ELSEVIER

Alcohol 45 (2011) 45–55

ALCOHOL

Ethanol induces second-order aversive conditioning in adolescent and adult rats

Ricardo Marcos Pautassi[a,b,*], Mallory Myers[a,1], Linda Patia Spear[a], Juan Carlos Molina[a,b], Norman E. Spear[a]

[a]*Department of Psychology, Center for Development and Behavioral Neuroscience, Binghamton University, Binghamton, New York, USA*
[b]*Laboratory of Alcohol and Development, Instituto de Investigación Médica M. y M. Ferreyra Medical Research Institute–Argentinian Nacional Council of Research (INIMEC—CONICET), Córdoba, CP 5016, Argentina*

Received 26 March 2009; received in revised form 15 September 2010; accepted 12 October 2010

Abstract

Alcohol abuse and dependence are considered public health problems, with an etiological onset often occurring during late childhood and adolescence, and understanding age-related differences in ethanol sensitivity is important. Low to moderate ethanol doses (0.5 and 2.0 g/kg, intragastrically [i.g.]) induce single-trial, appetitive second-order place conditioning (SOC) in adolescent, but not adult, rats. Recent studies have demonstrated that adolescents may be less sensitive than adults to the aversive properties of ethanol, reflected by conditioned taste aversion. The present study assessed the aversive motivational effects of high-dose ethanol (3.0 and 3.25 g/kg, i.g., for adolescents and adults, respectively) using SOC. Experiment 1 revealed similar blood and brain ethanol levels in adolescent and adult rats given 3.0 and 3.25 g/kg ethanol, respectively. In Experiment 2, animals received ethanol or vehicle paired with intraoral pulses of sucrose (conditioned stimulus 1 [CS1]). After one, two, or three conditioning trials, the rats were presented with the CS1 while in a distinctive chamber (CS2). When tested for CS2 preference, ethanol-treated animals exhibited reduced preference for the CS2 compared with controls. This result, indicative of ethanol-mediated aversive place conditioning, was similar for adolescents and adults; for females and males; and after one, two, or three training trials. In conjunction with previous results, the present study showed that, in adolescent rats subjected to SOC, ethanol's hedonic effects vary from appetitive to aversive as the ethanol dose increases. Adolescent and adult animals appear to perceive the postingestive effects of high-dose ethanol as similarly aversive when assessed by SOC.

Keywords: Adolescence; Adulthood; Ethanol; Reinforcement; Second-order conditioning

Introduction

Early initiation of alcohol consumption is associated with a greater likelihood of developing alcohol abuse and dependence (''early debut effect''; Pedersen and Skrondal, 1998). This relationship is not linear, nor is it necessarily causal. Alcohol initiation at certain developmental stages is critically important to determine the pattern of alcohol consumption at adulthood. Specifically, the risk of alcohol abuse and dependence is greater when the onset of alcohol intake occurs during early adolescence (13–14 years old; Anthony and Petronis, 1995). These findings have strong public health implications, particularly when viewed in conjunction with the fact that alcohol intake usually begins during adolescence, with 28% of underage drinkers in the United States having started at age 13 years (Johnston and O'Malley, 2007).

The use of animal models has identified factors that could help explain the avidity for alcohol during adolescence and the enduring consequences of such consumption. Ethanol intake in adolescent (postnatal days [PD] 28–42) and late-adolescent animals (until approximately PD55 or so; Spear, 2000) surpasses that observed in older animals (Doremus et al., 2005). Adolescents are also more sensitive than adults to the facilitating effects of low-dose ethanol on social behavior but are less sensitive to the disruptive effects that higher ethanol doses have on social behavior (Varlinskaya and Spear, 2002). Intriguingly, adolescents are remarkably resistant to several acute effects of ethanol (e.g., motor incoordination, hypothermia, narcosis; Spear, 2004; White et al., 2002) that normally should serve to preclude further engagement in alcohol intake.

* Corresponding author. Laboratory of Alcohol and Development, Instituto de Investigación Médica M. y M. Ferreyra Medical Research Institute–Argentinian Nacional Council of Research (INIMEC—CONICET), Córdoba, CP 5016, Argentina. Tel: 154-351-4681465, ext. 218; fax: 154-351-4695163.

E-mail address: rpautassi@gmail.com (R.M. Pautassi).

[1] Current address: Department of Psychology, LD 124, Indiana University–Purdue University, Indianapolis, 402 North Blackford Street, Indianapolis, IN 46202-3275, USA.

0741-8329/$ - see front matter
doi: 10.1016/j.alcohol.2010.10.004

The motivational effects of ethanol are critical in the modulation of drug seeking and self-administration (Cunningham et al., 2000). Adult rats readily detect an aversive component derived from alcohol intoxication. For example, they reject a taste that has been previously paired with ethanol's effects (conditioned taste aversion [CTA]; Davies and Parker, 1990). In contrast, evidence of the expression of ethanol-mediated conditioned preferences in adult rats has proven problematic. Unlike mice, rats tend to avoid locations or textures that signal the drug (conditioned place aversion; Cunningham et al., 1993). Some intriguing data suggest that adolescent rats may exhibit differential sensitivity to ethanol's motivational effects compared with their more mature counterparts. Philpot et al. (2003) found ethanol-induced conditioned place preference (CPP) at PD25 (0.2 g/kg) and late in adolescence (PD45, 0.5 and 1 g/kg, intraperitoneally [i.p.]), whereas a trend toward conditioned aversion was found in young adults (PD60).

A variation of the CPP procedure has provided another venue for the analysis of ethanol-mediated motivational learning. In this preparation, described as second-order conditioning (SOC; Molina et al., 2006, 2007), a gustatory stimulus (e.g., water or sucrose, conditioned stimulus 1 [CS1]) is paired with ethanol's pharmacological effects. Animals are then stimulated with the CS1 while placed in a visually and tactually distinctive chamber (CS2). Preference or aversion toward the CS2 is then assessed in a choice procedure (CS2 vs. CS novel). In other words, ethanol's motivational effects are assessed not through direct responsiveness to the taste CS1 but rather by assessing whether the ethanol-paired taste can transfer motivational information to the CS2.

The use of SOC proved as a valuable tool for detecting appetitive effects of ethanol in infant and adolescent rats (Molina et al., 2006, 2007; Pautassi et al., 2008b). The preweanling, 14-day-old rats were given pairings of an intraoral CS and either the early or late (5–15 min or 30–45 min postintubation, respectively) effects of intragastric (i.g.) administration of a low dose of ethanol (0.5 g/kg) or the early effects of a moderate dose (2 g/kg). This resulted in the gustatory CS becoming a positive second-order reinforcer. Interestingly, aversions emerged when the CS1 was paired with 2.0 g/kg, 30–45 min postadministration (Molina et al., 2006, 2007). A subsequent study assessed ethanol-mediated, one-trial SOC in adolescent and adult rats (PD32 and PD70, respectively; Pautassi et al., 2008b). The CS1 (a sucrose taste) was delivered through a surgically implanted catheter 5–15 min or 30–45 min after ethanol administration (0.5 or 2.0 g/kg, i.g.). The CS1 then acted as an appetitive second-order reinforcer in the adolescents, mediating the expression of CPP, which was particularly strong when the CS1 was originally paired with 2.0 g/kg ethanol. The adult rats did not exhibit changes in tactile preferences, thus suggesting the absence of ethanol-mediated learning. These results suggest greater sensitivity to ethanol's appetitive effects in adolescent than in adult rats assessed by SOC (Pautassi et al., 2008b). In a follow-up study, the second-order appetitive conditioning in adolescents was blocked after treatment with naloxone, a general opioid antagonist (Pautassi et al., 2010).

