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ETHANOL-INDUCED LOCOMOTOR ACTIVITY IN ADOLESCENT RATS AND THE RELATIONSHIP WITH ETHANOL-INDUCED CONDITIONED PLACE PREFERENCE AND CONDITIONED TASTE AVERSION

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Abstract

Adolescent rats exhibit ethanol-induced locomotor activity (LMA), which is considered an index of ethanol's motivational properties likely to predict ethanol self-administration, but few studies have reported or correlated ethanol-induced LMA with conditioned place preference by ethanol at this age. The present study assessed age-related differences in ethanol's motor stimulating effects and analysed the association between ethanol-induced LMA and conventional measures of ethanol-induced reinforcement. Experiment 1 compared ethanol-induced LMA in adolescent and adult rats. Subsequent experiments analyzed ethanol-induced conditioned place preference and conditioned taste aversion in adolescent rats evaluated for ethanol-induced LMA. Adolescent rats exhibit a robust LMA after high-dose ethanol. Ethanol-induced LMA was fairly similar across adolescents and adults. As expected, adolescents were sensitive to ethanol's aversive reinforcement, but they also exhibited conditioned place preference. These measures of ethanol reinforcement, however, were not related to ethanol-induced LMA. Spontaneous LMA in an open field was, however, negatively associated with ethanol-induced CTA.

Keywords

adolescence; rats; locomotor activation; ethanol reinforcement

Introduction

Adolescent consumption of alcohol is a significant public health problem. Lifetime prevalence of alcohol consumption in the US is 72.3% in 17 year olds (Johnston, O'Malley, Bachman & Schulenberg, 2009). Developing countries exhibit similar prevalence of alcohol use and abuse. Lifetime prevalence of alcohol consumption in adolescents 12–17 years old is around 50% in Brazil (Galduróz & Carlini 2007), Argentina (Alderete, Kaplan, Nah,

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Pérez-Stable, 2008) and México (Rojas-Guiot, Fleiz-Bautista, Medina-Mora Icaza, Móron & Domenech-Rodríguez., 1999). Alcohol intake during adolescence can lead to immediate negative consequences (e.g., poor academic performance; Cooper, 2002), and it has long-term detrimental consequences as well. An early onset of alcohol drinking is associated with heightened probability of alcohol-related problems later in life (DeWit, Adlaf, Offord & Ogborne, 2000).

Why adolescents exhibit such avidity for alcohol intake and why early alcohol exposure is likely to modulate later alcohol consumption are questions of utmost importance. Animal studies indicate that when adolescent and adults are given equivalent ethanol doses, the younger animal display less sedation, narcosis and ataxia (see Spear, 2000). These effects likely serve as barriers preventing the escalation in alcohol consumption in adulthood and, therefore, the relative insensitivity of adolescents may put them at risk for problematic alcohol consumption.

Alcohol also induces appetitive and aversive reinforcing effects. These effects are particularly important for understanding why some individuals progress quickly from the use of alcohol to abuse and dependence and can be measured through conditioned place preference (CPP) and conditioned taste aversion (CTA) models (Pautassi, Nizhnikov & Spear, 2009). In these preparations animals are given ethanol and then exposed to a tactile/visual cue (CPP) or they are given access to a salient taste (CTA). Subsequent preference or aversion for these conditioned stimuli is considered a measure of ethanol-induced reinforcement. Very little is known, however, about the sensitivity to these effects during adolescence. Several studies have indicated that ethanol usually produces place aversion or no place conditioning when given to adult rats, particularly at doses equal or higher than 2.0 g/kg. CPP has sometimes been observed, however, when ethanol is co-administered with stress (Matsuzawa, Suzuki, Misawa & Nagase, 1999) or other drugs (Marglin, 1988), and also when young adult rats are employed (i.e., Wistars weighing 200–250 grs; Kotlinska, Bochenski & Danysz, 2011). To our knowledge, there is only one study that has found ethanol-induced CPP in adolescent rats based on first-order conditioning (Philpot, Badanich & Kirstein, 2003), although younger, preweanling rats do seem to find ethanol rewarding when tested through first-order CPP (Nizhnikov et al., 2009) or a second-order variant of CPP (Molina, Pautassi, Truxell & Spear, 2007). Recent work also indicated that adolescents are more resistant to ethanol-induced conditioned taste aversion (Anderson, Varlinskaya & Spear, 2010) than adults.

Ethanol-induced locomotor activity (LMA) has been proposed as another measure of ethanol-induced reinforcement (Quoilin, Didone, Tirelli & Quertemont, 2010). Rats, however, have been deemed mostly insensitive to the motor activating effects of systemic or peripherally administered ethanol (Masur, Oliveira de Souza & Zwicker, 1986; Cunningham, Niehus & Noble, 1993; Chuck, McLaughlin, Arizzi-LaFrance, Salamone & Correa, 2006). Yet recent studies indicate that preweanling rats are highly sensitive to ethanol-induced LMA, particularly when tested during the initial, rising phase of the blood ethanol curve (BEC; Arias, Molina, Mlewski, Pautassi & Spear., 2008). Less is known about the stimulatory effects of ethanol in adolescent rats. Recent work, however, revealed that intragastric ethanol administration (2.0–2.5 g/kg) significantly increased LMA during the rising phase of the BEC, an effect diminished by naloxone administration (Acevedo, Molina, Nizhnikov, Spear, Pautassi, 2010; Pautassi, Nizhnikov, Acevedo & Spear, 2011).

