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The role of acetaldehyde in ethanol reinforcement assessed by Pavlovian conditioning in newborn rats

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Abstract
Rationale Animal studies indicate that central acetaldehyde, dependent on catalase metabolism of ethanol (EtOH), modulates ethanol reinforcement. Brain catalase activity and acetaldehyde (ACD) production are significantly higher in rat pups compare d with adults. Interestingly, infant rats show high EtOH affinity for alcohol consumption and are particularly sensitive to the drug's reinforcing effects.
Objectives We tested whether central ACD is necessary and sufficient to induce appetitive conditioning in newborn rats through the artificial nipple technique.
Methods Vehicle, EtOH (100 mg%), and acetaldehyde (0.35 μmol) were administered into the cisterna magna (1 μl). Half of the animals also received a central administration of 75 μg (experiment 1) or 40 μg of D-penicillamine (experiment 2). Afterwards, pups were exposed to an olfactory cue (conditioned stimulus). One hour later, neonates were tested with an artificial nipple in the presence of the conditioned cue. Nipple attachment duration, mean grasp duration, and number of nipple disengagements served as dependent variables.
Results Positive responses to the scented nipple occurred in neonates conditioned with EtOH or ACD (experiments 1 and 2). In experiment 1, there were indications that D-penicillamine weakened the reinforcing effects of EtOH and ACD. In experiment 2, D-penicillamine (40 μg) significantly inhibited appetitive conditioned responses dependent upon EtOH or ACD.
Conclusions Appetitive conditioning was observed when employing either central EtOH or ACD as unconditioned stimuli. Central abduction of ACD inhibited conditioned appetitive responsiveness to the surrogate nipple. Central ACD is involved in the determination or modulation of EtOH's motivational properties during early stages in development.

Keywords Ethanol · Acetaldehyde · D-Penicillamine · Neonate · Reinforcement · Ontogeny · Suckling

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Introduction

Acetaldehyde, the first metabolite in the chain of ethanol (EtOH) oxidation, is capable of inducing EtOH-like effects, such as motor stimulation and conditioned-place preferences (Correa et al. 2003a, b; Quertemont and De Witte 2001; Quertemont et al. 2005). Most EtOH-derived acetaldehyde (ACD) is produced by the enzyme alcohol dehydrogenase in the liver (Lieber 1999). Peripheral ACD reaches the brain only when very high arterial blood concentrations (>100 μM) are present (Petersen and Tabakoff 1979; Quertemont and Tambour 2004; Quertemont et al. 2005). However, local production of ACD in the brain has been observed and it is mediated by the catalase–H_2O_2 system (Aragon et al. 1992). Catalase inhibitors (sodium azide, cyanamide, or 3-amino-1,2,4-triazole) block the production of ACD (Gill et al. 1992) and attenuate EtOH-induced motor stimulation (Pastor and Aragon 2008), anxiolysis (Correa et al. 2008), conditioned aversions (Aragon et al. 1985), and conditioned preferences (Font et al. 2008).

The catalase system activity is more active soon after birth and shows a progressive fall of about 50 % during the first ten

postnatal days (Del Maestro and McDonald 1987). Brain catalase activity and ACD production are significantly higher in rat pups than adults (Gill et al. 1992). This observation, along with experimental evidence relevant to ACD's central reinforcing effects, suggests that the infant brain might be particularly sensitive to EtOH's motivational properties. Substantial susceptibility to EtOH reinforcement has been observed in early ontogeny (Molina et al. 2007b; Pautassi et al. 2009) in terms of operant conditioning (Bordner et al. 2008) and conditioned tactile preference (Pautassi et al. 2008). Additionally, spontaneous EtOH consumption drops with advancing ontogeny (Truxell et al. 2007), a phenomenon more related with the drug's motivational effects rather than its sensory properties (Kozlov et al. 2008).