The previous studies underscore an important advantage of SOC, namely, it can be used with minimal modification across ontogeny. Because of inherent developmental changes, the ontogeny of ethanol reinforcement has been studied through different tests for infant (Pautassi et al., 2002); adolescent (Ristuccia and Spear, 2008); and adult (Bienkowski et al., 1999) subjects. The development of SOC has provided a single benchmark to study ethanol's motivational effects. SOC has also proven useful to detect "silent" (i.e., not detectable through first-order conditioning) associations in young rats. A study conducted with 4-day-old rats revealed a lack of aversion for an odor CS previously paired with lithium chloride (LiCl), an emetic, nonaddictive substance. The conditioned aversion was observed, however, after pups were provided subsequent second-order pairings between the odor and a novel texture (Miller et al., 1990). The SOC procedure may be more likely to reveal these seemingly elusive associations, because it minimizes the effects of conditioned responses often emitted in the presence of the CS after first-order conditioning—conditioned behaviors that may compete with the target response used to reflect conditioning. This property of SOC may be particularly valuable for analyzing ethanol-mediated place conditioning, given previous studies revealing first-order, motor conditioned responses in response to a taste CS previously paired with ethanol (Molina et al., 2006; Pautassi et al., 2008b).

Age-specific predisposition in terms of sensitivity to ethanol's motivational effects may render adolescents at risk of ethanol-related problems. Adolescents may be more sensitive to ethanol's appetitive effects (Pautassi et al., 2008b) but less sensitive to the aversive consequences of ethanol. The latter effects are easily observed in both adult and infant rats, particularly at doses greater than or equal to 2.0 g/kg. Adolescent rats are less susceptible to CTA induced by psychoactive drugs (e.g., cocaine, amphetamine, and nicotine) and also by LiCl (Schramm-Sapyta et al., 2006). Less is known, however, about ethanol's ability to induce aversive learning in adolescent rats. A recent series of studies (Anderson et al., 2008; Varlinskaya and Spear, 2008; Vetter-O'Hagen et al., 2009) assessed age- and sex-related differences in terms of ethanol-mediated CTA in adult and adolescent rats (PD32 and PD74, respectively). Ethanol-induced CTA was evident in the adolescents but at higher doses than those in adults. The older animals showed CTA at i.p. doses of 1.0 and 1.5 g/kg, whereas CTA in adolescents was evident only at 2.0 g/kg. These results suggest that, when assessed by CTA, adolescents may be less sensitive than their older counterparts to the aversive properties of ethanol. To date, responsiveness to the aversive properties of ethanol as a function of age and sex has only been studied using the

CTA paradigm (Anderson et al., 2008; Varlinskaya and Spear, 2008; Vetter-O'Hagen et al., 2009) and, to a lesser extent, by means of CPP (Philpot et al., 2003). The study of sex-related differences during adolescence is important, because epidemiological and preclinical data indicate sex differences in alcohol consumption (Chester et al., 2006; Doremus et al., 2005; Greenfield, 2002). Little is known, however, about sex differences in terms of motivational learning promoted by ethanol during adolescence (but see Varlinskaya and Spear, 2008). The present study further explored these age- and sex-related differences using alternative learning preparations. SOC has already proven sensitive to the detection of the appetitive motivational effects of ethanol in adolescent rats (Pautassi et al., 2008b). Therefore, the aim of the present study was to assess the expression of ethanol-mediated aversive learning during adolescence and adulthood using a SOC procedure. Specifically, we explored whether adolescents and adults differ in SOC when trained with a high ethanol dose (3.0 or 3.25 g/kg i.g.; see Experiment 1) in either one-trial training (Experiment 2a) or after more prolonged training (two or three daily conditioning sessions, Experiments 2a and 2b, respectively). The possibility of SOC differing across males and females was also fully assessed.

Pharmacokinetic differences could be major determinants of age-related differences in behaviors (Walker and Ehlers, 2008). We recently observed significantly higher blood ethanol concentrations (BECs) in adolescent rats than that in adult rats 30 min after administration of 2.0 g/kg ethanol i.g. (Pautassi et al., 2008b). Therefore, equating the level of intoxication at both ages (Experiment 1) before proceeding with the analysis of potential age-related differences in SOC was important. Our underlying hypothesis was that high-dose ethanol would result in conditioned aversion, and that adolescents may be less likely to show such an aversion, particularly when multiple conditioning trials are given. These expectations were based on previous data gathered with CTA (Varlinskaya and Spear, 2008) and on the possibility that daily training with ethanol intubations would differentially facilitate the development of tolerance to the aversive effects of ethanol in adolescent rats. In the present study, the timing of exposure to the CS (30–45 min postadministration) was selected to maximize the chances of ethanol inducing conditioned aversion.

The assessment of ontogenetic differences in the development of tolerance to the hypnotic and sedative effects of ethanol has produced inconsistent results. Tolerance to the hypothermic effects of ethanol, as delivered through vapor inhalation, developed faster in adult than in adolescent rats (Ristuccia and Spear, 2005). A different profile was observed in a study that administered 4 g/kg ethanol i.g. twice a day for 7 days (Swartzwelder et al., 1998) and found that tolerance to ethanol-induced sedation and hypothermia was greater in adolescent than in adult rats. In a study where adolescent and adult rats were equated for their initial level of ethanol-induced motor impairment, equivalent levels of tolerance after chronic ethanol were found across age (Silveri and Spear, 2001). Less is known about the development of tolerance to ethanol's hedonic effects.

General methods

Subjects

A total of 228 Sprague–Dawley rats were used. These animals were derived from 55 litters born and reared at the Center for Development and Behavioral Neuroscience (Binghamton University, Binghamton, NY). Births were examined daily, and the day of parturition was considered PD0. Pups were housed with the dam in standard maternity cages with free access to water and food. The colony was maintained at 22–24°C with a 12-h light–dark cycle. Weaning was performed at PD21. On that day, eight animals from each litter (four males and four females) were assigned to these studies and transferred to clean tubs lined with pine shavings. On PD28, males and females from a given litter were transferred to clean tubs in same-sex groups of four. Animals that were tested during adulthood were further separated into pairs at PD60.

One hundred nine rats were tested during adolescence (Experiment 1, 12 subjects; Experiment 2a, 61 subjects; Experiment 2b, 36 subjects), and 118 rats were tested at PD67–71 (i.e., adults: Experiment 1, 30 animals; Experiment 2a, 58 animals; Experiment 2b, 30 subjects). Both males and females were tested. The litter representation was as follows: Experiment 1, 9 litters; Experiment 2a, 30 litters; Experiment 2b, 16 litters. To eliminate confounds between litter and treatment effects, no more than one subject per litter was assigned to the same condition (Holson and Pearce, 1992). Experimental procedures complied with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and were approved by the Institutional Animal Care and Use Committee within a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Surgical procedures

Animals were implanted with polyethylene tubing cannulae on P30 (adolescents) or P68 (adults). This device allowed control over the amount and timing of the intraoral stimulation provided by sucrose infusion (CS1). The surgical procedures were similar to those described in Pautassi et al. (2008b) (also see Kiefer, 1995; Kiefer et al., 2005). Briefly, animals were anesthetized with isoflurane (by means of 2.5% vapor, oxygen carrier: 55 psi). After appropriate antiseptic procedures, an incision was made in the cheek using a thin-walled 14-gauge disposable needle (Harvard Instruments, Columbus, OH). PE10 polyethylene tubing (10-cm section; Clay-Adams, Parsippany, NJ) was run through the needle, which was then removed. Heat was applied to one end of the tubing, thus creating a small flange. The tubing

was pulled through the medial internal surface of the cheek such that the flanged end of the cannula rested over the oral mucosae while the remainder exited from the mouth. Another needle was subsequently inserted at the back of the neck and was guided subcutaneously to exit close to the tubing site. The tubing was then run through the needle, thus exiting on the top of the neck where it was secured with a fast-acting adhesive (SuperGlue, Santa Anita, CA). Completion of the procedure took approximately 10 min per animal. Animals remained isolated thereafter to avoid damage to the tubing.