The present study assessed whether adolescent and adult rats differ in their susceptibility to ethanol-induced LMA. In Experiment 1 there were no age-related differences in ethanol-induced LMA. Subsequent experiments (Experiments 2 and 3) focused on adolescent rats

and analysed ethanol-induced CPP and CTA in animals previously evaluated for ethanol-induced or spontaneous LMA.

One of the aims was to detect appetitive place conditioning to ethanol in adolescent rats. It is important to develop rat models of ethanol reinforcement during adolescence, modelling a period when ethanol initiation and escalation usually occurs in humans. The availability of these models should allow further scrutiny of mechanisms underlying the ontogeny of ethanol reinforcement. Given the apparent difficulty in finding CPP in rats, our CPP procedure was based on those previous studies (Nizhnikov et al., 2009; Philphot et al., 2003) that successfully revealed conditioned tactile preference in infant or juvenile rats. Specifically, a salient tactile conditional stimulus was paired with the early phase of the blood ethanol curve, a period in which the appetitive reinforcing effects of ethanol are supposedly maximal.

The assessment for spontaneous or ethanol-induced LMA was meant to provide information to analyze the association between ethanol's stimulant effects and later susceptibility to ethanol appetitive or aversive reinforcement (i.e., CPP and CTA, respectively). The aim was to explore -- through a correlational approach -- possible behavioral differences that would distinguish between rats predisposed and those not predisposed to ethanol reinforcement. The rationale for choosing locomotor activity as the behavioral predictor was that it has been positively associated with ethanol intake (Acevedo et al., 2010). Rats genetically selected for high-alcohol intake -- such as Sardinian alcohol-preferring and Warsaw High Preferring rats -- display greater ethanol-induced stimulation than unselected controls (Colombo et al., 2006; Dyr & Kostowski, 2004). Sensitivity to ethanol-induced LMA has also been linked with sensitivity to ethanol reinforcement, which has, in turn, been associated with ethanol intake. Moreover, delayed habituation or enhanced locomotor activity in an open field after habituation has been associated with enhanced ethanol intake (Bisaga & Kostowski, 1993) and initial operant self-administration (Nadal, Armario & Janak, 2002). Also, the recent study by Acevedo et al. (2010) suggested that sensitivity to ethanol-induced LMA may predict ethanol intake during adolescence. In Acevedo et al. (2010) adolescent Wistar rats were divided between high- and low-responders in terms of ethanol-induced LMA or received exposure to only vehicle. When later tested for ethanol intake, ethanol-exposed animals drank more than vehicle-exposed controls. Perhaps more importantly, high-responder females exhibited an even greater increase in ethanol self-administration.

Less is known about the relationship between ethanol-induced LMA and ethanol reinforcement. An association between them, however, is predicted by theories of Wise and Bozarth (1987) and Robinson and Berridge (2008). The psychomotor stimulant theory of addiction (Wise & Bozarth, 1987) suggests that all addictive substances have the ability to evoke motor activity. These stimulant actions have a shared biological mechanism, homologous to that underlying the perception of appetitive reinforcement. In other words, drug-induced motor activation and reinforcement would be homologous phenomena, resulting from the activation of a shared brain mechanism, the mesocorticolimbic pathway. The theory, thus, predicts that drug-induced forward locomotion could be a predictor of sensitivity to drug-induced reinforcement. Some studies that employed ip ethanol, however, indicated a dissociation between the two variables (e.g. Chester & Cunningham, 1999; Risinger y cols, 1992). The theory proposed by Robinson & Berridge (2008) suggests that the compulsive pattern of drug seeking observed in addiction results from the sensitization of a motivational system that provides incentive salience to reward-related stimuli. The theory does not equate "incentive sensitization" to "locomotor sensitization". Psychomotor sensitization, however, can be considered an indirect proxy of the development of hypersensitivity in the system attributing incentive value to stimuli. It has also been observed that rats classified as high-responders as a function of activity in a novel

environment exhibited less sensitivity to ethanol-induced CTA than low-responders (Arias, Molina, Spear, 2009). Based on these theories and studies, our expectation was that spontaneous and alcohol-induced LMA would be positively and negatively associated with CPP and CTA scores, respectively.

Methods

General procedures

Subjects—A total of 179 Wistar rats [Wistar-King Aptekman Hokkaido (WKAH/Hok) inbred strain, 119 males and 60 females], representative of 40 litters, was employed in Experiments 1, 2 and 3 (Experiment 1: 39 animals, 8 litters; Experiment 2a: 49 animals; 7 litters; Experiment 2b: 20 animals; 12 litters; Experiment 3: 71 animals; 13 litters). Rats were born and reared at the Instituto Ferreyra (INIMEC-CONICET, Córdoba, Cba, Argentina). Births were examined daily and the day of parturition was considered postnatal day 0 (PD0). Pups were kept with their dam in standard maternity cages until weaning day at PD21. Weaned animals were housed in standard cages (45 × 30 × 20 cm) with continuous free access to water and food (four animal per cage) until the beginning of procedures on postnatal day 28 (PD28, adolescents) or PD74 (adults).

