in all parameters: group: $F[1,14] = 9.40$, $p < 0.01$, drug: $F[1,14] = 18.78$, $p < 0.001$, and group x drug: $F[1,14] = 9.42$, $p < 0.01$, product of a $p < 0.01$ difference between the Pb-SAL group and all other groups. Table 1 also shows data from ALDH2 activity in response to 0.3 mg CY microinfused in the brain of animals that had consumed ethanol and their non-ethanol counterparts. Although all four CY doses (0, 0.1, 0.2, and 0.3 mg i.c.v.) were analyzed, only ALDH2 activity for the vehicle and the 0.3 mg dose are shown here, while the 0.1 and 0.2 mg CY data are included in Figs. S2B and S3B, respectively. No significant differences emerged at the 0.3 mg dose analysis for the ethanol animals. In the case of the non-ethanol animals, significant effects resulting from the 0.3 mg dose were found in two areas (a drug effect: $F[1,19] = 20.30$, $p < 0.001$ for the cerebellum, and group: F

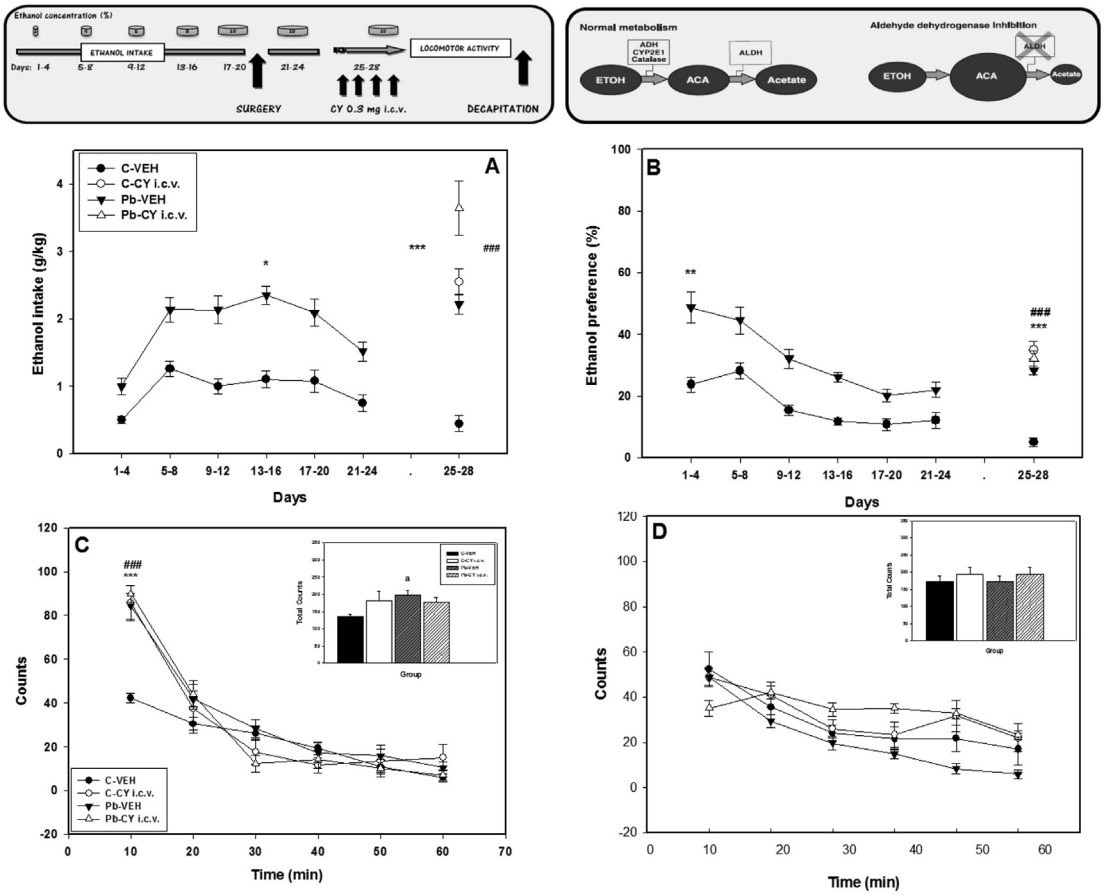

Fig. 2. Behavioral responses to central CY (0.3 mg i.c.v.) administration. The upper left panel corresponds to the protocol employed in this study with increasing ethanol concentrations over time represented in cylinders, while the upper right panel depicts normal (left) and ALDH inhibited (right) ethanol metabolism *(adapted from* Quertemont & Didone, 2006*)*. C = control; Pb=Pb-exposed; VEH = vehicle; CY i.c.v. = intracerebroventricular cyanamide. **A-B. Voluntary ethanol intake expressed as g ethanol/kg body weight (A) and percentage of preference (B)**. Data (mean ± SE) grouped in 4-day blocks along the horizontal axis correspond to ethanol intake in response to increasing ethanol concentrations (days 1–4: 2%; days 5–8: 4%; days 9–12: 6%; days 13–16: 8%, and days 17–24: 10%). *Baseline: *denotes differences compared to controls at *p < 0.05 and **p < 0.01. CY administration: *denotes differences between the C and Pb-exposed animals injected with VEH at ***p < 0.001; #denotes differences between the VEH and corresponding CY groups for both C and Pb-exposed animals at ###p < 0.001.* C-VEH = 11; C-CY i.c.v. = 14; Pb-VEH = 8; Pb-CY i.c.v. = 8 animals per group. The arrows indicate CY administration. **C-D. Locomotor activity assessed immediately after the ethanol/water free choice test in ethanol (C) and non-ethanol (D) animals**. **indicates differences between the C and the Pb group injected with VEH at ***p < 0.001. #indicates differences between the C-VEH and the C-CY groups at ###p < 0.001. Insert: the letter "a" indicates differences between the C group injected with VEH and all other groups at **p < 0.01.* Total animals in the ethanol group: C-VEH = 22; C-CY i.c.v. = 10; Pb-VEH = 20; Pb-CY i.c.v. = 14; and in the non-ethanol group: C-VEH = 9; C-CY i.c.v. = 9; Pb-VEH = 19; Pb-CY i.c.v. = 7.