Drug preparation and administration procedures

The 3.0, 3.25, and 0.0 g/kg ethanol doses were achieved by i.g. administration of 0.015 mL/kg of 25.2, 27.3, or 0.0% ethanol solutions, respectively (190-proof ethanol; Pharmaco, Brookfield, CT). For i.g. administration of ethanol, a 20-cm section of Clay-Adams polyethylene-10 or 50 tubing (for adolescent and adult rats, respectively) was connected to a 5-mL syringe through a 27½-gauge needle, inserted into the oral cavity and gently guided into the rat's stomach before the actual delivery of ethanol. The intubation took about 5 s, and ethanol was then delivered in 3–4 s.

Conditioning and testing procedures

Experiment 2 used the SOC protocol developed by Molina et al. (2006, 2007) and later adapted for use in adolescent rats by Pautassi et al. (2008b). In this preparation, animals are given pairings of ethanol and intraoral infusion of sucrose (CS1, first-order conditioning phase), followed by pairings of sucrose and a chamber lined with sandpaper (CS2, SOC). Preference or aversion for the CS2 is then assessed in a two-way location preference paradigm (Fig. 1). An important difference between this and previous (Molina et al., 2006, 2007; Pautassi et al., 2008b) SOC protocols is that control animals were intubated with vehicle instead of being given unpaired exposure to ethanol and CS1. The unpaired control condition addresses several caveats, such as the possibility of nonspecific changes (i.e., habituation, sensitization) caused by the mere exposure to the unconditioned stimulus (US) or the CS. The "vehicle + CS exposure" control accounts only for nonspecific changes caused by handling and for CS pre-exposure. The rationale for using the vehicle control condition was that Pautassi et al. (2008b) treated unpaired controls with both low (0.5 g/kg) and high (2.0 g/kg) doses of ethanol and found no evidence for unspecific or deleterious effects of ethanol on SOC. Specifically, the level of preference for sandpaper, after sandpaper–sucrose pairings, was similar in unpaired groups given low- or high-dose ethanol, with paired animals expressing first-order preferences for sucrose and transferring that learning to sandpaper (Pautassi et al., 2008b). A more detailed account of conditioning procedures used in the present study is as follows.

Phase 1 (first-order conditioning)

This phase occurred on PD32, PD33, and PD34 for adolescent subjects given three conditioning trials (Experiment 2b). Rats that received only one or two trials (Experiment 2a) were conditioned on PD32 or on PDs32–33, respectively. Adult animals were given one (PD70), two (PD70–71), or three (PD70–72) conditioning trials. During each daily session, animals were individually placed in a square-shaped chamber (sides and height: 23 cm, lined with cotton). They remained in the chambers for 10 min (habituation phase). The animals were then weighed to the nearest 0.01 g (Sartorius, Gottingen, Germany) and were given ethanol i.g. at a dose of 3.25 or 3.0 g/kg for adults and adolescents, respectively, or its vehicle (tap water; i.e., 0.0 g/kg ethanol). The rats were then returned to their individual holding chambers until 30 min postadministration. Exposure to the CS1 occurred in the square-shaped chambers. Specifically, animals received intraoral pulses of sucrose (CS1; 10% vol/vol; 9 μL per pulse; pulse duration, 5 s; interpulse interval, 55 s) 30–45 min postadministration. This postadministration time was selected on the basis of a previous study conducted in infant rats (Molina et al., 2007). Delivery of sucrose was conducted by slipping the free end of the cannula inside a second polyethylene tube (PE10), which, in turn, was connected to a Gilmont syringe (Barnant Co., Barrington, IL) mounted on a rotary microsyringe infusion pump. Sucrose (Sigma-Aldrich, St. Louis, MO) was prepared daily using distilled water as a vehicle. The experiment was conducted under bright room illumination (four 25-W fluorescent lamps located in the wall opposite the chambers).

Phase 2 (second-order conditioning)

Twenty-four hours after termination of the last conditioning session, animals were confined, by means of an acrylic barrier, to one section of the box used for evaluation of CPP (see Phase 3 later). This section (CS2) was made distinctive by visual and tactile cues. Specifically, the compartment ($28 \times 20 \times 21$ cm) had alternating vertical black and white stripes and was lined with a rough sandpaper sheet (Gatorgrit, 60 grit; Ali Industries, Fairborn, OH). While in this compartment, animals were briefly stimulated with 10% sucrose (four 9-μL pulses; interstimulus interval, 55 s; trial duration, 4 min).

Phase 3 (location preference test)

Thirty minutes after Phase 2, animals were tested in a 12-min location preference test. The apparatus consisted of three interconnected chambers ($28 \times 20 \times 21$ cm each) with a central area made of white Plexiglas and lined with Formica and two end compartments. The animals had already experienced being in one of these compartments that contained a vertical striped pattern on the walls with a sandpaper floor (CS2), during Phase 2. The other compartment had a horizontal striped diagram on the walls with a smooth floor (the reverse side of a sandpaper sheet). Sandpaper sheets

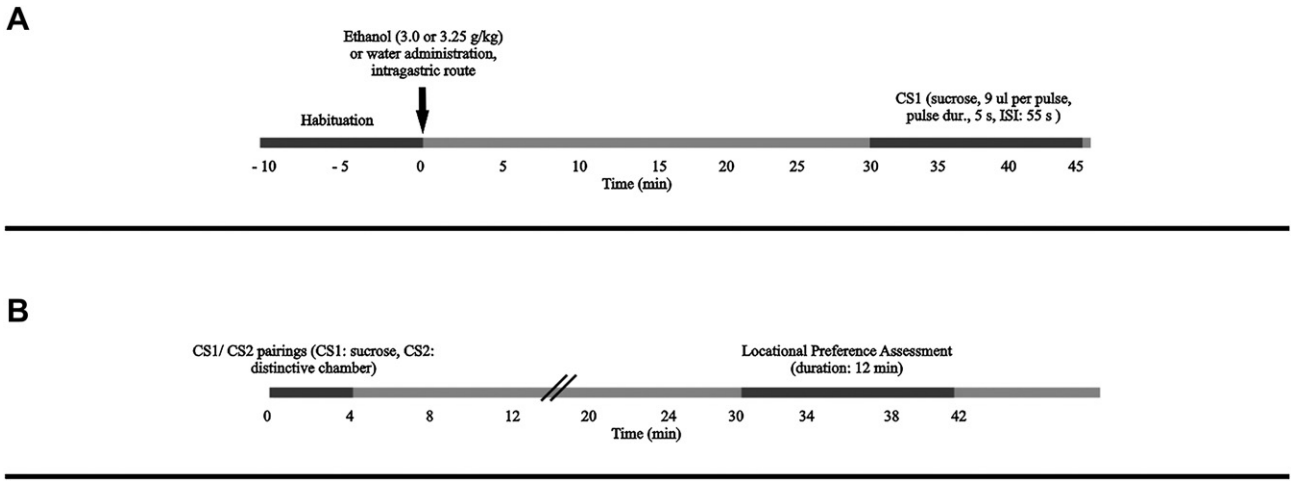

Fig. 1. Methods for the analysis of the motivational properties of high-dose ethanol in adolescent and adult rats. A. *Phase 1: first-order conditioning*—Animals underwent an initial, nonreinforced habituation phase (10-min duration). After habituation, they were administered ethanol (3.0 or 3.25 g/kg, i.g., for adolescent and adult rats, respectively) or its vehicle (tap water) and were then given CS1 consisting of intraoral pulses of sucrose. CS1 delivery occurred 30–45 min after the i.g. administration. B. *Phase 2: second-order conditioning*—Animals were briefly stimulated with 10% sucrose (4-min duration) while placed in a visually and tactilely distinctive chamber (CS2). B. *Phase 3: locational preference test, PD33 or PD71*—The time spent in the CS2 chamber was recorded in a 12-min place preference test. i.g. = intragastric, ISI = interstimulus interval, PD = postnatal day, CS = conditioned stimulus.

were replaced for each new test. During this phase, barriers separating the compartments were absent; therefore, animals could freely explore the three-chamber test box. The position of the test box during testing was the same as that in Phase 2 of conditioning to keep potential distal spatial cues constant, which could signal CS1 delivery (Cunningham et al., 2006). Preference assessment began by placing the animal in the central portion of the neutral compartment. The time spent over each end compartment of the apparatus was recorded. A subject was considered to be in a particular compartment when two paws and the head were over that section. Time spent in the middle section of the apparatus (i.e., start box) was not considered in the calculation of percentage time. The apparatus was cleaned with distilled water after each animal was tested.