The colony was kept on a 12 hr. light/dark cycle (0800) at an ambient temperature of 22 ± 1 °C. During PD25–27 or PD72-73, animals were handled twice per day for 2 min. The handling was aimed at reducing the behavioral activation caused by the experimenter's manipulation. Across experiments and to eliminate confounds between litter and treatment effects, no more than one male and one female per litter were assigned to each particular cell of the experimental design. All experimental procedures were in compliance with the Guide for Care and Use of Laboratory Animals (National Research Council, 1996) and the guidelines indicated by the Ministry of animal care of INIMEC-CONICET.

Drug preparation and administration procedures—Ethanol was administered intragastrically (i.g.) via a 12-cm length of polyethylene-50 tubing (PE-50 Clay Adams, Parsippany, New Jersey, USA) attached to a 23-gauge needle on a 3 ml syringe (Becton Dickinson, Rutheford, NJ). Ethanol doses of 3.0, 2.5 or 1.0 g/kg resulted from the administration of a volume equivalent to 0.015 ml per gram of body weight of 25.2%, 21% or 8.4% v/v ethanol solutions, respectively (Porta Hnos., Córdoba, Argentina). An equivalent volume of tap water was administered as vehicle. All animals were gently intubated in about 5 sec, and solutions were then slowly delivered during 3 or 4 sec into the stomach.

Conditioning and Testing Procedures

Assessment of ethanol-induced LMA in adolescent and adult rats (Experiment 1): On PD28 or PD74, males rats were weighed (portable Ohaus L2000; Ohaus, Pine Brook, NJ) and given ethanol (2.5 g/kg) or vehicle. Rats were then returned to standard cages with pine shavings, where they remained until the beginning of the evaluation. Five minutes later animals were evaluated for locomotor activity during 7 minutes (post-administration time of 5–11 min; bins 1–7) in square chambers made of opaque black wood (30 × 30 × 30 cm or 50 × 50 × 50 cm; adolescent and adult rats, respectively) and lined with black rubber. The dosage and post-administration time was selected based on a study carried out in our laboratory (Acevedo et al., 2010). This study also indicated that sex did not affect the magnitude of ethanol-induced LMA. This served as the rationale for employing only males in this Experiment. The rationale for not starting LMA measurements immediately upon intubation was that it has been suggested (Cunningham, Tull, Rindal, & Meyer; 2002; Cunningham, Smith & McCullin, 2003) that ethanol administration generates a short-lived

initial aversive effect, probably related to the novelty of the transition from the sober to the intoxicated state.

All evaluations were conducted in a room illuminated by two fluorescent lamps situated in the centre, about 250 cm above the testing chambers. The dependent variables (total duration of horizontal forward locomotion and wall climbing) were hand-timed by a single experimenter who was unaware of the training conditions of the animals. This experimenter also assessed locomotor activity in the subsequent experiments. Locomotion was defined as the coordinated movement of the four paws. Wall climbing was recorded when the animals stood on their rear limbs with the forepaws placed on the walls of the chamber. Evaluation began by gently placing each rat in the center of the chamber.

Conditioned place preference and testing (Experiment 2a, 2b)

Locomotion: In Experiment 2a, male and female adolescent rats were given 2.5 g/kg ethanol or vehicle at PD28 and evaluated for motor activation in an open field, as described in Experiment 1. Motor activity tests were not conducted in Experiment 2b.

Conditioning and Testing Apparatus: A rectangular black Plexiglas chamber (20 × 24 × 70 cm) was utilized during conditioning sessions and at test. During conditioning sessions the chamber was divided into two equally sized compartments (20 × 24 × 35 cm) by a removable guillotine door made of Plexiglas. Animals were confined to one of these compartments, which were lined with either sandpaper (coarse: 60, Norton, Rio Grande do Sul, Brazil) or with a smooth surface made of copolymer of Ethylene Vinyl Acetate (EVA, Cordoba, Argentina).

During assessment of tactile preferences the guillotine door was removed and the floor of the chamber was divided into three sections, a middle section featuring an opaque Plexiglas (13 × 20 cm) surface and two end areas (20 wide × 28.5 cm long) that differed according to the distinct tactile cues used during conditioning (sandpaper or EVA). Each evaluation began by placing the animal in the central area. Training and testing sessions were conducted using a red 40-W electric bulb positioned on a table 50 cm above the chambers.

Conditioning and Testing Procedure: Animals were trained for 4 or 2 days (PDs 30–33 or 30–31; Experiment 2a and Experiment 2b, respectively) and were tested a day after the last conditioning session. In Experiment 2a, animals were confined to a tactile surface (EVA) after receiving an intragastric administration of vehicle (tap water). Five hours later animals were given daily pairings of 1.0 g/kg ethanol (i.g.) and a rough surface (sandpaper). Thus, sandpaper and EVA served as excitatory (CS+) and inhibitory (CS−) conditioned stimuli, respectively. In other words, the training involved a biased subject assignment. In each training trial animals were given sandpaper paired with ethanol and the rubber-like texture was paired with vehicle.