A constraint in testing the reinforcing properties of drugs during early ontogeny is the limited behavioral repertoire of the newborn. In mammals, suckling is indispensable for survival. Within the nursing context, neonates acquire information about the environment such as food safety (Blass 1990; Blass and Teicher 1980). Considering the relevance of suckling behavior during early ontogeny, Smotherman and colleagues developed the artificial nipple technique (Robinson et al. 1993; Smotherman et al. 1993). This technique is useful to understand the mechanisms underlying suckling (Petrov et al. 1998) and for the analysis of early EtOH's reinforcing properties and consumption patterns (Petrov et al. 2003).

Centrally injected (intracisternal, IC) EtOH concentrations of 25–200 mg% support neonatal appetitive conditioning, as assessed by the artificial nipple technique (Nizhnikov et al. 2006b). This effect is blocked in rat neonates pretreated with sodium azide (Nizhnikov et al. 2007). The use of catalase inhibitors impedes certain levels of data interpretation since along with inhibition of ACD formation, an accumulation of EtOH levels may also occur. Additionally, most catalase inhibitors have undesirable effects such as learning impairments caused by sodium azide (Lalonde et al. 1997).

A sequestering agent of ACD has been utilized to unravel its role in the modulation of EtOH's behavioral and motivational effects (Font et al. 2005, 2006a; Peana et al. 2008). D-penicillamine (d-p) lowers ACD blood levels without altering EtOH levels in vivo (Nagasawa et al. 1975, 1977). It is a metabolically inert thiol amino acid that forms a stable adduct with ACD. The condensation product (2,5,5-trimethylthiazolidine-4-carboxylic acid) is excreted in urine (Cohen et al. 2000; Nagasawa et al. 1975, 1978). d-p administration prevents EtOH-induced behavioral stimulation (Font et al. 2005; Pautassi et al. 2011), conditioned place preference (Font et al. 2006a; Peana et al. 2008), and voluntary EtOH consumption (Font et al. 2006b) and inhibits EtOH-induced stimulation of the mesolimbic dopaminergic transmission (Enrico et al. 2009). d-p doses of 37.5, 75, 150, and 300 mg/kg (i.p.) inhibit EtOH's activating effects. The high dose (150 and 300 mg/kg) exert by itself depressant motor activity effects (Font et al. 2005).

The aim of the present work was to extend the study of Nizhnikov et al. (2007), by testing if the direct administration of ACD into the cisterna magna is sufficient to support appetitive conditioning. We also tested if ACD is necessary to support EtOH reinforcement instead of blocking ACD formation through sodium azide (which blocks catalase activity and hence may lead to EtOH accumulation), and we used a sequestering agent of ACD (d-p). In experiment 1, the d-p dose (75 μg) was selected in accordance with the studies conducted by Font et al. (2006a, b). In experiment 2, we tested if a lower dose of d-p (40 μg) successfully blocked appetitive conditioning induced by EtOH or ACD.

Perinatal rats were utilized since at this age: (1) brain catalase activity is high relative to subsequent developmental stages (Del Maestro and McDonald 1987; Gill et al. 1992), (2) experiences with peripheral and central EtOH administration promote appetitive conditioning (Bordner et al. 2006; Nizhnikov et al. 2006b; Petrov et al. 2003), (3) pups show affinity to EtOH in terms of drug consumption via the AN technique (Petrov et al. 2001), and (4) early EtOH experiences affect subsequent EtOH's acceptance and reinforcement (Molina et al. 2007b).

Materials and methods

Subjects

Animals were born and reared at the vivarium of the Instituto de Investigaciones Médicas Mercedes y Martín Ferreyra. Temperature was kept at 22–24 °C with a 12-h light/12-h dark cycle. Vaginal smears of Wistar-derived female adult rats (prepregnancy weight, 230–300 g) were microscopically analyzed. On the day of proestrus, three females were housed during the dark cycle with one male. The morning after mating, females were withdrawn from the presence of males, and this day was considered as gestational day 0 (GD0). Pregnant females were housed in standard maternity cages (three dams per cage) with access to rat chow and tap water delivered through automatic dispenser valves.