[1,1] = 4.92, $p < 0.05$ and drug: F[1,13] = 6.49, $p < 0.05$ effects for the hippocampus), although the Tukey's test revealed only a drug statistical significance for the hippocampus ($p < 0.05$).

4.4. CAT activity

Fig. 4A represents blood CAT activity for the rats submitted to the ethanol intake protocol, while Fig. 4B depicts their non-ethanol counterparts' data. Reproducing previous results (Mattalloni et al., 2013), a significant effect of the group variable F[1,32] = 56.18, $p < 0.001$ revealed a higher blood CAT activity in the Pb-treated animals (in this case regardless of the drug treatment), while no differences emerged in the non-ethanol groups in any condition. In contrast, in the liver, although a significant group × drug interaction F[1,15] = 5.51, $p < 0.05$ was evident in the ethanol animals (Fig. 4C), no effect in CAT activity emerged in the Tukey's test; similarly, no differences were present in the non-ethanol animals (Fig. 4D). Furthermore, from the observation of Fig. 4E&F, it can be inferred that brain CAT activity was not modified as a result of a systemic CY injection, while the same is evident after 0.3 mg CY i.c.v. (Fig. 4G&H), regardless of the animal's history of ethanol consumption. Regional brain CAT activity data resulting from peripheral or central (0.3 mg i.c.v.) CY administration is presented in Table S1 (results for 0.1 and 0.2 mg CY i.c.v. are plotted in Figs. S2D and S3D, respectively). No differences were evident in any area in animals submitted to the ethanol intake protocol. However, significant differences emerged in animals that had not consumed ethanol in the NAc (drug: F[1,11] = 7.42, $p < 0.05$) and in the frontal cortex (group: F[1,13] = 5.02, $p < 0.05$, and drug: F[1,13] = 5.47, $p < 0.05$). Additionally, Table S1 shows brain region CAT activity for the animals microinfused with CY into the lateral ventricle that had or had not consumed ethanol. The statistical analysis revealed a drug effect: F[3,51] = 24.37, $p < 0.001$, and group × drug interaction: F[3,51] = 8.58, $p < 0.001$ in the NAc, as well as a drug effect in the striatum (F[3,49] = 3.56, $p < 0.05$), the cerebellum (F[3,48] = 3.73, $p < 0.05$), and in the frontal cortex (F[3,52] = 16.84, $p < 0.001$) in the ethanol animals while, in the hypothalamus, differences in the group: F[1,24] = 30.64, $p < 0.001$ and drug: F[3,24] = 7.85, $p < 0.001$ variables, and in their interaction: F[3,24] = 5.60, $p < 0.01$ were evident. In the case of their non-ethanol counterparts, the interaction group x drug (F[1,18] = 11.75, $p < 0.01$) was found in the striatum, with a significant