Conditioning session started 5 minutes after the corresponding i.g. administration and lasted for 15 min (i.e., 5–20 minutes post-administration). At test adolescents were allowed to move freely in the apparatus for 12 min. The specific combinations of ethanol dose, post-administration time of conditioning and route of drug administration were chosen on the basis of a previous study that revealed ethanol-induced conditioned preference in infant rats (Nizhnikov et al., 2009). The duration of the test, on the other hand, was chosen based on previous conditioning studies with ethanol in adolescents (Pautassi, Myers, Spear, Molina & Spear, 2008). The testing chamber featured a section lined with the drug-paired surface (sandpaper, CS+), a section lined with the smooth stimulus (EVA, CS−) and an intermediate zone (about 20% of the total surface) made of black Plexiglas. Time spent in each end compartment was recorded whenever the animal placed its forepaws on one of the two

surfaces. The relative position of the floors (sandpaper vs. EVA) was counterbalanced within each animal. Time spent in the intermediate, neutral zone, was not taken into account for data collection or analysis. Just before the present study a preliminary experiment determined that, when employing the two-way tactile preference test, adolescent naive rats showed approximately 50% selection of the sandpaper surface.

Experiment 2b aimed at replicating the acquisition of ethanol-mediated appetitive learning in adolescents (see Experiment 2a). It also provided a more stringent control condition and shortened the CPP training phase. The vehicle-treated conditions employed in Experiment 2a do not control for nonspecific changes -- such as habituation or sensitization -- as a result of mere exposure to the CS or the unconditional stimulus (US). Therefore, in Experiment 2b the vehicle control was replaced by an unpaired control condition.

Specifically, at the beginning of each conditioning session subjects (paired and unpaired animals alike) were exposed to the smooth CS− for fifteen minutes. No vehicle administration was conducted at this time. Five hours later paired animals were administered ethanol and exposed to sandpaper for 15 min (i.e., post-administration interval 5–20 min). Unpaired animals were also given a 15-min exposure to the sandpaper CS+ five hours after CS−. Ethanol administration in unpaired rats, however, occurred 90 min after termination of CS+ exposure. In other words, unpaired controls lacked CS-US temporal contingency, as they were given explicitly unpaired presentations of the tactile CSs and ethanol's unconditional effects. Due to the absence of sex-related differences in ethanol-mediated learning in Experiment 2a (see Results section) this replication only employed males. The experiment also tested whether less training (2 sessions, as opposed to 4 in Experiment 2a) would be sufficient to yield CPP in adolescents. Use of a short training period provides higher throughput and is preferred in studies aimed at analysing the effects of ethanol during restricted ontogenetic periods, such as adolescence.

Conditioned taste aversion procedures (CTA, Experiment 3)

LMA evaluation: Male and female adolescent rats were given ethanol (3.0 g/kg) or vehicle on PD28 and evaluated for motor activation in an open field, as described in Experiment 1. The rationale for increasing the ethanol dose was twofold. First, we sought to extend the motor assessment to a higher ethanol dose, which potentially could induce some degree of motor depression. Also, we had previously found that pre-treatment with 2.5 g/kg ethanol is not sufficient to alter subsequent acquisition of ethanol-induced CTA in adolescents (Acevedo et al., 2010). Therefore, an additional aim was to assess if prior exposure to a slightly higher dose would be effective in preventing later conditioning to ethanol's aversive properties (i.e., a US pre-exposure effect; Randich & LoLordo, 1979). An untreated group (UT) was also included during LMA evaluation. These animals were removed from their housing cages and assessed for activity in the test arena, yet received no ethanol or vehicle intubations. The purpose was to control for potential locomotor-activating effects of these manipulations. After testing animals were returned to their home cages (30 × 20 × 45 cm) and housed with a same-sex companion.

Conditioned Taste Aversion: We closely followed the procedure employed by Anderson et al. (2010), although some new features were added, such as an additional intake test session to assess the expression of aversive learning after repeated extinction. On Day 1 of the protocol (PD29), the adolescents were housed in individual cages (22 × 20 × 30 cm) and given ad libitum access to food and water. On the morning of PD30, the water bottle was replaced by a graded tube containing 50% of the water (±0.2 ml) they had ingested during the previous 24-hr period. On Day 3 (PD31), animals were weighed and returned to their cages. The water tube was then replaced by a tube containing a 0.1% saccharin solution

(Parker Davis, Buenos Aires, Argentina). Animals were given 30 min access to the solution, saccharin intake was measured and animals were immediately administered ethanol (2.5 g/kg) or vehicle. Water and food was available ad libitum after the administration of ethanol. On Day 4 (PD32), rats were given 50% of the volume of water they had ingested on PD29. On Day 5 (PD33, extinction test 1) CTA was assessed. Subjects were given 60 minutes access to a graded tube containing a 0.1% saccharin solution. Saccharin intake was recorded at the termination of this test and then animals were offered 80% of the volume of water they ingested on DP31. Finally, on day 6 (PD34, extinction test 2) animals were again given 60 minutes access to 0.1% saccharin solution and intake was recorded for a second time. Saccharin intake was expressed as millilitres consumed per 100 grams of body weight (ml/100g).