Animals were maintained and treated in compliance with the guidelines for animal care established by the Institute of Laboratory Animal Resources, National Research Council, USA (National Institute of Health and Institute of Laboratory Animal Resources, Commission on Life Sciences 1996) and were approved by the Animal Care and Use Committee at INIMEC-CONICET.

Cesarean delivery

During GD21, pups were delivered by cesarean section. Procedures involved in cesarean delivery have been detailed elsewhere (Abate et al. 2002, 2004). Two hours after birth,

pups were randomly assigned to one of six possible postnatal conditions. Cesarean delivery was used to avoid suckling experiences with the dam. It has been shown that appetitive behavior towards the surrogate nipple increases over the first 3 h after birth (Smotherman et al. 1997a) and that suckling from an artificial nipple is more vigorous when pups lack experience with the maternal nipple (Petrov et al. 2001).

Central drug administration procedures

Learning and central administration procedures replicated those employed by Nizhnikov et al. (2006b, 2007). EtOH (100 mg%), ACD (0.35 μmol), phosphate buffer (PB 0.1M), and d-p (75 μg in experiment 1 or 45 μg in experiment 2) were administered into the cisterna magna (IC). The dosage of each particular drug was selected in accordance with previous literature. The 100-mg% EtOH dose acts as an effective central reinforcer in newborns (Nizhnikov et al. 2007) while central injections of 0.35 μmol ACD exert stimulatory motor effects in adult rats (Arizzi-LaFrance et al. 2006; Correa et al. 2009). The 75-μg d-p dose and the interval between drug injections were chosen since this d-p dose has been observed to inhibit EtOH ingestion in adults (Font et al. 2006b). Pups were centrally injected with either PB (vehicle) or d-p and placed in a temperature-controlled incubator for 5 min. Then, EtOH, ACD, or PB was centrally injected and subjects were individually placed in the conditioning chamber to acclimate. Drugs were administered into the cisterna magna following the procedures described by Petrov et al. (1998). The corresponding solution (1 μl) was injected for a period of 10 s using a micrometer gastight syringe (Hewlett-Packard, USA). Following each IC administration, the needle remained in position for 30 s and then removed to minimize leaking of cerebrospinal fluid. It has been observed that an inert substance administered into the cisterna magna (inulin) follows a caudal-to-rostral and ventral-to-dorsal pattern of distribution and preferred entry of tracer from ventral surfaces of the ventral forebrain—particularly hypothalamus—and brain stem (Proeschtoldt et al. 2000).

Conditioning and testing procedure

Following drug administrations, pups were suited in a restriction vest and maintained in a supine position. Pups were conditioned and tested in a Plexiglas chamber equipped with a heated Styrofoam container (internal base diameter, 9 cm; volume capacity, 750 cm^3, temperature kept at 35 °C). One minute after placement in the conditioning chamber, lemon odor (conditioned stimuli (CS)) was presented using a cotton applicator scented with 0.1 cm^3 of lemon oil (Montreal, Argentina) for 5 min. This conditioning procedure implies temporal contiguity between IC drug administration and presence of a salient odorant. This temporal pattern of stimuli presentation was selected based on previous studies showing that EtOH and ACD central administration induces rapid behavioral effects that seem to fade around 10 min postadministration (Correa et al. 2003b). Video recordings for 6 min during which pups stayed in the conditioning chamber were obtained. Pups were then returned to the incubator where they remained for 1 h until commencement of the nipple attachment test.