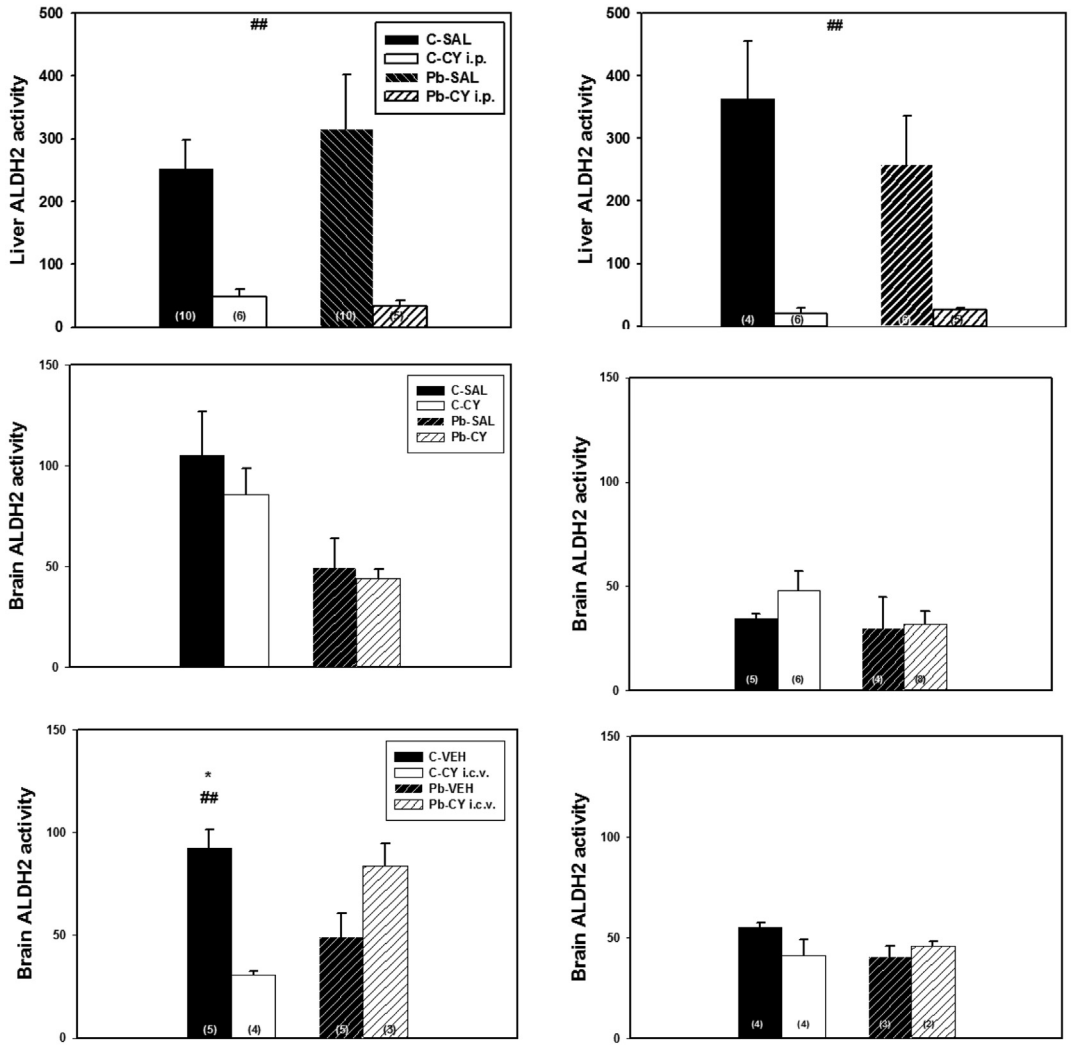

Fig. 3. ALDH2 activity in response to CY administration in animals that have consumed ethanol (left panels) and their non-ethanol counterparts (right panels). C = control; Pb= Pb-exposed; SAL = saline; CY i.p. = systemic cyanamide; VEH = vehicle; CY i.c.v. = intracerebroventricular cyanamide. The number of animals per group is indicated between parentheses. **A-B. Liver and C-D whole brain ALDH2 activity in response to systemic CY (25 mg/kg i.p.).** ***Liver***: *$^{\#}$denotes a significant effect of the drug variable (SAL vs. CY at $^{\#\#}p < 0.01$) with no statistical difference between C and Pb-exposed animals for both the ethanol and non-ethanol conditions.* ***Brain***: **indicates differences between the C and the Pb groups at $^{**}p < 0.01$.* **E-F. whole brain ALDH2 activity in response to central CY (0.3 mg i.c.v.) administration.** ***Brain***: **indicates differences between the C and the Pb group injected with VEH at $^{*}p < 0.05$; $^{\#}$indicates differences between the C-VEH and the C-CY groups at $^{\#\#}p < 0.01$.*

effect of the drug parameter in several areas ($F[1,21] = 13.07$, $p < 0.01$ for the cerebellum, $F[1,18] = 15.97$, $p < 0.001$ for the striatum, $F[1,18] = 4.94$, $p < 0.05$ for the hippocampus, and $F[1,21] = 10.94$, $p < 0.01$ for the hypothalamus. In the cases in which the Tukey's test revealed significant differences, they are indicated in the table.

5. Discussion

The present study provides evidence of opposing behavioral consequences of peripheral and central ALDH2 inhibition (and thereby ACD accumulation) in control and Pb-exposed animals. The results demonstrate that liver (not brain) ALDH2 activity was completely inhibited in both groups as a consequence of systemic CY administration. However, at the behavioral level, only the elevated ethanol intake and hyperlocomotion previously described in the Pb-exposed group (Mattalloni et al., 2013) was reversed by CY. In contrast, central ALDH2 inhibition augmented ethanol intake in both groups, while subsequent locomotor activity was increased only in control animals, both events in the absence of brain ALDH2 inhibition in the Pb-exposed group. In addition, the lack of effects on CAT activity (Fig. 4) revealed that the CY effect was restricted to ALDH2 inhibition.

It is well known that peripheral ACD accumulation is a deterrent for alcohol consumption. Animal models using genetic manipulations demonstrated that an ALDH antisense (Garver et al., 2001; Ocaranza et al., 2008) or an adenoviral vector that encoded for a mutated high-activity ADH either alone or in combination with an inactive ALDH2 (ALDH2*2) (Rivera-Meza et al., 2010, 2012; respectively) reduced ethanol intake. Furthermore, although our results match reports of a reduction in ethanol-induced locomotor activity by peripheral CY administration in high ethanol-consuming animals (Escarabajal & Aragon, 2002; Sanchis-Segura et al., 1999; Tambour et al., 2007), they contrast with those of Critcher and Myers (1987), who reported an enhancement in ethanol intake in ethanol-*naive* animals. While, in our study, CY was administered 30 min *before* the chronic ethanol intake test, in Critcher's, the drug effect was evaluated on *subsequent* ethanol preference in ethanol-

Table 1
Brain ALDH activity expressed as nmol NADH/min/mg protein. * *In the cerebellum: of the non-ethanol Pb-SAL group is significantly different to all other groups ($p < 0.01$).* #*In the hippocampus: the non-ethanol C and Pb-VEH-injected animals are significantly different from the i.c.v.CY-injected group ($p < 0.05$).* C = control; Pb= Pb-exposed; SAL = saline; CY i.p. = systemic cyanamide; VEH = vehicle; CY i.c.v. = intracerebroventricular cyanamide; EOTH = ethanol; non-ETOH = non-ethanol. Between parentheses is indicated the number of animals per group.