Experimental designs

Experiment 1 assessed age-related differences in ethanol's motor stimulating effects in adolescent and adult male rats. A 2 (age: adult or adolescent) × 2 (ethanol treatment on PDs 28 or 74: 0.0 or 2.5 g/kg) factorial design was used, with 9–11 animals in each group. On PD28 or PD74, animals were tested for ethanol-induced behavioural activation in an open field.

Experiments 2 and 3 assessed the relationship between ethanol's motor stimulating effects and the appetitive and aversive effects of ethanol, as measured through CPP (Exp. 2) and CTA (Exp. 3). A 2 (sex) × 2 (ethanol treatment on PD28: 0.0 or 2.5 g/kg) × 2 (ethanol treatment on PDs 30–33: 0.0 or 1.0 g/kg) factorial was employed in Exp. 2a, with 5–7 animals in each group. Experiment 2b aimed at replicating the ethanol-induced CPP found in Exp. 2a and employed only two groups: paired animals (n = 9) experienced the sandpaper while intoxicated with ethanol, whereas Unpaired controls (n = 11) experienced the sandpaper in the same way but were not given ethanol until 90 min later. No motor activity tests were conducted in Experiment 2b. Experiment 3 was defined by a 2 (sex) × 3 (treatment on PD28: untreated, 3.0 or 0.0 g/kg) × 2 (ethanol treatment on PD31: 2.5 or 0.0 g/kg) factorial, with 5–7 subjects per group.

Data analysis

Ethanol-induced forward locomotion and wall climbing were analyzed by separate factorial ANOVAs. In Experiment 1 the between group factors were ethanol dose (0.0 or 2.5 g/kg) and age at testing (adulthood or adolescence). Similar ANOVAs were run for Experiment 2a, where the between group factors were ethanol dose and sex (males or females). These same between group factors were considered in Experiment 3, although ethanol dose in that experiment was higher (3.0 g/kg) and an untreated (UT) condition was included. The minute-by-minute profile of ethanol-induced LMA in Experiment 1 was assessed by a three-way ANOVA (between factors: age at testing and ethanol dose; within factor: bin of assessment: minutes 1 to 7).

Absolute time spent in the compartments equipped with sandpaper (CS+) or EVA (CS-) floor was analyzed with either a four (Experiment 2a) or two-way (Experiment 2b) mixed factor ANOVA. In Experiment 2a CPP was evaluated as a function of the rat's prior experience with ethanol treatment during initiation on PD28. The between group factors were sex (male or female), ethanol dose on PD28 (0.0 or 2.5 g/kg, Exp 3a), treatment during conditioning (0.0 or 1.0 g/kg and paired or unpaired group; Experiments 2a and 2b, respectively). Following the approach employed in previous studies (e.g., Molina et al., 2007), total time spent on the drug-paired and unpaired compartment (s) was considered as the within-group measure.

Following previous studies (e.g., Davis & Riley, 2010), reduced saccharin intake in animals given saccharin- ethanol pairings, as compared with counterparts given vehicle, was considered an index of ethanol-mediated CTA. CTA was also evaluated in relation to the rat's prior experience with ethanol during the LMA test on PD28. Conditioning and testing sessions differed in length (30 and 60 min, respectively). Therefore, saccharin intake (ml/100 g) during conditioning and testing sessions was analysed separately using a three and a four-way mixed factor ANOVA, respectively. Treatment on PD28 (UT, 0.0 or 3.0g/kg) and PD31 (0.0 or 2.5 g/kg) and sex (male or female) were between-group factors. For the analysis of testing scores, sessions (1 and 2) served as within-group factor.

The locus of significant main effects or significant interactions was further analysed using Fisher's *post hoc* test (alpha level was set at 0.05). Across variables and experiments, sex did not exert significant main effects or significantly interact with the remaining factors. Therefore, the data were collapsed across sex for representation in the figures.

We further analyzed the motor activity measures recorded on PD28, time spent in the ethanol-paired compartment (Experiment 3a) and saccharin consumption (Experiment 3c) data by means of a correlational approach. The specific question was to what extent spontaneous or ethanol-induced activity in the open field predicted CTA or CPP scores. These correlations (Pearson's r product-moment) were between groups given ethanol or vehicle (or left untreated) at LMA assessment and then conditioned (i.e., given CS-US pairings) to avoid saccharin or prefer sandpaper.

Results

Ethanol-induced LMA in adolescent and adult rats (Experiment 1)

Ethanol-induced motor activating effects were similar for adolescents and adults. Figure 1 depicts horizontal activity (left panel) and wall-climbing (right panel) after ethanol or water administration at both ages. The ANOVA for wall climbing yielded neither significant main effects nor significant interactions. The ANOVA for locomotor activity indicated a significant main effect of ethanol treatment, $F_{1, 35} = 41.98$ $p < .001$. No other significant main effects or significant interactions were observed. Ethanol-induced motor activation was similar for adolescents and adults. No significant main effect or significant interaction involving age was observed. The latter result indicates that adolescents and adult animals in the control condition exhibited similar overall levels of motor activity.