Data analysis

The main dependent variable in Experiment 2 was absolute time spent in the sandpaper-lined compartment (CS2) during the location preference test. Additionally, the percent time spent in the compartment was analyzed. The latter variable measured time in CS2 compared with time spent in the smooth-floor compartment. The time spent in the central compartment was not taken into account. Percent time was calculated as follows: *(total time spent over sandpaper* $\times$ *100)/(total time spent over sandpaper* $+$ *total time spent over smooth)*.

These variables were analyzed by separate three-way (Experiment 2b) or four-way (Experiment 2a) mixed-factor analyses of variance (ANOVAs). The between-group factors were age (adolescence or adulthood); sex (male or female); and drug treatment during conditioning (ethanol or its vehicle, tap water). In Experiment 2a, the ANOVA also included the factor length of training (i.e., number of conditioning trials conducted during the first-order conditioning phase: one or two conditioning trials). The loci of significant main effects or interactions were further examined using follow-up ANOVAs and post hoc comparisons (Fisher least significant difference tests). Values of $P < .05$ were considered statistically significant. BECs and brain ethanol concentrations (BrECs) were analyzed separately using two-way ANOVAs (see Experiment 1 for details).

Experiment 1

When assessing ontogenetic differences in ethanol's behavioral effects, verifying that similar alcohol levels are achieved across age after drug administration is critical (Walker and Ehlers, 2008). The objective of this first experiment was to find equivalence of ethanol levels across ages. To achieve this aim, adolescent rats were given 3.0 g/kg ethanol, and adults were administered 3.0, 3.25, or 3.5 g/kg ethanol. BECs and BrECs were measured at a postadministration time (32.5 min) that represented a time point close to the onset of the conditioning trial that was used in the SOC procedure. Specifically, during Phase 1 of conditioning, sucrose exposure occurred 30–45 min after ethanol intubation. This experiment was a necessary step for the subsequent study aimed at analyzing the expression of ethanol-mediated SOC.

Experimental design and procedures

The design consisted of eight independent groups defined by the age and sex of the animals and the ethanol dose

administered. Adolescents (PD32) were given 3.0 g/kg ethanol, whereas adults (PD70) were administered 3.0, 3.25, or 3.50 g/kg ethanol. Both males and females were used. Groups were composed of 8–12 subjects each. Animals were individually placed in pine-shaving-lined containers, with brain and blood ethanol samples taken 32.5 min after ethanol administration. The procedure was similar to that described in Pautassi et al. (2008b). Briefly, blood samples obtained through decapitation were centrifuged at high speed (3000 rpm, 15 min, Micro-Haematocrit Centrifuge; Hawksley & Sons, Sussex, England) and then processed using an AM1 Alcohol Analyzer (Analox Instruments, Lunenburg, MA, USA). Whole brains were collected, sonicated in a water solution (Silveri and Spear, 2000), and analyzed for ethanol using a head-space gas chromatograph (Hewlett Packard 5890 series II; Hewlett Packard, Wilmington, DE). BECs and BrECs were expressed as milligrams of ethanol per deciliter of body fluid (mg/dL = mg%).

Results

Figure 2 depicts BECs and BrECs in adolescent and adult animals. BECs and BrECs were analyzed using separate two-way ANOVAs [(sex male or female) × group assignment (PD32/3.0, PD70/3.0, PD70/3.25, or PD70/3.50, in which the decimal numbers indicate the ethanol doses received by the subjects)]. The BEC and BrEC data yielded very similar results (Fig. 2). A significant main effect of group assignment was found (BEC: $F_{3,32} = 6.42$, $P < .005$; BrEC: $F_{3,32} = 6.36$; $P < .005$). Subsequent post hoc tests revealed that adults given 3.0 g/kg ethanol had lower BECs and BrECs than adolescents administered 3.0 g/kg ethanol. In contrast, BECs and BrECs assessed in the latter group (PD32/3.0) were equivalent (i.e., not statistically different) to those found in adult rats administered 3.25 g/kg ethanol. Post hoc analysis also indicated that the PD70/3.50 group had higher BECs and BrECs than any other group. The analyses showed no significant main effects of sex or significant interactions with this factor.

Consistent with other reports (Pautassi et al., 2008b; Walker and Ehlers, 2008), these results suggest faster ethanol metabolism after i.g. ethanol administration in mature rats than that in juvenile rats. Considering these results, subsequent experiments tested motivational consequences associated with i.g. administration of 3.0 and 3.25 g/kg in adolescent and adult rats, respectively.

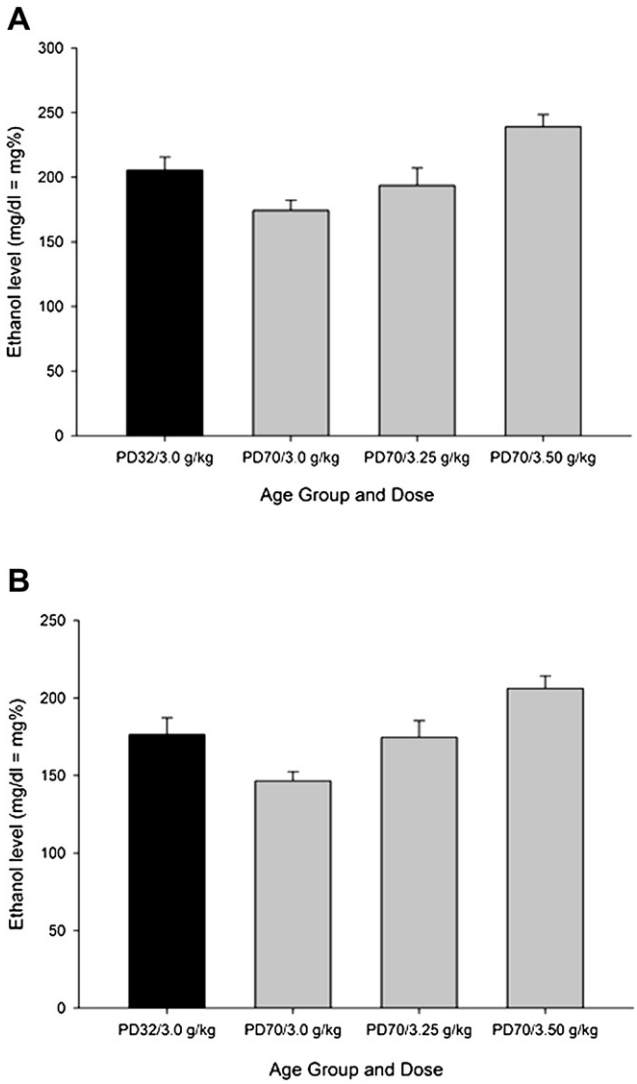

Fig. 2. Blood and brain ethanol levels ([A] blood ethanol concentrations and [B] brain ethanol concentrations; mg/dL = mg%) in adolescent rats given 3.0 g/kg ethanol (Group PD32/3.0) and adult rats given 3.0, 3.25, or 3.50 g/kg ethanol (Groups PD70/3.0, PD70/3.25, and PD70/3.5, respectively). Blood and brain samples were collected 32.5 min postadministration of ethanol (intragastric). Data were collapsed across sex (male or female). The sex factor did not exert a significant main effect or significantly interact with the remaining variables. Vertical bars indicate the standard error of the mean. PD = postnatal day.