		ETOH				non ETOH			
		SAL	CY i.p.	VEH	CY i.c.v.	SAL	CY i.p.	VEH	CY i.c.v.
Cerebellum	C	17.55 ± 2.59(6)	3.41 ± 1.18(4)	10.74 ± 2.27(8)	9.58 ± 2.74(6)	14.18 ± 4.22(4)	6.11 ± 3.22(4)	18.74 ± 1.77(4)	5.13 ± 1.37(10)
	Pb	16.12 ± 6.84(4)	9.23 ± 0.98(5)	7.73 ± 0.72(6)	13.70 ± 6.88(3)	29.72 ± 9.10(2)*	6.10 ± 0.90(5)	16.78 ± 5.43(4)	8.10 ± 0.89(5)
Frontal Cortex	C	7.94 ± 5.38(4)	14.21 ± 4.84(4)	6.91 ± 4.91(3)	0.00 ± ----(1)	2.20 ± 1.57(4)	26.18 ± 1.63(2)	13.69 ± 3.25(2)	22.76 ± 0.00(1)
	Pb	7.73 ± 3.50(2)	9.29 ± 4.58(5)	5.10 ± 3.30(2)	6.62 ± 0.77(2)	12.47 ± 6.50(4)	4.31 ± 2.25(4)	2.73 ± ----(1)	6.06 ± ----(1)
Hippocampus	C	5.41 ± 0.89(6)	9.74 ± 2.00(5)	3.24 ± 0.94(5)	2.40 ± 1.03(5)	9.59 ± 2.70(5)	6.78 ± 2.70(4)	7.81 ± 7.81(3)#	5.37 ± 1.88(5)
	Pb	12.39 ± 3.54(5)	11.32 ± 3.06(7)	4.66 ± 1.43(5)	6.81 ± 1.06(5)	13.98 ± 4.50(8)	2.66 ± 1.20(6)	27.06 ± 8.45(3)#	6.32 ± 2.43(6)
Striatum	C	9.27 ± 5.06(3)	3.34 ± 2.00(3)	4.28 ± 2.14(3)	8.74 ± ----(1)	4.02 ± ----(1)	2.73 ± 1.38(4)	5.21 ± 2.53(2)	11.52 ± 2.06(2)
	Pb	6.77 ± 2.57(3)	6.83 ± 1.54(3)	4.14 ± 2.51(3)	19.97 ± ----(1)	0.00 ± ----(1)	6.31 ± 2.52(4)	2.47 ± ----(1)	8.60 ± ----(1)
Hypothalamus	C	0.00 ± 0.00(3)	6.26 ± 3.00(2)	9.11 ± 3.76(2)	7.38 ± 1.95(3)	14.95 ± 6.93(5)	1.18 ± 0.71(3)	---- ± ----(0)	5.68 ± 1.64(2)
	Pb	12.56 ± 5.94(5)	8.77 ± 6.43(4)	0.00 ± 0.002)	16.70 ± 0.21(2)	20.21 ± 5.44(2)	4.93 ± 3.15(3)	---- ± ----(0)	5.39 ± ----(1)
N. Accumbens	C	16.18 ± 12.00(3)	6.38 ± 1.60(3)	13.67 ± 8.34(2)	3.31 ± ----(1)	8.68 ± ----(1)	9.28 ± 3.53(2)	5.20 ± 2.61(2)	---- ± ----(0)
	Pb	10.24 ± 6.99(3)	24.46 ± 7.63(3)	3.84 ± 0.12(2)	5.78 ± 1.18(2)	2.78 ± 0.66(3)	8.57 ± 3.00(3)	14.76 ± 9.85(2)	---- ± ----(0)

naive animals. It has been reported that CY requires CAT and H_2O_2 to be bioactive by converting itself to HNO; therefore, when incubated together, the peroxidatic activity of CAT toward alcohols can be inhibited by CY, an effect that is prevented when ethanol was added before or at the same time as CY (Cederbaum & Dicker, 1985; DeMaster, Shirota, & Nagasawa, 1984). According to these reports, our protocol design would prevent CY-induced CAT inhibition, as is also demonstrated by the absence of CY effects on CAT activity (Fig. 4). The lack of Pb effects in our experimental conditions on whole brain CAT activity that have been reported by others (Correa, Miquel, Sanchis-Segura, & Aragon, 1999a, 2000) may respond to a compensatory effect due to differential region-specific activity (Mattalloni et al., 2013). In addition, given that the brains were not perfused previous to enzymatic determinations, contamination of the neural tissue with blood, even in the absence of hemoglobin content in the tissue should be considered. We thus propose that the aversive consequences of peripheral ACD accumulation resulting from systemic ALDH2 inhibition (Figs. 1A and 3A) overcome its central reinforcing effects, leading to the drastic reduction of ethanol intake in Pb-exposed animals, providing predictive validity to this model. The absence of such an effect in control animals despite ALDH2 inhibition may respond to their minimal ethanol intake and consequently low peripheral ACD accumulation, thus preventing the manifestation of its aversive effects.