The minute-by-minute profile of ethanol-induced activation was analysed through a three-way ANOVA (age x ethanol dose x bin of assessment). The analysis indicated similar ethanol-induced LMA in adolescent and adults across testing bins. Specifically, the analysis yielded a significant interaction between ethanol dose and bin of assessment ($F_{6, 210} = 5.69$ $p < 0.0001$) and a significant interaction between age and bin, ($F_{6, 210} = 2.20$ $p < 0.05$). Post-hoc comparisons indicated that, in adolescents and adults, ethanol induced stimulant motor effects in testing bins 1 to 6, but not in the last bin. Also, overall locomotion scores (i.e., across ethanol- and vehicle-treated animals) were similar in adolescent and adults in all bins, except in bins 3 and 6, in which adults exhibited slightly more locomotion. Perhaps more important, the interaction between age at testing, ethanol dose and bin of assessment did not achieve significance, ($F_{6, 210} = 3.72$ $p > 0.80$). The latter result indicates that the minute-by-minute profile of ethanol-induced LMA did not differ as a function of age. Mean and SEM (s) in adolescent and adult rats administered ethanol or vehicle for LMA scores during the 7-min assessment is presented in Table 1.

Ethanol-induced LMA and Ethanol-induced CPP (Experiment 2)

Experiment 2a—As in the previous experiments ethanol administration on PD28 reliably induced LMA (significant main effect of ethanol treatment; $F_{1, 44} = 21.91$; $p < .001$) without altering wall climbing. Absolute LMA and wall climbing scores, in terms of mean and SEM for each condition, are presented in Table 2.

The ANOVA for time spent over each texture (sandpaper and the smooth EVA) at test revealed a significant interaction between type of texture and conditioning treatment, $F_{1,41} = 4.74$, $p < .05$. Post-hoc tests indicated that animals given sandpaper-ethanol pairings spent more time over sandpaper than over smooth EVA. These animals also exhibited greater sandpaper preference than those given sandpaper-vehicle pairings (data not shown).

Pre-treatment with ethanol on PD28 did not alter place conditioning scores. Moreover, there was no significant correlation between spontaneous or ethanol-induced LMA at PD28 and CPP scores in animals given ethanol-sandpaper pairings. In other words, the magnitude of ethanol-induced LMA did not predict later appetitive conditioning to ethanol.

Experiment 2b—This experiment aimed at replicating the ethanol-induced CPP found in Experiment 2a, yet employed a more stringent control condition and a shorter length of training. In this Experiment adolescents were not evaluated for ethanol-induced motor activation prior to CPP training. The ANOVA analyzing differences in time spent in each texture at test between paired and unpaired groups yielded a significant conditioning treatment x texture interaction, $F_{1, 18} = 4, 70$ $p < .05$. Post hoc tests indicated that paired animals spent significantly more time over sandpaper than they did over smooth cardboard. Post-hocs also indicated greater sandpaper preference in paired than in unpaired adolescents. Unpaired rats, in turn, exhibited similar predilection for both textures (see Figure 2). These data replicate the main result of the previous Experiment and strengthen this conclusion in terms of the additional, more stringent control condition. To better illustrate the consistency of these results, indicative of ethanol-mediated conditioned place preference, Figure 2 (lower panel) depicts time spent over each surface on a minute-by-minute basis.

Ethanol-induced LMA and Ethanol-induced CTA (Experiment 3)

The ANOVA for LMA revealed a significant main effect of ethanol dose ($F_{2, 65} = 34.05$; p<. 001). The post-hoc tests indicated that animals given ethanol (3.0 g/kg) on PD28 exhibited significantly more LMA than animals treated with vehicle or given no administration (untreated group). The latter two groups did not differ. Sex did not exert a significant main effect nor did it significantly interact with the remaining variables. Wall climbing was not affected by the variables under consideration. Absolute LMA scores (mean +/− SEM) were as follows: ethanol group, 29.57 +/− 1.35 s; vehicle group, 16.07 +/−1.36 s, untreated group, 16.29 +/− 1.11 s.

With regards to CTA scores, the ANOVA yielded no significant differences in saccharin consumption during training as a function of sex or prior ethanol experience on PD28. Overall drinking scores during conditioning can be found in Table 3.

The ANOVA for saccharin intake at test, on the other hand, revealed significant main effects of ethanol treatment at PD31 and day of assessment, as well as a significant treatment at PD28 x treatment at PD31 interaction, $F_{1,59} = 16.61$; p<.001, $F_{1,59} = 7.99$; p<.01, and $F_{2,59} = 5.22$; p<.01, respectively. Post-hoc tests indicated that overall saccharin intake across groups decreased significantly from one testing day to the next testing session (overall drinking scores on testing sessions 1 and 2 were 5.08 +/− 0.31 ml/kg and 4.31 +/−0.27 ml/ kg, respectively). More importantly, animals given saccharin-ethanol pairings at training

drank significantly less saccharin than vehicle-treated controls, but only if they had not been treated with ethanol on PD 28. Ethanol pre-exposure on PD 28 completely inhibited ethanol-induced CTA. Sex did not exert a significant main effect nor did it interact with the other factors. Figure 3 depicts the mean saccharin intake across testing days at test as a function of ethanol treatment on PD28 and PD31.

Our hypothesis of an association between sensitivity to ethanol-induced behavioural activation and CTA for ethanol was not confirmed. The correlation between ethanol-induced LMA scores on PD28 and saccharin intake during extinction tests 1 or 2 was not significant. Interestingly, there was a positive, significant association between LMA at PD28 and saccharin intake at extinction test 1 in untreated animals (i.e., UT group on PD28) given pairings of saccharin and ethanol on PD31 ($r = .64$; $p < .05$). Higher ambulation on the open field predicted higher consumption of the ethanol-paired CS, i.e., a diminished expression of ethanol-induced CTA. These results are depicted in Figure 4.