During testing, pups were presented with an artificial nipple that delivered water and was scented with lemon oil (for a detailed description of the artificial nipple, see Petrov et al. 1997). Water availability through the nipple facilitates attachment behavior but does not induce conditioning in these circumstances (Smotherman et al. 1993). For providing the CS odor, an alligator clip with a cotton ball scented with 0.1 cm^3 lemon oil was attached to the handle of the surrogate nipple (distance between the clip and the nipple, 2 cm). An experimenter, blind to treatments, tested the pups. Exposure to the surrogate nipple involved gentle contact between the tip of the nipple and the oral area. The subject was completely free to grasp the nipple or to disengage from it. Attachment consisted of an active movement of the head and mouth toward the nipple that resulted in the nipple entering the oral cavity and the mouth closing around the tip (Robinson et al. 1992). The nature of the procedure excluded any compulsion because an attempt to force the nipple into the mouth evokes vigorous nipple rejection and choking and asphyxiation (Smotherman et al. 1997b). Attachment was confirmed by periodic gentle attempts to withdraw the nipple from the pup (every 30 s) and was regarded as sustained if the pup resisted withdrawal of the nipple. An active release of the nipple was considered a disengagement response (Nizhnikov et al. 2006b).

Experimental design and data analysis

Each experiment included a 2 (d-p dose)×3 (unconditioned stimulus (US) drug) between-subject factorial design. To eliminate confounding of litter with treatment effects, no more than one subject from a given litter was assigned to the same treatment condition (Holson and Pearce 1992).

During conditioning, duration and frequency of forelimb and hind limb movements were registered. In order to consider a new behavioral bout, limb activity had to be completely absent at least for 1 s. During testing, the suckling response was assessed in terms of total attachment (summation of the duration of all grasps), mean attachment duration (total time divided by number of grasps), and total number of disengagements. Latencies to display forelimb and hind limb movements were also assessed. All behavioral scores were analyzed using separate between-groups ANOVAs. Orthogonal planned comparisons were used if a significant

main effect or interaction was found ($p<0.05$). Data shown in figures have been depicted as mean values and standard error of the means.

Experiment 1

The aim of the experiment was to assess possible central reinforcing effects of EtOH and its principal metabolite, ACD, in newborn rats. To further assess the role of ACD in EtOH's motivational effects, we also included newborns treated with a sequestering agent of the metabolite (d-p).

Subjects and procedures

A total of 57 male and female pups from 13 cesarean section deliveries were tested. Pups were assigned to one of six treatment conditions (d-p 0 μg/US vehicle, d-p 0 μg/US EtOH, d-p 0 μg/US ACD, d-p 75 μg/US vehicle, d-p 75 μg/US EtOH, and d-p 75 μg/US: ACD) defined by the factorial design which took into account central drug injection of d-p and US (i.e., EtOH or ACD). The number of pups in each group ranged between 7 and 11 (Table 1). In prior studies, it was observed that sex systematically failed to exert significant effects or to interact with EtOH reinforcement (Nizhnikov et al. 2012; Pautassi et al. 2012a, b, c). For this reason, inferential processing of the data was performed by collapsing sex across treatments.

Results

Forelimb and hind limb activity displayed during conditioning did not differ across treatments. Pertinent ANOVAs showed no significant effects on these behaviors of the main factors under consideration (d-p dose or US drug) or a significant interaction between them. Limb activity scores during conditioning are also summarized in Table 1.

EtOH and ACD exerted appetitive motivational effects in newborns as assessed through mean attachment duration (Fig. 1a). Indeed, the ANOVA showed a significant effect of US drug ($F(2, 51)=11.84$, $p<0.0001$). The interaction between this factor and d-p dose did not achieve significance. Relative to total attachment duration (Fig. 1b), the ANOVA also showed a significant main effect of US drug effect ($F(2, 51)=3.25$, $p<0.05$). Finally, when considering disengagement from the nipple, fewer disengagements were observed in pups that were administered with EtOH or ACD ($F(2, 51)=11.97$, $p<0.0001$; Fig. 1c). In each of these analyses, pups administered with EtOH as well as ACD differed from vehicle-treated siblings (p values <0.05).