It has been reported that brain (rather than liver) ALDH2 activity closely correlates with ethanol preference (Amir, 1978), adding further support to the concepts stated in the introduction that ACD is a centrally-active metabolite involved in the positive reinforcing properties of ethanol (Aragon & Amit, 1985; Quertemont et al., 2005). We thus assume that either CAT overactivation (Mattalloni et al., 2013) or ALDH2 inactivation (this study) will increase brain ACD, promoting its accumulation and eliciting enhanced ethanol intake and hyperlocomotion. We report here that control animals showed a dramatic increase in ethanol intake and subsequent locomotion associated with brain ALDH2 inhibition. Pb-exposed animals, on the other hand, exhibited an increase in ethanol consumption in the absence of subsequent hyperlocomotion and brain ALDH2 inhibition. This effect can be ascribed to either a deficient CY inhibition, the product of Pb interaction with the thiol derivates resulting from ALDH2 bioactivation (Koppaka et al., 2012), or to a dysfunctional ALDH2. Remarkably, brain ALDH2 activity in Pb-exposed animals that had consumed ethanol was lower than their control counterparts (Fig. 3C&E), an effect not evidenced in the corresponding non-ethanol respective groups (Fig. 3D&F). Given the metalloenzyme nature of ALDH and Pb's ability to compete with essential metals for their insertion sites (Flora, Gautam, & Kushwaha, 2012; Flora, Gupta, & Tiwari, 2012), a competition between Pb^{2+} and Mg^{2+} for the ALDH2 site is a scenario that deserves further study.

On the other hand, it should be noted that most of the discussion above assumed that systemic CY administration primarily affected liver (not brain) ALDH2 activity (Fig. 3A–D), indicating that CY would not cross the blood-brain barrier, and that, under these conditions, brain and liver ACD would be locally formed with compartmentalization-restricted effects. Unquestionably, these assumptions are controversial, making it possible that peripheral ACD at high concentrations can readily cross the blood-brain barrier (Heap et al., 1995; Tabakoff, Anderson, & Ritzmann, 1976), or have been reported to be elevated after CY administration (Jamal et al., 2003; Martí-Prats et al., 2013).

Another aspect that deserves discussion is based on reports from both human populations (Hense, Filipiak, Novak, & Stoeppler,

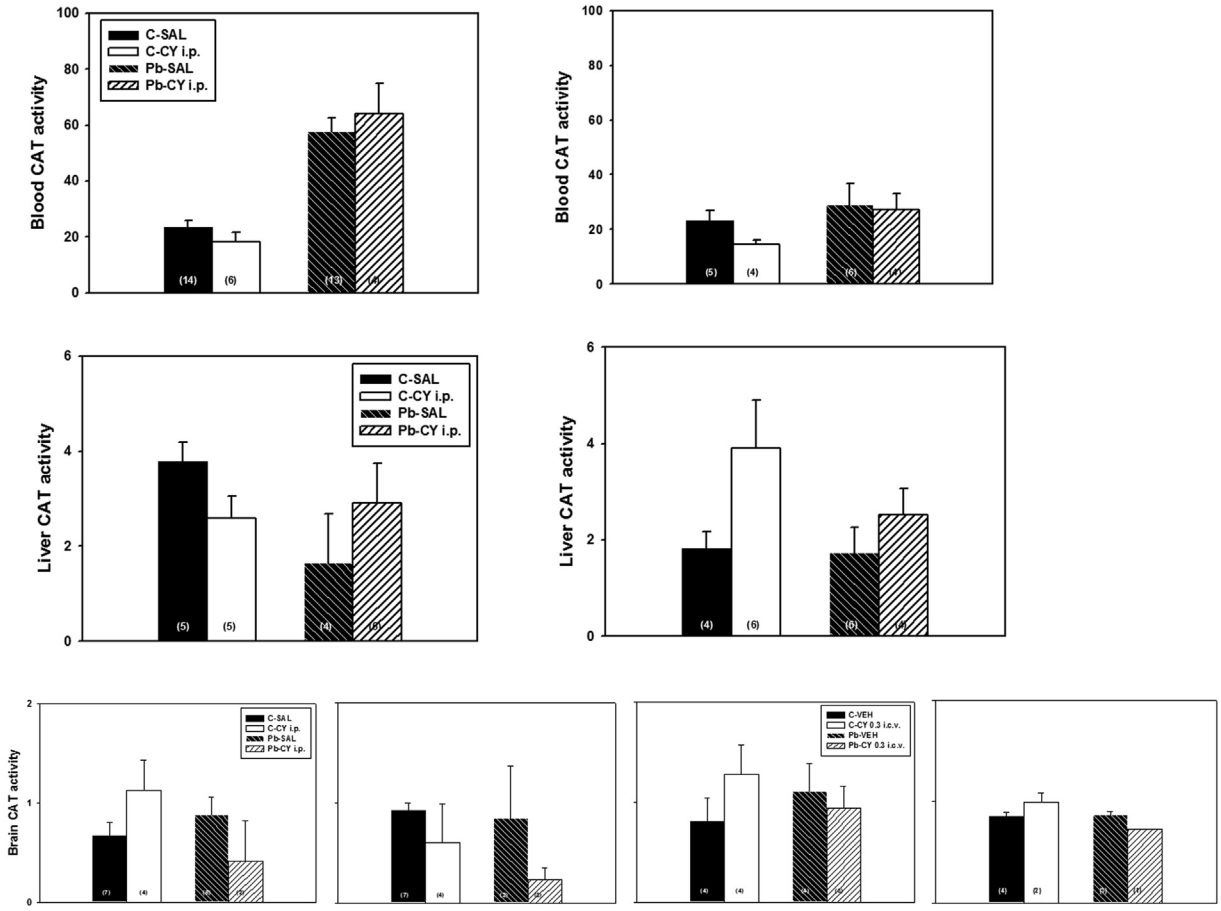

Fig. 4. CAT activity in response to CY administration. C = control; Pb= Pb-exposed; SAL = saline; CY i.p. = systemic cyanamide; VEH = vehicle; CY i.c.v. = intracerebroventricular cyanamide. The number of animals per group is indicated between parentheses. **A-B. Blood, C-D. Liver and E-F whole brain CAT activity in response to systemic CY (25 mg/kg i.p.).** ***Blood***: **denotes differences in the animals that had consumed ethanol between the C and Pb groups regardless of the drug treatment at ***p < 0.001.* ***Liver and Brain***: *no differences emerged among any conditions.* **G-H. whole brain CAT activity in response to central CY (0.3 mg i.c.v.) administration** *no differences were evident among any conditions.*