Experiment 2

The present experiment assessed the motivational effects of high-dose ethanol (3.0 and 3.25 g/kg for adolescent and adult rats, respectively) in adolescent and adult rats using a similar SOC procedure. The preparation was similar to that previously used to detect the appetitive effects of low- and moderate-dose ethanol in adolescent and infant rats (Molina et al., 2006, 2007; Pautassi et al., 2008b). Animals were given one, two (Experiment 2a), or three (Experiment 2b) pairings of an intraoral tastant (sucrose, CS1) with ethanol's effects. Ethanol doses were derived from Experiment 1, which found similar BECs and BrECs in adolescent and adult rats given 3.0 and 3.25 g/kg, respectively.

Experiment 2a

Experimental design

The design was a 2 × 2 × 2 × 2 factorial. Experimental subjects were divided into 16 groups defined by age

(adolescence or adulthood); sex (male or female); drug treatment (ethanol or its vehicle, tap water); and length of training (one or two conditioning trials). Each condition consisted of six to nine subjects.

Conditioning and testing procedures

A full account of the second-order procedure has been provided in the General methods section (Fig. 1). Briefly, rats were given one or two pairings of water or ethanol (3.0 and 3.25 g/kg for adolescent and adult rats, respectively) and a novel tastant (sucrose, CS1). Sucrose was delivered 30–45 min postadministration in a pulsate pattern by means of an infusion pump connected to an intraoral cannula. Twenty-four hours after the last conditioning session, animals were briefly re-exposed to the taste CS1 while confined to a distinctive chamber (CS2). Thirty minutes thereafter, the animals were tested for their preference for CS2 in a three-way preference test. The preference/avoidance observed toward the section of the test cage originally paired with the ethanol-related CS1 was considered an index of the motivational properties of ethanol.

Results

As depicted in Fig. 3A, animals treated with ethanol during conditioning spent less absolute time on the sandpaper-lined compartment (CS2) of the testing cage than animals that had been given vehicle. The corresponding ANOVA indicated that this pattern, indicative of ethanol-mediated aversive place learning, was similar across age (adolescence or adulthood), sex (female or male), and length of training (one or two trials). Specifically, the ANOVA revealed a significant main effect of drug treatment ($F_{1,103} = 6.56$; $P < .05$) that failed to significantly interact with the remaining variables. Percent preference for the CS2 is shown in Fig. 3B. Visual inspection of Fig. 3 appears to indicate that the length of training had a differential effect on conditioned aversion in adolescent versus adult rats. This impression received some support from the inferential analyses. The ANOVA for percent preference yielded a complex pattern of results. Significant main effects of gender and drug treatment were observed ($F_{1,103} = 5.10$ and 7.63; $P < .05$ and $< .001$, respectively). The following interactions also achieved significance: length of training × drug treatment ($F_{1,103} = 5.02$, $P < .05$) and length of training × drug treatment × age ($F_{1,103} = 5.66$, $P < .05$). The four-way interaction (gender × length of training × drug treatment × age) yielded a trend toward significance ($F_{1,103} = 3.10$, $P = .081$). To better understand these interactions, follow-up ANOVAs (gender × drug treatment × length of training) were performed for each age group. With adult animals, only significant main effects of drug treatment and gender were observed ($F_{1,50} = 11.91$ and 4.37; $P < .005$ and $< .05$, respectively). Females spent more percent time in CS2 (46.13 ± 2.26) than males (40.18 ± 1.92). More importantly, ethanol treatment in adults resulted in significantly less time on CS2 (sandpaper), indicating ethanol-induced second-order aversion for the texture.

When considering adolescent rats, the ANOVA revealed a significant drug treatment × length of training interaction ($F_{1,53} = 8.22$, $P < .01$). Subsequent post hoc analysis revealed that percent time spent in the sandpaper-lined chamber (CS2) was lower in ethanol-treated adolescents given one, but not two, conditioning trials compared with their appropriate vehicle-treated controls, with adolescents given one conditioning trial exhibiting a significantly lower predilection for CS2 than their counterparts conditioned with ethanol in two conditioning trials.

Visual inspection of Fig. 3 may suggest that differences occurred between control groups in time spent in CS2 as a function of age and length of the first-order training. This supposition, however, was not corroborated by the inferential analysis. Specifically, two-way ANOVAs conducted on animals that received vehicle indicated a lack of significant main effects or significant interactions. This is an important result indicating that, at this level of training, no age-related differences were observed in terms of basal level of responsiveness toward the target CS or, looking at it from the converse perspective, for the novel portion of the apparatus.

Experiment 2b

The main result of Experiment 2a was that after one or two pairings of high-dose ethanol and a distinctive taste (CS1), the ethanol-paired tastant endowed a paired tactile/spatial cue (CS2) with aversive, second-order reinforcing capabilities. Results of the ANOVA for percentage or absolute time spent on CS2 indicated that age did not affect selection of CS2. In other words, conditioned aversion was not statistically different for adolescents and adults. However, within-age analyses conducted on the percent preference data revealed a potential ontogenetic difference as a function of length of training, with less evidence of aversion in adolescents, but not in adults, after two conditioning trials than after only one. Experiment 2b was conducted to assess whether age-related differences in ethanol-mediated conditioned aversion might emerge after more prolonged training. Adolescents and adults were given three conditioning trials, that is, three pairings of an intraoral tastant and the postabsorptive effects of ethanol. Animals were then briefly exposed to the CS1–CS2 pairing followed shortly thereafter by assessment of CS2 preference. By replicating a substantial part of the procedures of Experiment 2a, we also sought to assess the stability and reliability of the SOC preparation for detecting aversive, ethanol-mediated learning.

Experimental design and procedures

A 2 (age: adolescence or adulthood) × 2 (sex: male or female) × 2 (drug treatment: ethanol or water) design was