Discussion

The present work assessed relative susceptibility to the motor stimulant effect of high-dose ethanol among adolescent and adult rats, and tested CPP and CTA in adolescent rats previously evaluated for spontaneous or ethanol-induced locomotor activity. The study indicated that, at the dose tested, the behavioural stimulant effect of ethanol was similar for adolescents and adults. Adolescents were sensitive to ethanol's aversive and appetitive reinforcing properties, although the hypothesis of these measures being related to ethanol-induced LMA was not corroborated. There was, however, a significant association between spontaneous motor activity in the open field and ethanol-induced CTA.

Ethanol induced reliable LMA even when the dosage was as high as 3.0 g/kg (Experiment 3). Ethanol's behavioural stimulant effects were specific to forward locomotion. Wall climbing was not affected by the drug, suggesting that ethanol-induced LMA is not just a manifestation of an overall increase in general activity. It has been considered that increased wall climbing may be indicative of negative hedonic effects (Arias, Pautassi, Molina, Spear, 2010), while increased drug-induced locomotion is often interpreted as reflecting positive hedonic reactions and exploration (Quoilin et al., 2010).

Adult rats exhibited a significant increase in LMA after 2.5 g/kg ethanol. This finding is apparently at odds with previous rat studies that revealed either no effect or motor depression after ethanol administration (e.g., Chuck et al., 2006; Sanchis-Segura, Grisel, Olive, Ghozland, Koob, Roberts & Cowen, 2005). Several procedural differences may explain this discrepancy. These previous studies employed an intraperitoneal (i.p.) route of administration, which has been suggested to exert a short-lived aversive effect (Cunningham et al., 2002; 2003). The absorption-distribution-clearance profile of the blood ethanol curve, which changes the expression of ethanol's motivational effects, is also significantly affected by the route of ethanol administration (Walker & Ehlers, 2009). Moreover, the present study employed a relatively large testing arena (when compared, for instance, to the one used by Chuck et al., 2006), a fact that might have favored the expression of ethanol's activating effects. Also, it is important to note that unlike previous studies that tested rats for a relatively long period after ethanol administration (e.g., 20 min, Sanchis-Segura et al., 2005; 60 min, Rezvani & Levin, 2004) we focused on the initial interval of the blood ethanol curve, when blood alcohol levels were rising and ethanol's appetitive effects are presumably maximal (Nizhnikov et al., 2009).

The present study did not control for pharmacokinetic differences between adolescent and adult rats after the intubation with 2.5 g/kg ethanol. A previous study that employed 2.0 g/kg

(i.g.), however, found similar blood and brain ethanol concentrations in animals 28 or 70 days old, at a time period (7.5 min after the intubation) that coincides with our testing interval (Pautassi et al., 2008).

There was no indication of age-related differences in ethanol-induced LMA, with adolescents and adults exhibiting similar scores. Although our expectation was that adolescent rats would show higher sensitivity to this effect of ethanol, the result is not inconsistent with previous reports. Previous studies have often (Pautassi et al., 2008; Anderson et al., 2010) but not always (Redolat, Pérez-Martínez, Carrasco & Mesa, 2009) revealed higher sensitivity to ethanol's motivational effects in adolescents than in adults. Rezvani and Levin (2004) observed similar magnitudes of motor activation in adolescent and adult rats after 2.5 g/kg ethanol (i.p.). It is also important to acknowledge that although we and others (e.g., Rodd, Bell, McKinzie, Webster, Murphy, Lumeng, Li & McBride, 2004) expect a meaningful relationship between LMA and conventional measures of reinforcement, this relationship is still uncertain. Ethanol-induced LMA may reflect, at least partially, a negative reinforcing (i.e., anxiolytic) effect of ethanol. Under this framework, the functional significance of ethanol-induced LMA may be different across ontogeny.

Adolescents readily exhibited ethanol-mediated CTA (2.5 g/kg) and also exhibited a subtle, yet significant, CPP (1.0 g/kg). The latter effect is in itself relevant because very few studies have found such an outcome in adult rats given first-order conditioning (e.g., Peana, Enrico, Assaretti, Pulighe, Muggironi & Nieddu, 2004), let alone during adolescence. Ethanol usually induces conditioned place aversion in adult rats unless given in a prolonged treatment likely to induce tolerance or co-administered with other drugs or stressors (Tzschentke, 2007; Matsuzawa et al., 1999). Notably, the specific combination of training parameters for dosing and conditioning that led to the expression of ethanol-mediated CPP in Experiments 2a and 2b was derived from a previous study that found ethanol-mediated appetitive learning in younger, preweanling rats. Also important are the studies by Philpot et al. (2003) and Fernandez-Vidal, Spear & Molina (2003). The latter study observed heightened sucrose preference in 28–32 day old rats following pairings of sucrose and low-dose ethanol (0.5 g/kg), whereas Philpot et al. found conditioned preference in early and late adolescent rats (PD25, 0.2 g/kg; PD45, 0.5–1.0 g/kg). When taken together these rat studies suggest that ethanol is more likely to induce conditioned preference at relatively low doses and while blood alcohol levels are rising, i.e, when the CS signals the initial phase of the intoxication. Human studies have often linked the positive appetitive effects of ethanol to the rising phase of the blood ethanol curve (e.g., Conrod, Peterson, Pihl, & Mankowski, 1997).