1992; Lopez et al., 2002; Sharper et al., 1982) and laboratory animals (Flora, Kumar, Sachan, & Das Guota, 1991) indicating that alcohol consumption may increase Pb body burden, making the organism more vulnerable to the neurochemical and neurobehavioral toxicity induced by the metal (Flora & Gautam et al., 2012; Flora & Gupta et al., 2012; Gupta & Gill, 2000). Moreover, both acute and chronic Pb administration have been used as a mechanism to activate CAT, a strategy that has been studied in several ethanol-induced behaviors (Correa et al., 1999a, 1999b, 2000) indicating CAT as a common site of action between Pb and ethanol. Thus, the results presented here, along with those of other authors, provide further evidence of Pb/ethanol synergism, in this case at the level of the enzymes involved in ethanol metabolism leading to brain ACD accumulation, with direct implications in the increased vulnerability of the Pb-exposed animals to acquire ethanol-addiction-related behaviors. A major question that remains to be elucidated is whether the relationship between both neurotoxicants is the result of their direct interaction in the brain (as Pb toxicokinetics reveals that, at the time of the behavioral studies, the metal is present in the organism (Flora et al., 1991), or the consequence of a neurological imprint resulting from developmental Pb exposure that may appear later in life. As Pb can affect the blood-brain barrier (Bressler & Goldstein, 1991), the answer would have important implications for Pb-related industrial workers that may also show concomitant excessive alcohol consumption, or for Pb-impregnated pregnant women that may give birth to children with an innate vulnerability to develop alcohol-related disorders.

In summary, these results highlight the importance of considering ethanol metabolism in its motivational and stimulant effects when the interplay between peripheral (aversive) and centrally-formed (positive reinforcing) ACD accumulation is under study. They also evidence the modulation that Pb exerts on these effects as a developmental neurotoxicant.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.alcohol.2016.11.002.

References

Aebi, H. (1984). Catalase in vitro. *Methods in Enzymology, 105*, 121–126.