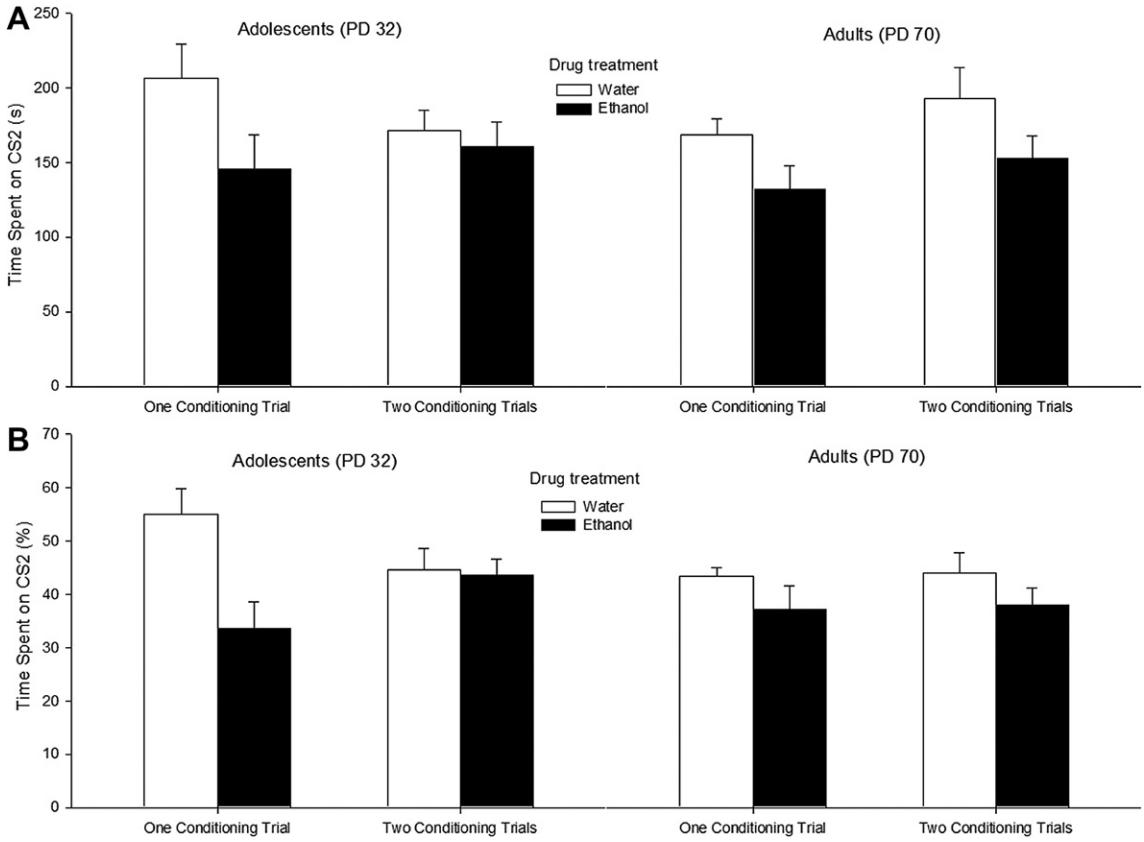

Fig. 3. Total time (s) spent in the sandpaper-lined chamber ([A] CS2) and the corresponding percent preference for the chamber (B) during the 12-min test session as a function of drug treatment during conditioning (ethanol or water), length of training (one or two first-order conditioning trials), and age of the subjects (adolescence or adulthood). Data were collapsed across sex (male or female). Vertical bars indicate the standard error of the mean. CS = conditioned stimulus.

used. Each of the eight groups consisted of 7–10 subjects each.

Procedures

Conditioning and testing procedures followed those described in Experiment 2a, with the exception that the first-order conditioning phase had one more training episode: adolescents were given daily pairings of ethanol and intraoral sucrose on PD32, PD33, and PD34, whereas adults received similar training on PD70, PD71, and PD72. The SOC phase (i.e., CS1–CS2 pairings) and the location preference assessment were conducted 24 h after the last conditioning session.

Results

Figure 4 illustrates ethanol-induced conditioned place aversion, with the magnitude of this avoidance response similar across age groups. These observations were supported by the corresponding statistical analysis. The ANOVAs for absolute and relative time spent in the sandpaper-lined chamber yielded very similar results. Age (absolute time: $F_{1,58} = 8.51$, $P < .01$; percent time: $F_{1,58} = 5.06$, $P < .05$) and dose (absolute time: $F_{1,58} = 13.03$, $P < .001$; percent time: $F_{1,58} = 8.98$, $P < .005$) exerted independent significant main effects. Adult animals spent significantly more time in CS2 than their younger counterparts, regardless of the condition. Furthermore, absolute and percent time in CS2 was significantly lower in ethanol-treated subjects than in controls given CS1–water pairings, thus revealing aversive conditioning supported by high-dose ethanol.

Discussion

The present study assessed the motivational effects of high-dose ethanol using SOC. Before comparing adolescent and adult subjects in ethanol-mediated learning, ethanol doses yielding equivalent BECs and BrECs across age were determined. Generally, ethanol elimination rates tend to increase across ontogeny when ethanol is infused i.g. For example, Kelly et al. (1987) found an increase from 7.5 mg/dL/h in neonatal stage to 42.2 mg/dL/h by young adulthood after i.g. challenges with ethanol. Moreover, adult Wistar rats appear to metabolize ethanol faster than the adolescent rats (Walker and Ehlers, 2008). Adolescent rats were recently shown to exhibit significantly higher BECs than adult rats 30 min after being administered 2.0 g/kg ethanol i.g. (Pautassi et al., 2008b). Consistent

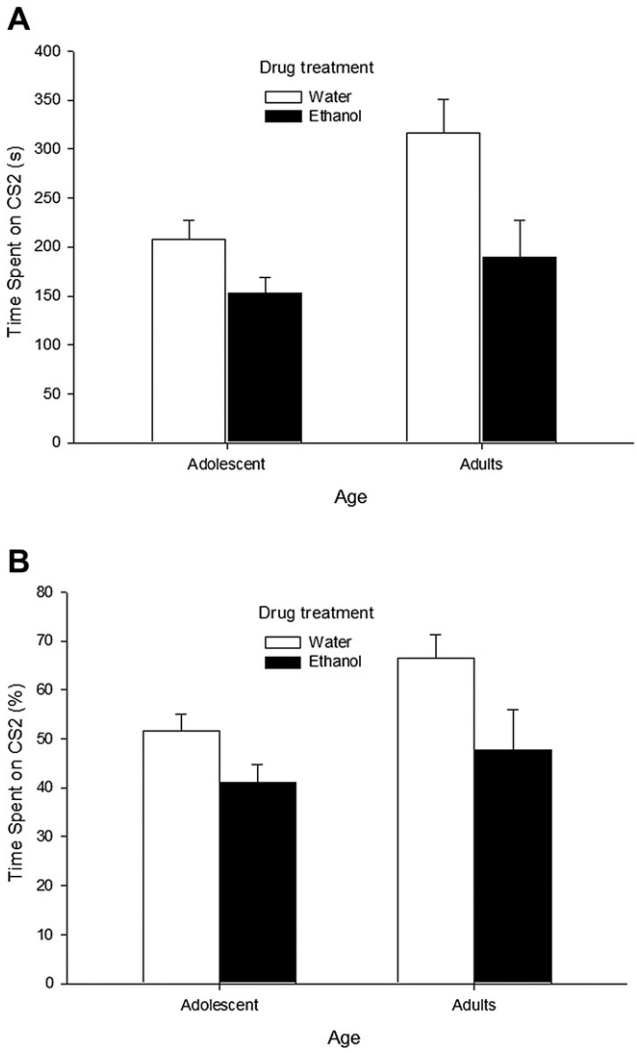

Fig. 4. Total time (s) spent in the sandpaper-lined chamber ([A] CS2) and the corresponding percent preference for the chamber (B) during the 12-min test session after three first-order-conditioning sessions. Data are plotted as a function of drug treatment during conditioning (ethanol or water) and age of the subjects (adolescence or adulthood). Data were collapsed across sex (male or female). The sex factor did not exert a significant main effect or significantly interact with the remaining variables. Vertical bars indicate the standard error of the mean.

with these previous reports, the present study found greater BECs and BrECs in adolescent rats than in adult Sprague–Dawley rats 32.5 min after administration of 3.0 g/kg ethanol (BEC: 205 vs. 174 mg%; BrEC: 176 vs. 146 mg %; adolescents and adults, respectively; Fig. 1). In contrast, BECs and BrECs did not differ statistically across age when adolescent and adult rats were given 3.0 and 3.25 g/kg ethanol, respectively. The latter doses were used in the subsequent behavioral experiment.

In Experiment 2, animals were trained in an SOC procedure, with high-dose ethanol as the US. Ethanol-treated animals exhibited reduced preference for the target CS at test compared with the controls. This result, indicative of ethanol-mediated second-order aversive place learning, was substantially similar across age (adolescence or adulthood) and length of training (one, two, or three trials). Important information provided by the present study was that sex did not appear to affect the development of ethanol-induced aversions.