Appetitive conditioning resulted from the central administration of EtOH or its principal metabolite. From a

Table 1 Limb activity (forelimb and hind limb) during conditioning

	Experiment 1									Experiment 2								
	Forelimb activity: frequency		Forelimb activity: duration (s)		Hind limb activity: frequency		Hind limb activity: duration (s)		*N* (males/females)	Forelimb activity: frequency		Forelimb activity: duration (s)		Hind limb activity: frequency		Hind limb activity: duration (s)		*N* (males/females)
d-p dose (μg)	0	75	0	75	0	75	0	75		0	75	0	75	0	75	0	75	
US drug																		
Vehicle	9.11 (0.99)	5.29 (1.54)	35.20 (10.10)	15.75 (3.20)	8.11 (1.37)	4.86 (1.47)	33.43 (9.89)	14.58 (3.13)	5/5	6.40 (1.64)	4.80 (1.30)	24.26 (7.37)	23.48 (8.18)	6.10 (1.05)		24.98 (6.65)	25.81 (7.80)	5/5 5/5
EtOH	7.78 (1.22)	7.54 (1.61)	29.75 (7.03)	19.98 (6.87)	7.67 (1.19)	4.00 (1.37)	25.69 (6.70)	12.13 (5.81)	5/5	3.90 (0.93)	6.40 (1.11)	21.26 (8.76)	36.70 (7.39)	5.09 (0.82)		24.79 (8.35)	37.93 (7.71)	5/6 5/5
ACD	5.86 (1.50)	8.36 (1.43)	20.22 (9.14)	24.95 (7.80)	4.71 (1.32)	6.18 (1.07)	17.10 (8.05)	21.54 (7.02)	4/4	5.00 (0.71)	6.55 (1.09)	26.21 (7.34)	26.95 (5.48)	5.82 (1.24)		30.06 (8.33)	21.59 (3.56)	5/6 4/5

Values represent mean±standard errors of the mean and number of pups per group

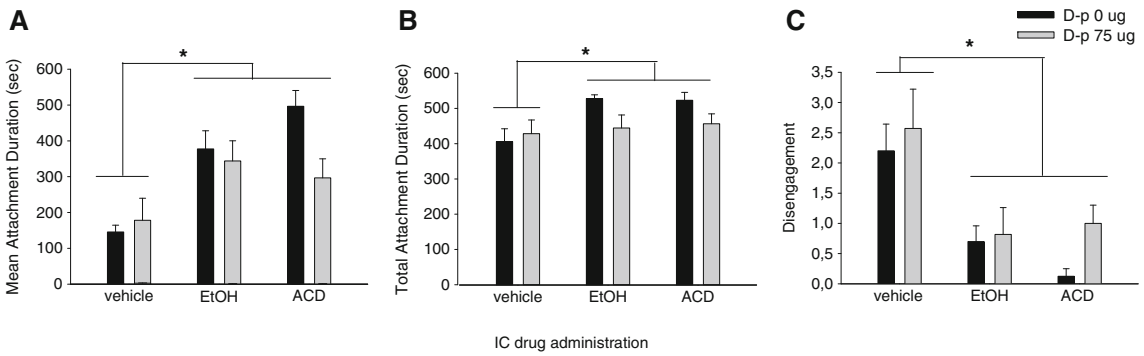

Fig. 1 **a** Mean attachment duration, **b** total time attached, and **c** total number of disengagements from a surrogate nipple in the presence of lemon odor as a function of d-p treatment (vehicle or 75 μg) and US drug (vehicle, EtOH 100 mg%, or ACD 0.35 μmol). *$p<0.05$, significant difference from US vehicle control group

descriptive perspective, it appeared that d-p partially inhibited this effect. Nevertheless, in no case, a significant interaction between US and d-p was found.