Amir, S. (1978). Brain and liver aldehyde dehydrogenase activity and voluntary ethanol consumption by rats: Relations to strain, sex, and age. *Psychopharmacology (Berl), 57*, 97–102.
Anacker, A. M. J., & Ryabinin, A. E. (2010). Biological contribution to social influences on alcohol Drinking: Evidence from animal models. *International Journal of Environmental Research and Public Health, 7*, 473–493.
Aragon, C. M., & Amit, Z. (1985). A two dimensional model of alcohol consumption: Possible interaction of brain catalase and aldehyde dehydrogenase. *Alcohol, 2*, 357–360.
Becker, H. C. (2013). Animal models of excessive alcohol consumption in rodents. *Current Topics in Behavioral Neuroscience, 13*, 355–377.
Bressler, J. P., & Goldstein, G. W. (1991). Mechanisms of lead neurotoxicity. *Biochemical Pharmacology, 41*, 479–484.
Cederbaum, A. I., & Dicker, E. (1985). Inhibition of the peroxidatic activity of catalase towards alcohols by the aldehyde dehydrogenase inhibitor cyanamide. *Toxicology Letters, 29*, 107–114.
Correa, M., Miquel, M., & Aragon, C. M. G. (2000). Lead acetate potentiates brain catalase activity and enhances ethanol-induced locomotion in mice. *Pharmacology Biochemistry and Behavior, 66*, 137–142.
Correa, M., Miquel, M., Sanchis-Segura, M., & Aragon, C. M. (1999a). Acute lead acetate administration potentiates ethanol-induced locomotor activity in mice: The role of brain catalase. *Alcoholism Clinical and Experimental Research, 23*, 799–805.
Correa, M., Miquel, M., Sanchis-Segura, M., & Aragon, C. M. G. (1999b). Effects of chronic lead administration on ethanol-induced locomotor and brain catalase activity. *Alcohol, 19*, 43–49.
Correa, M., Salamone, J., Segovia, K., Pardo, M., Longoni, R., Spina, L., et al. (2012). Piecing together the puzzle of acetaldehyde as a neuroactive agent. *Neuroscience and Biobehavioral Reviews, 36*, 404–430.
Crabb, D. W., Matsumoto, M., Chang, D., & You, M. (2004). Overview of the role of alcohol dehydrogenase and aldehyde dehydrogenase and their variants in the genesis of alcohol-related pathology. *Proceedings of the Nutrition Society, 63*, 49–63.
Critcher, E. C., & Myers, R. D. (1987). Cyanamide given ICV or systemically to the rat alters subsequent alcohol drinking. *Alcohol, 4*, 347–353.
Deitrich, R. A., Troxell, P. A., & Worth, W. S. (1976). Inhibition of aldehyde dehydrogenase in brain and liver by cyanamide. *Biochemical Pharmacology, 25*, 2733–2737.
DeMaster, E. G., Shirota, F. N., & Nagasawa, H. T. (1984). The metabolic activation of cyanamide to an inhibitor of aldehyde dehydrogenase is catalyzed by catalase. *Biochemical and Biophysical Research Communications, 122*, 358–365.
Diana, M., Peana, A. T., Sirca, D., Lintas, A., Melis, M., & Enrico, P. (2008). Crucial Role of acetaldehyde in alcohol activation of the mesolimbic dopamine system. *Annals of the New York Academy of Sciences, 1139*, 307–317.
Drabkin, D. L., & Austin, J. H. J. (1935). *Biological Chemistry, 112*, 51.
Escarabajal, M. D., & Aragon, C. M. G. (2002). The effect of cyanamide and 4-methylpyrazole on the ethanol-induced locomotor activity in mice. *Pharmacology Biochemistry and Behavior, 72*, 389–395.
Escrig, M. A., Pardo, M., Aragon, C. M., & Correa, M. (2012). Anxiogenic and stress-inducing effects of peripherally administered acetaldehyde in mice: Similarities with the disulfiram-ethanol reaction. *Pharmacology Biochemistry and Behavior, 100*, 404–412.
Flora, S. J. S., Gautam, P., & Kushwaha, P. (2012). Lead and ethanol co-exposure lead to blood oxidative stress and subsequent neuronal apoptosis in rats. *Alcohol and Alcoholism, 47*, 92–101.
Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead: A review with recent updates. *Interdisciplinary Toxicology, 5*, 47–58.
Flora, S. J. S., Kumar, D., Sachan, S. R. S., & Das Guota, S. (1991). Combined exposure to lead and ethanol on tissue concentration of essential metals and some biochemical indices in rat. *Biological Trace Elements Research, 28*, 157–164.
Foddai, M., Dosia, G., Spiga, S., & Diana, M. (2004). Acetaldehyde increases dopaminergic neuronal activity in the VTA. *Neuropsychopharmacology, 29*, 530–536.
Garver, E., Tu, G. C., Cao, Q. N., Aini, M., Zhou, F., & Israel, Y. (2001). Eliciting the low-activity aldehyde dehydrogenase Asian phenotype by an antisense mechanism results in an aversion to ethanol. *The Journal of Experimental Medicine, 194*, 571–580.
Gill, K., Amit, Z., & Smith, B. R. (1996). The regulation of alcohol consumption in rats: The role of alcohol-metabolizing enzymes-catalase and aldehyde dehydrogenase. *Alcohol, 13*, 347–353.
Gupta, V., & Gill, K. D. (2000). Lead and ethanol coexposure: Implications on the dopaminergic system and associated behavioral functions. *Pharmacology Biochemistry and Behavior, 66*, 465–474.
Heap, L., Ward, R. J., Abiaka, C., Dexter, D., Lawlor, M., Pratt, O., et al. (1995). The influence of brain acetaldehyde on oxidative status, dopamine metabolism and visual discrimination task. *Biochemical Pharmacology, 50*, 263–270.
Hense, H. W., Filipiak, B., Novak, L., & Stoeppler, M. (1992). Nonoccupational determinants of blood lead concentrations in a general population. *International Journal of Epidemiology, 21*, 753–762.
Higuchi, S., Matsushita, S., Murayama, M., Takagi, S., & Hayashida, M. (1995). Alcohol and aldehyde dehydrogenase polymorphisms and the risk for alcoholism. *The American Journal of Psychiatry, 152*, 1219–1221.
Israel, Y., Quintanilla, M. E., Karahanian, E., Rivera-Meza, M., & Herrera-Marschitz, M. (2015). The "first hit" toward alcohol reinforcement: Role of ethanol metabolites. *Alcoholism Clinical and Experimental Research, 39*, 776–786.
Jamal, M., Ameno, K., Kumihashi, M., Ameno, S., Kubota, T., Wang, W., et al. (2003). Microdialysis for the determination of acetaldehyde and ethanol concentrations in the striatum of freely moving rats. *Journal of Chromatography B, 798*, 155–158.
Koppaka, V., Thompson, D. C., Chen, Y., Ellermann, M., Nicolaou, K. C., Juvonen, R. O., et al. (2012). Aldehyde dehydrogenase inhibitors: A comprehensive review of the pharmacology, mechanism of action, substrate specificity, and clinical application. *Pharmacological Reviews, 64*, 520–539.