It is conceivable that the reduced preference for CS2 after CS1–ethanol pairings reflect causes other than ethanol-induced aversive learning. Repeated ethanol exposure could have resulted in nonspecific changes (i.e., sensitization, habituation) that affected the motivational value of sucrose or the ability to learn from second-order pairings. Also, prior treatment with ethanol could have resulted in hangover effects 24 h after the last ethanol administration. If so, the conditioned aversion could have been favored, because the CS2 would have been paired with hangover, and perhaps, differentially across age. The use of vehicle-treated instead of unpaired controls implies that these possibilities cannot be ruled out. Yet, it would be expected that the magnitude of the hangover would be greater as a function of repeated treatment. Evidence for aversive conditioning was, however, as robust after one training trial as three trials at both ages. Studies suggest that ethanol is more likely to exert detrimental effects on hippocampus- than in non-hippocampus-dependent tasks (Hunt et al., 2009), and that these inhibitory effects are greater in adolescents than in adults (Markwiese et al., 1998; White and Swartzwelder, 2005). If ethanol exposure has nonspecific detrimental effects on the encoding of secondary conditioned aversion, age differences in the expression of this learning would be expected. That was not the case in the present set of experiments. Also, the rat studies revealing ethanol-induced cognitive impairment usually use drug exposure schedules that are longer and more concentrated that the one used in the present work. For instance, Obernier et al. (2002) observed cognitive and neural deficits in rats given ethanol three times daily for 4 days.

It is useful to consider the present findings within the framework of Varlinskaya and Spear's (2008) study (also see Anderson et al., 2008; Vetter-O'Hagen et al., 2009). These authors observed age-related differences in ethanol-mediated CTA. Adult, but not adolescent, rats expressed CTA after 1.0 and 1.5 g/kg ethanol i.p., whereas adolescent rats showed CTA only at a higher dose (2.0 g/kg). The fact that the present SOC experiment does not report age-related differences, but rather similar aversive conditioning at both ages, might be explained by procedural differences. One obvious difference is the higher dose used in the present study. Age-related differences in SOC can be postulated to emerge at low or moderate, but not high, ethanol doses. Previous data support this possibility. As stated earlier, adolescent, but not adult, rats show appetitive SOC when using 0.5 or 2.0 g/kg ethanol (Pautassi et al., 2008b).

With regard to the generality of the SOC results, in the present experiments, animals are first intubated with ethanol and later (30–45 min postintubation) exposed to

the CS1. This CS–US design could be argued to involve backward conditioning (i.e., CS follows the US; Minnier et al., 2007). Thus, the sensitivity of SOC for detecting ethanol-induced learning may be related to possible age-related differences in backward conditioning. One could also wonder what would have happened if the order of presentation of CS and US had been reversed. This is an interesting, empirical question that would require to systematically vary the interval between intubation and CS onset (e.g., −60; −30; 0 [i.e., simultaneous conditioning]; 10; 20; or 30 min; for a similar strategy, see Bormann and Cunningham (1998)). Previous mice studies indicate that manipulation of injection timing can alter the direction of CPP by ethanol (Cunningham et al., 2002). The present study, however, was based on the assumption that the unconditional stimulus being conditioned was probably not the intubation in itself but rather the aversive, postabsorptive effects of ethanol. Under this assumption the study can be better described as using a simultaneous or delayed conditioning. More in detail, the postadministration timing of exposure to the CS (30–45 min) was selected to maximize contiguity between the CS and an interval characterized by peak BEC and, therefore, more likely to induce conditioned aversion than earlier stages of the intoxication. It has been found that the hedonic effects of ethanol vary from appetitive to aversive as a function of the course of the toxic process (Molina et al., 2006; Pautassi et al., 2002, 2006).

Experiment 2b also indicated that adult animals spent significantly more time in CS2 than their younger counterparts, a result that was not affected by dose or sex. This result is consistent with our earlier work that revealed less preference for the sandpaper-lined floor in adolescents than adults (Pautassi et al., 2008b). We discussed in that article that rats have ''natural preference scales'' (Rakover-Atar and Weller, 1997) for tactile and odor stimuli that may vary ontogenetically. It could also be the case that this reflected heightened novelty preference among adolescents relative to adults. Yet, in Experiment 2a, there were no differences in basal sandpaper preferences across age in the basic control conditions (i.e., water-treated animals), suggesting that this difference emerged across exposures.

It is notable that more extensive training (i.e., three first-order trials) in Experiment 2b relative to 2a resulted in higher levels of baseline preference for the sandpaper texture. This is an interesting phenomenon, particularly when considering that all groups were equivalent in terms of their experience with sandpaper, suggesting that the animals may have developed a pre-exposure effect toward the CS1 (i.e., an increase in preference for a given stimulus after repeated nonreinforced exposure; Chotro and Alonso, 1999). Subsequently, during the CS1–CS2 transfer phase, this increased preference may have transferred to the sandpaper texture (CS2). It should be noted, however, that this change in overall preference for the target CS did not affect the detection of significant ethanol-mediated aversion in adolescent and adult rats.

The main result of this report is that adolescent and adult rats appear to perceive the effects of high-dose ethanol as similarly aversive when assessed by SOC. Appetitive effects of ethanol in adolescent rats assessed by SOC have been associated with BECs in the range of 50–137 mg% (Pautassi et al., 2008b). This, together with the present study, suggests that, in adolescents assessed with SOC, ethanol's hedonics may fluctuate from appetitive to aversive as the ethanol dose increases. This switch from appetitive to aversive in this learning context appears to occur when BECs reach approximately 150–200 mg%.

Acknowledgments

This work was supported by NIAAA grants AA011960, AA013098, and AA015992 to N.E.S.; NIMH grant MH035219 to N.E.S.; NIAAA grants AA012525, AA16887, and AA018036 to L.P.S.; the Agencia Nacional de Promocion Cientifica y Tecnologica (PICT 05-14024) to J.C.M.; and grant PIP CONICET 2010-2012 (Argentina), PICT PRH Project 246 and SECYT-UNC to R.M.P. The authors wish to express their gratitude to Teri Tanenhaus, Carlos Martínez, and Judy Sharp.

References

Anderson, R.I., Varlinskaya, E.I., and Spear, L.P. (2008). Isolation stress and ethanol-induced conditioned taste aversion in adolescent and adult male rats. Paper presented at the 41st Annual Meeting of the International Society for Developmental Psychobiology, Washington DC, November 12–15.

Anthony, J. C., and Petronis, K. R. (1995). Early-onset drug use and risk of later drug problems. Drug Alcohol Depend. *40*, 9–15.

Bienkowski, P., Kostowski, W., and Koros, E. (1999). Ethanol-reinforced behaviour in the rat: effects of naltrexone. Eur. J. Pharmacol. *374*, 321–327.

Bormann, N., and Cunningham, C. (1998). Ethanol-induced conditioned place aversion in rats: effect of interstimulus interval. Pharmacol. Biochem. Behav. *59*, 427–432.

Chester, J. A., de Paula Barrenha, G., DeMaria, A., and Finegan, A. (2006). Different effects of stress on alcohol drinking behaviour in male and female mice selectively bred for high alcohol preference. Alcohol Alcohol. *41*, 44–53.

Chotro, M., and Alonso, G. (1999). Effects of stimulus preexposure on the generalization of conditioned taste aversions in infant rats. Dev. Psychobiol. *35*, 304–317.

Cunningham, C., Clemans, J., and Fidler, T. (2002). Injection timing determines whether intragastric ethanol produces conditioned place preference or aversion in mice. Pharmacology Biochemistry and Behavior *72(3)*, 659–668.

Cunningham, C. L., Fidler, T. L., and Hill, K. G. (2000). Animal models of alcohol's motivational effects. Alcohol Res. Health *24*, 85–92.

Cunningham, C. L., Niehus, J. S., and Noble, D. (1993). Species difference in sensitivity to ethanol's hedonic effects. Alcohol *10*, 97–102.