Experiment 2

According to experiment 1, central EtOH and ACD supported appetitive conditioning. In terms of possible inhibitory effects of d-p, the data were not conclusive. It is important to note that d-p administration can modify EtOH effects by other mechanisms aside from ACD adduction. d-p affects nitric oxide (NO) production and release (Wigley and Sule 2001) and has been found to act as a NO donor (Feelisch 1998; Lakatos and Oroszlan 1994). The inhibition of NO synthesis reduces voluntary EtOH consumption (Calapai et al. 1996; Rezvani et al. 1995). Additionally, male knockout mice, genetically engineered to lack a gene involved in the neural nitric oxide synthase, do not develop EtOH-induced conditioned place preference as effectively as wild-type counterparts (Itzhak et al. 2009). Therefore, NO plays a role in EtOH effects and has also been shown to modulate morphine (Manzanedo et al. 2004) and nicotine (Martin and Itzhak 2000) reinforcement. It is possible that the d-p dose used in this experiment might be above threshold to alter NO activity. For this reason, experiment 2 was undertaken using a lower d-p dose (40 μg) than the one previously employed (75 μg). A second goal of the present experiment was to increase the internal validity of the results previously obtained with respect to EtOH or ACD central reinforcing effects.

Subjects and procedures

A total of 61 neonates derived from 12 litters were utilized. Procedures were similar to those described for experiment 1, with the difference that the d-p dose was lower (40 μg). Each group consisted of 8–11 pups (Table 1).

Results

As in experiment 1, limb activity during conditioning was not different across groups (Table 1). Mean attachment duration (Fig. 2a) indicated clear central reinforcement of EtOH and ACD. Pups given centrally administrated ACD or EtOH increased their mean attachment duration in comparison to controls given only with vehicle. d-p (40 μg) preadministration completely inhibited ACD's and EtOH's positive reinforcing effects. The ANOVA showed a main effect of d-p dose ($F(1, 55)=21.50$, $p<0.0001$), a main effect of US ($F(2, 55)=8.91$, $p<0.001$), and a significant interaction between these factors ($F(2, 55)=3.38$, $p<0.05$). Planned comparisons showed that the higher levels of mean attachment duration were found in groups d-p 0 μg/US ACD and d-p 0 μg/US EtOH, which differed from all remaining groups, but not from each other.

In terms of total attachment duration (Fig. 2b), the ANOVA indicated a significant main effect of US drug ($F(2, 55)=5.30$, $p<0.01$) and d-p ($F(1, 55)=16.12$, $p<0.001$) treatments. EtOH and ACD induced similar duration of attachment which, in both cases, was longer than the response observed in vehicle groups ($p<0.001$). Additionally, d-p reduced total attachment duration ($p<0.001$). Although the interaction between these factors did not achieve statistical significance, the reduction in attachment duration by d-p seems to be mainly driven by the reduction observed in groups given EtOH and ACD as USs.

The inverse profile was observed when analyzing disengagements from the nipple (Fig. 2c). Pups showing minimal detachments were those exposed to EtOH (d-p 0 μg/US) or ACD (d-p 0 μg/US ACD). Blocking ACD effects through its sequestration by d-p increased the number of disengagements from the nipple in pups that also experienced the contingency between central administered EtOH or ACD and lemon odor. The ANOVA showed a significant main effect of d-p dose ($F(1, 55)=13.45$, $p<0.001$), a significant main effect of US drug ($F(2, 55)=3.78$, $p<0.05$), and an

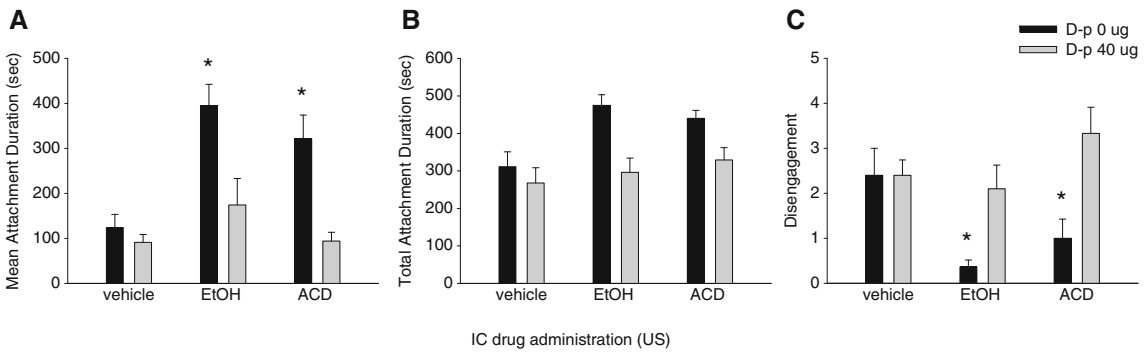

Fig. 2 **a** Mean attachment duration, **b** total time attached, and **c** total number of disengagements from a surrogate nipple in the presence of lemon odor as a function of d-p treatment (vehicle or 40 μg) and US drug (vehicle, EtOH 100 mg%, or ACD 0.35 μmol). *$p<0.05$, significant difference from control groups