Lopez, C. M., Vallejo, N. E., Piñeiro, A. E., Uicich, R., Damin, C. F., Sarchi, M. I., et al. (2002). Alteration of biochemical parameters related with exposure to lead in heavy alcohol drinkers. *Pharmacological Research, 45*, 47–50.
Marchitti, S. A., Brocker, C., Stagos, D., & Vasiliou, V. (2008). Non-P450 aldehyde oxidizing enzymes: The aldehyde dehydrogenase superfamily. *Expert Opinion on Drug Metabolism & Toxicology, 4*, 697–720.
Martí-Prats, L., Sánchez-Catalán, M. J., Orrico, A., Zornoza, T., Polache, A., & Granero, L. (2013). Opposite motor responses elicited by ethanol in the posterior VTA: The role of acetaldehyde and the non-metabolized fraction of ethanol. *Neuropharmacology, 72*, 204–214.
Mattalloni, M. S., De Giovanni, L. N., Molina, J. C., Cancela, L. M., & Virgolini, M. B. (2013). Participation of catalase in voluntary ethanol consumption in perinatally low-level lead-exposed rats. *Alcoholism Clinical and Experimental Research, 37*, 1632–1642.
Maurissen, J. (2010). Practical considerations on the design, execution and analysis of developmental neurotoxicity studies to be published in Neurotoxicology and Teratology. *Neurotoxicology and Teratology, 32*, 121–123.
McBride, W. J., Le, A. D., & Noronha, A. (2002). Central nervous system mechanisms in alcohol relapse. *Alcoholism Clinical and Experimental Research, 26*, 280–286.
Melis, M., Enrico, P., Peana, A. T., & Diana, M. (2007). Acetaldehyde mediates alcohol activation of the mesolimbic dopamine system. *European Journal of Neuroscience, 26*, 2824–2833.
Nation, J., Baker, D., Fantasia, M., Ruscher, A., & Clark, D. (1987). Ethanol consumption and free operant avoidance performance following exposure to dietary lead. *NeuroToxicology, 8*, 561–568.
Nation, J. R., Baker, D. M., Taylor, B., & Clark, D. E. (1986). Dietary lead increases ethanol consumption in the rat. *Behavioral Neuroscience, 100*, 525–530.
Nation, J., Dugger, L., Dwyer, K., Bratton, G., & Grover, C. (1991). The effects of dietary lead on ethanol-reinforced responding. *Alcohol and Alcoholism, 26*, 473–480.
Nation, J., Grover, C., & Bratton, G. (1991). Lead attenuates the antipunishment effects of ethanol. *Alcohol, 8*, 1–5.
Ocaranza, P., Quintanilla, M. E., Tampier, L., Karahanian, E., Sapag, A., & Israel, Y. (2008). Gene therapy reduces ethanol intake in an animal model of alcohol dependence. *Alcoholism Clinical and Experimental Research, 32*, 52–57.
Paxinos, G., & Watson, C. (2009). *The Rat brain in stereotaxis coordinates* (Compact 6th ed.).
Peana, A. T., Enrico, P., Assaretti, A. R., Pulighe, E., Muggironi, G., Nieddu, M., et al. (2008). Key role of ethanol-derived acetaldehyde in the motivational properties induced by intragastric ethanol: A conditioned place preference study in the rat. *Alcoholism: Clinical and Experimental Research, 32*(2), 249–258.
Peana, A. T., Muggironi, G., & Diana, M. (2010). Acetaldehyde-reinforcing effects: A study on oral self-administration behavior. *Frontiers in Psychiatry, 1*, 1–6.
Quertemont, E., & Didone, V. (2006). Role of acetaldehyde in mediating the pharmacological and behavioral effects of alcohol. *Alcohol Research and Health, 29*, 258–265.
Quertemont, E., Tambour, S., & Tirelli, E. (2005). The role of acetaldehyde in the neurobehavioral effects of ethanol: A comprehensive review of animal studies. *Progress in Neurobiology, 75*, 247–274.
Rivera-Meza, M., Quintanilla, M. E., & Tampier, L. (2012). Reduction of ethanol consumption in alcohol-preferring rats by dual expression gene transfer. *Alcohol and Alcoholism, 47*, 102–108.
Rivera-Meza, M., Quintanilla, M. E., Tampier, L., Mura, C. V., Sapag, A., & Israel, Y. (2010). Mechanism of protection against alcoholism by an alcohol dehydrogenase polymorphism: Development of an animal model. *The FASEB Journal, 24*, 266–274.
Rodd-Henricks, Z. A., Melendez, R., Zaffaroni, A., Goldstein, A., McBride, W. J., & Li, T. K. (2002). The reinforcing effects of acetaldehyde in the posterior ventral tegmental area of alcohol-preferring rats. *Pharmacology, Biochemistry and Behavior, 72*, 55–64.
Sanchis-Segura, C., Miquel, M., Correa, M., & Aragon, C. M. G. (1999). Cyanamide reduces brain catalase and ethanol-induced locomotor activity: Is there a functional link? *Psychopharmacology, 144*, 83–89.
Sharper, A. G., Pocock, S. J., Walker, M., Wale, C. J., Clayton, B., Delves, H. T., et al. (1982). Effects of alcohol and smoking on blood lead in middle-aged British men. *British Medical Journal, 284*, 299–302.
Sinclair, J. D., Lindros, K. O., & Terho, K. (1980). Aldehyde dehydrogenase inhibitors and voluntary ethanol drinking by rats. *Advances in Experimental Medicine and Biology, 132*, 481–487.
Smith, R. F. (2003). Animal models of periadolescent substance abuse. *Neurotoxicology and Teratology, 25*, 291–301.
Spivak, K., Aragon, C. M., & Amit, Z. (1987). Alterations in brain aldehyde dehydrogenase activity modify ethanol-induced conditioned taste aversion. *Alcoholism Clinical and Experimental Research, 11*, 513–517.
Tabakoff, B., Anderson, R. A., & Ritzmann, R. F. (1976). Brain acetaldehyde after ethanol administration. *Biochemical Pharmacology, 25*, 1305–1309.
Tambour, S., Closon, C., Tirelli, E., & Quertemont, E. (2007). Effects of cyanamide and acetaldehyde accumulation on the locomotor stimulant and sedative effects of

ethanol in mice. *Behavioral Pharmacology, 18*, 777–784.
Vinci, S., Ibba, F., Longoni, R., Spina, L., Spiga, S., & Acquas, E. (2010). Acetaldehyde elicits ERK phosphorylation in the rat nucleus accumbens and extended amygdala. *Synapse, 64*(12), 916–927.
Virgolini, M. B., & Cancela, L. M. (2014). Exposición a plomo y adicción a drogas. *Revista de Farmacologia de Chile, 7*, 26–38.
Virgolini, M. B., Cancela, L. M., & Fulginiti, S. (1999). Behavioral responses to ethanol in rats perinatally exposed to low lead levels. *Neurotoxicology and Teratology, 21*, 551–557.
Zimatkin, S. M., Pronko, S. P., Vasiliou, V., Gonzalez, F. J., & Deitrich, R. (2006). Enzymatic mechanisms of ethanol oxidation in the brain. *Alcoholism Clinical and Experimental Research, 30*, 1500–1505.