Cunningham, C. L., Patel, P., and Milner, L. (2006). Spatial location is critical for conditioning place preference with visual but not tactile stimuli. Behav. Neurosci. *120*, 1115–1132.

Davies, B. T., and Parker, L. L. (1990). Novel versus familiar ethanol: a comparison of aversive and rewarding properties. Alcohol *7*, 523–529.

Doremus, T. L., Brunell, S. C., Rajendran, P., and Spear, L. P. (2005). Factors influencing elevated ethanol consumption in adolescent relative to adult rats. Alcohol. Clin. Exp. Res. *29*, 1796–1808.

Greenfield, S. F. (2002). Women and alcohol use disorders. Harv. Rev. Psychiatry *10*, 76–85.

Holson, R. R., and Pearce, B. (1992). Principles and pitfalls in the analysis of prenatal treatment effects in multiparous species. Neurotoxicol. Teratol. *14*, 221–228.

Hunt, P. S., Levillain, M. E., Spector, B. M., and Kostelnik, L. A. (2009). Post-training ethanol disrupts trace conditioned fear in rats: effects of timing of ethanol, dose and trace interval duration. Neurobiol. Learn. Mem. *91*, 73–80.

Johnston, L. D., and O'Malley, P. M. (2007). *Monitoring the Future Study: National Survey Results on Drug Use, 1975-2006: Vol. 1, Secondary School Students* (NIH Pub. No. 07-6206). Bethesda, MD: National Institute on Drug Abuse.

Kelly, S. J., Bonthius, D. J., and West, J. R. (1987). Developmental changes in alcohol pharmacokinetics in rats. Alcohol. Clin. Exp. Res. *11*, 281–286.

Kiefer, S. (1995). Alcohol, palatability, and taste reactivity. Neurosci. Biobehav. Rev. *19*, 133–141.

Kiefer, S. W., Hill, K. G., Coonfield, D. L., and Ferraro, F. M. III (2005). Ethanol familiarity and naltrexone treatment affect ethanol responses in rats. Alcohol *37*, 167–172.

Markwiese, B., Acheson, S., Levin, E., Wilson, W., and Swartzwelder, H. (1998). Differential effects of ethanol on memory in adolescent and adult rats. Alcohol. Clin. Exp. Res. *22*, 416–421.

Miller, J. S., Molina, J. C., and Spear, N. E. (1990). Ontogenetic differences in the expression of odor-aversion learning in 4- and 8-day-old rats. Dev. Psychobiol. *23*, 319–330.

Minnier, E., Misanin, J. R., and Hinderliter, C. F. (2007). Age and interstimulus interval in forward and backward long-trace taste-aversion conditioning. Percept. Mot. Skills *105*, 1223–1226.

Molina, J. C., Ponce, L. F., Truxell, E., and Spear, N. E. (2006). Infantile sensitivity to ethanol's motivational effects: ethanol reinforcement during the third postnatal week. Alcohol. Clin. Exp. Res. *30*, 1506–1519.

Molina, J. C., Pautassi, R. M., Truxell, E., and Spear, N. (2007). Differential motivational properties of ethanol during early ontogeny as a function of dose and postadministration time. Alcohol *41*, 41–55.

National Research Council (1996). *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academy Press.

Obernier, J., White, A., Swartzwelder, H., and Crews, F. (2002). Cognitive deficits and CNS damage after a 4-day binge ethanol exposure in rats. Pharmacol. Biochem. Behav *72*, 521–532.

Pautassi, R., Godoy, J., Spear, N., and Molina, J. C. (2002). Early responsiveness to stimuli paired with different stages within the state of alcohol intoxication. Alcohol. Clin. Exp. Res. *26*, 644–654.

Pautassi, R. M., Myers, M., Spear, L. P., Molina, J. C., and Spear, N. E. (2008b). Adolescent but not adult rats exhibit ethanol-mediated appetitive second-order conditioning. Alcohol. Clin. Exp. Res. *32*, 2016–2027.

Pautassi, R. M., Nizhnikov, M. E., Acevedo, M. B., and Spear, N. E. (2011). Naloxone blocks ethanol-mediated appetitive conditioning and locomotor activation in adolescent rats. Behav. Brain. Res. *216*, 262–269.

Pautassi, R., Sanders, S., Miller, S., Spear, N. E., and Molina, J. C. (2006). Early ethanol's anxiolytic effects assessed through an unconditional stimulus revaluation procedure. Alcohol. Clin. Exp. Res. *30*, 448–459.

Pedersen, W., and Skrondal, A. (1998). Alcohol consumption debut: predictors and consequences. J. Stud. Alcohol *59*, 32–42.

Philpot, R. M., Badanich, K. A., and Kirstein, C. L. (2003). Place conditioning: age-related changes in the rewarding and aversive effects of alcohol. Alcohol. Clin. Exp. Res. *27*, 593–599.

Rakover-Atar, S., and Weller, A. (1997). The influence of natural preference for tactile stimuli on appetitive learning in rat pups. Dev. Psychobiol. *30*, 29–39.

Ristuccia, R. C., and Spear, L. P. (2005). Sensitivity and tolerance to autonomic effects of ethanol in adolescent and adult rats during repeated vapor inhalation sessions. Alcohol. Clin. Exp. Res. *29*, 1809–1820.

Ristuccia, R. C., and Spear, L. P. (2008). Adolescent and adult heart rate responses to self-administered ethanol. Alcohol. Clin. Exp. Res. *32*, 1807–1815.

Schramm-Sapyta, N. L., Morris, R. W., and Kuhn, C. M. (2006). Adolescent rats are protected from the conditioned aversive properties of cocaine and lithium chloride. Pharmacol. Biochem. Behav. *84*, 344–352.

Silveri, M. M., and Spear, L. P. (2000). Ontogeny of ethanol elimination and ethanol-induced hypothermia. Alcohol *20*, 45–53.

Silveri, M. M., and Spear, L. P. (2001). Acute, rapid, and chronic tolerance during ontogeny: observations when equating ethanol perturbation across age. Alcohol. Clin. Exp. Res. *25*, 1301–1308.

Spear, L. P. (2000). The adolescent brain and age-related behavioral manifestations. Neurosci. Biobehav. Rev. *24*, 417–463.

Spear, L. P. (2004). Adolescence and the trajectory of alcohol use: introduction to part VI. Ann. N.Y. Acad. Sci. *1021*, 202–205.

Swartzwelder, H. S., Richardson, R. C., Markwiese-Foerch, B., Wilson, W. A., and Little, P. J. (1998). Developmental differences in the acquisition of tolerance to ethanol. Alcohol *15*, 311–314.

Varlinskaya, E. I., and Spear, L. P. (2002). Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. Alcohol. Clin. Exp. Res. *26*, 1502–1511.

Varlinskaya, E. I., and Spear, L. P. (2008). Attenuated aversive effects of ethanol among adolescent rats are diminished further in adolescent males by the presence of a social partner. Alcohol. Clin. Exp. Res. *32(Suppl 1)*, 94A.

Vetter-O'Hagen, C., Varlinskaya, E., and Spear, L. (2009). Sex differences in ethanol intake and sensitivity to aversive effects during adolescence and adulthood. Alcohol Alcohol. *44*, 547–554.

Walker, B. M., and Ehlers, C. L. (2008). Age-related differences in the blood alcohol levels of Wistar rats. Pharmacol. Biochem. Behav. *91*, 560–565.

White, A. M., and Swartzwelder, H. S. (2005). Age-related effects of alcohol on memory and memory-related brain function in adolescents and adults. Recent Dev. Alcohol. *17*, 161–176.

White, A. M., Truesdale, M. C., Bae, J. G., Ahmad, S., Wilson, W. A., Best, P. J., et al. (2002). Differential effects of ethanol on motor coordination in adolescent and adult rats. Pharmacol. Biochem. Behav. *73*, 673–677.