interaction between these factors ($F(2, 55)=3.53$, $p<0.05$). Specific comparisons indicated that experimental groups d-p 0 μg/US ACD and d-p 0 μg/US EtOH differed from all other groups but not from each other ($p<0.001$).

Discussion

The present study confirms appetitive reinforcement when EtOH is intracisternally administrated to the newborn rat (Nizhnikov et al. 2006b, 2007). This is, to our knowledge, the first study to show that a relatively low dose of ACD promotes similar appetitive conditioning when directly administrated into the newborn's brain. In both experiments, there were clear indications that pairing EtOH or ACD with an odorant (lemon) resulted in heightened acceptance of a lemon-scented artificial nipple as operationalized through higher levels of attachment and grasping and lower levels of disengagements.

It could be argued that the increase in suckling behavior following EtOH or ACD administration could be explained through a pseudoconditioning effect, due to the stimulation of the opioid system. It has been observed that the present USs (EtOH and ACD) stimulate opiates. The artificial nipple technique involves activation of this neurobiological system (Robinson and Smotherman 1995, 1997). Perhaps previous stimulation of the opioid system facilitated oral grasping because this system was already prompted. However, previous studies in which unpaired and US-only control groups have been included have consistently supported the notion that an associative learning mechanism underlies later increases in responses to lemon–nipple–water CS when EtOH is used as the US (Nizhnikov et al. 2006a, b; Petrov et al. 2003).

One weakness derived from the present methodological strategy is the impossibility to determine if the ACD dose administered is similar to the ACD levels formed following an IC administration of EtOH in the range of doses observed to produce reinforcement (25–200 mg%, Nizhnikov et al. 2006b) or if ACD brain levels reached after peripheral EtOH administration are also similar. This issue can be framed into the actual controversy about the effect of central ACD in mediating EtOH effects. Uncertainty originates from the observation that very low ACD is detected in the brain after EtOH administration (Gill et al. 1992; Hunt 1996). However, the participation of ACD has been observed not only when directly administering this substance in the brain (Correa et al. 2003b, 2009; Rodd-Henricks et al. 2002) but also by manipulating EtOH metabolism (Arizzi-LaFrance et al. 2006; Correa et al. 2008; Enrico et al. 2009; Font et al. 2006a; Pastor and Aragon 2008). In the present study, from a behavioral perspective, EtOH and ACD exerted a similar magnitude of conditioning. Also, EtOH and ACD reinforcements were similarly inhibited when the metabolite was abducted by d-p (see experiment 2).

It is interesting to note that within a critical period (4–10 days of age), nociceptive stimuli tend to establish conditioned preferences when paired with salient odorants. This paradoxical learning appears vital to conserve and enhance mother–infant interactions (Sullivan et al. 2000). Within this sensitive period, high alcohol doses, known to act as aversive stimuli in older animals, appear to promote conditioned chemosensory preferences (Arias and Chotro 2006). In light of these observations, it could be argued that central EtOH and ACD are aversive events that, under the frame of a particular neurobiological context, serve to establish conditioned olfactory preferences. Nevertheless, before and during this ontogenetic window, aversive conditioning has been described when lithium chloride acts as an US (Gruest et al. 2004; Miller et al. 1990; Molina et al. 1986). This substance induces internal malaise and disgust reactions similar to those observed following EtOH intoxication and shares common biological substrates with EtOH's aversive effects (Arias et al. 2010).

A matter of special concern when addressing EtOH-derived reinforcing effects is the drug's potential to also alter

motor activity. In infant rats, moderate to high EtOH doses result in biphasic motor effects (behavioral stimulation followed by depression). The adult preclinical literature also reveals the likelihood of biphasic motor effects of ACD (Quertemont et al. 2005). These effects of EtOH or its metabolite cannot be dismissed when analyzing possible motivational properties. In preweanlings, motor conditioned responses arising from the use of EtOH as an US can confound interpretations in other tests of reinforcing effects (Molina et al. 2007a). In the present study, we explicitly examined if EtOH, ACD, or d-p had specific motor effects during conditioning and found that all groups, independently of the drug administered, exhibited similar motor scores during conditioning. Yet, it can be argued that, since newborns are fitted in a vest to facilitate odor exposure, the procedure restricts possibilities of detecting a more ample pattern of spontaneous or induced activity. In recent experiments, we have tested freely moving neonates confronted with a similar odorant and under the effects of EtOH (100 mg%) or ACD (0.35 μmol). There was no indication that these USs altered motor activity (March et al. in preparation).

In the present study, a somewhat paradoxical effect of d-p was observed. A lower d-p dose (40 μg) was more successful in blocking EtOH and ACD reinforcement than a 75-μg dose. As mentioned, this unexpected effect may be related to d-p capability to alter the NO system (Feelisch 1998; Lakatos and Oroszlan 1994; Wigley and Sule 2001). Nitric oxide synthesized in the central nervous system produces a myriad of effects. For example, it plays a role in the control of blood flow, learning and memory, neurotransmitter release, gene expression, immune responsiveness, and cell survival. It is also implicated in numerous pathologies such as Alzheimer's disease, Huntington's disease, and cerebral ischemia (Snyder 1992). It is not possible to discard the possibility that d-p's effects upon appetitive conditioning are due to another mechanism different from ACD abduction. Nevertheless, in studies in which brain ACD formation has been blocked by catalase inhibition through aminotriazole or sodium azide, the appetitive properties of EtOH during conditioning have also been blocked (Font et al. 2008; Nizhnikov et al. 2007). Beyond this observation, it is clear that further studies are required to unravel possible NO intervention on motivational effects of EtOH and ACD, and the effects of d-p upon NO availability. Certainly, there is also a need for systematic dose-response studies concerning d-p central administration and EtOH or ACD reinforcement.

Most findings regarding ACD's behavioral or motivational effects were derived from studies conducted with adult animals. These studies show that ACD has differential motivational effects when its administration is peripheral (mainly aversive) versus central (primarily reinforcing, Quertemont et al. 2005). We should be cautious in extrapolating findings in adult animals to those obtained in newborns since there are marked differences in metabolic systems (both peripheral and central) across ontogeny. Catalase concentrations in cerebellum, striatum, cerebral hemispheres, and brain stem of the newborn rat are about eight times higher than those observed in adults (Del Maestro and McDonald 1987). Additionally, preweanlings have slower rates of EtOH metabolism after peripheral administration compared to older animals (Silveri and Spear 2000). Probably, the balance between peripheral–central levels of this metabolite might be critical in explaining notable differences in alcohol affinity across ontogeny. Lower accumulation of peripheral ACD due to hepatic immaturity in the infant rat (Kelly et al. 1987) might help explain early resistance to EtOH's aversive properties (Arias and Chotro 2006). On the other hand, central catalase activity might represent a neurobiological mechanism mediating alcohol acceptance and reinforcement during infancy (for reviews, see Molina et al. 2007b; Pautassi et al. 2009). The present results, coupled with those of Nizhnikov et al. (2007), strongly endorse a marked central reinforcing effect of central ACD in the newborn rat. When considering the possibility that the balance between central and peripheral actions of this metabolite determines the final outcome in EtOH's motivational properties, it appears that ontogenetic studies based on differential metabolic processes are needed to evaluate such a hypothesis.

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AUTHOR QUERIES

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