It is important to identify subjects susceptible to ethanol reinforcement before they are exposed to ethanol. It has been found that high-responder rats are more resistant to the aversive effects of amphetamine, as indexed by CTA (Kunin, Gaskin, Borjas, Smith & Amit, 2001). We also assessed the relationship between spontaneous or ethanol-induced LMA and two measures of motivational sensitivity to alcohol: CPP and CTA. The hypothesis that ethanol-induced LMA would be positively and negatively associated with ethanol-induced CPP and CTA, respectively, was not supported. The hypothesis was grounded in theoretical proposals (Wise & Bozarth, 1987; Robinson & Berridge, 2008) as well as in empirical findings (Rodd et al., 2004; Risinger, Malott, Prather, Niehus & Cunningham, 1994). Other studies, however, had also found dissociation between ethanol-induced LMA and alternatives measures of ethanol reinforcement. Deletion of μ-opiate receptors blocked the expression of ethanol-induced CPP but did not affect ethanol-induced LMA (Hall, Sora & Uhl, 2001), and haloperidol inhibited ethanol-induced LMA but did not affect ethanol-mediated CPP (Cunningham, Malott, Dickinson & Risinger, 1992).

Among subjects that remained untreated during the LMA evaluation there was a negative association between open field ambulation and later expression of ethanol-induced CTA (i.e., the higher the LMA scores, the lower the CTA). This result suggests that subjects with higher response to novelty-induced activation may be less sensitive to the aversive effects of ethanol and, hence, more vulnerable to addiction. The result is consistent with the notion that individual differences in behavioural response to a novel environment reflect different levels of novelty seeking, which in turn predict vulnerability to addiction (Redolat et al., 2009).

Despite the apparent vulnerability of high-responding untreated animals, the lowest level of sensitivity to ethanol's aversive attributes was found in ethanol-pretreated rats. These subjects exhibited no decrease whatsoever in saccharin intake after saccharin-ethanol pairings. Experiment 3 indicated that adolescents acquired a single-trial, ethanol-induced CTA. A more important finding, however, was that adolescents exhibited a US pre-exposure effect (Randich & LoLordo, 1979). The otherwise reliable ethanol-induced CTA was inhibited if adolescents had been given a high dose of ethanol (3.0 g/kg) a few days before conditioning. Employing similar procedures, Acevedo et al. (2010) observed that a lower pre-exposure dose (2.5 g/kg) was not sufficient to significantly affect the acquisition of ethanol-induced CTA. Therefore, it seems that 3.0 g/kg ethanol induces a toxic state that is just above the threshold necessary to evoke a US pre-exposure effect in this preparation.

In drug-related studies the US pre-exposure effect can be accounted for by development of tolerance to the drug's effects (Davis & Riley, 2007; Camarini and Hodge, 2004). Initial exposure to the drug makes subjects less sensitive to the effects of subsequent administrations. It has been suggested (Arias et al., 2010) that ethanol-induced CTA is mediated by the activation of emetic areas at the brainstem, much like lithium chloride – the prototypical CTA-inducing agent – does. Therefore, it could be postulated that exposure to 3.0 g/kg ethanol induces a toxic state that enables the development of tolerance in terms of activation of these brain areas. An alternative explanation is that cues that had been paired with initial ethanol exposure (e.g., those from the testing environment) would come to predict the US (i.e., ethanol's malaise-inducing effects) and, therefore, make the putative CS less able to acquire conditioned reinforcement later on (de Brugada et al., 2003). Exteroceptive cues, however, are not very likely to serve as CSs in taste conditioning preparations.

There are important caveats in the experiments that examined the correlations between LMA and CPP or CTA in adolescent rats. Each experiment examined only a single dose for initial LMA and a single dose for establishing CPP or CTA, which certainly limits the generality of the null outcomes (i.e., lack of correlation between ethanol-induced LMA and CPP or CTA). Also, the WKAH/HoK rats employed in the present study are inbred and exhibit significantly less genetic variability than those derived from outbred strains. A more conclusive answer on the nature of the association between LMA and indices of ethanol-mediated reinforcement will have to wait, therefore, for follow-up studies that employ an experimental (not correlational) approach with outbred rats. It is interesting to note, however, that even under this limited methodological approach a negative association between spontaneous ambulation and CTA was found. This result is consistent with studies conducted in outbred Wistar rats (Arias et al., 2009) and fits well with theories indicating that novelty-seeking predicts drug effects (Redolat et al., 2009).

Overall, the present results confirm that adolescent rats readily exhibit LMA after high-dose ethanol. Under the present circumstances, adolescents and adults exhibit similar levels of ethanol-induced LMA. Adolescents were sensitive to ethanol's aversive reinforcement and

they also exhibited a subtle, yet significant, ethanol-induced conditioned place preference. These measures of learning, however, were not related to ethanol-induced LMA.

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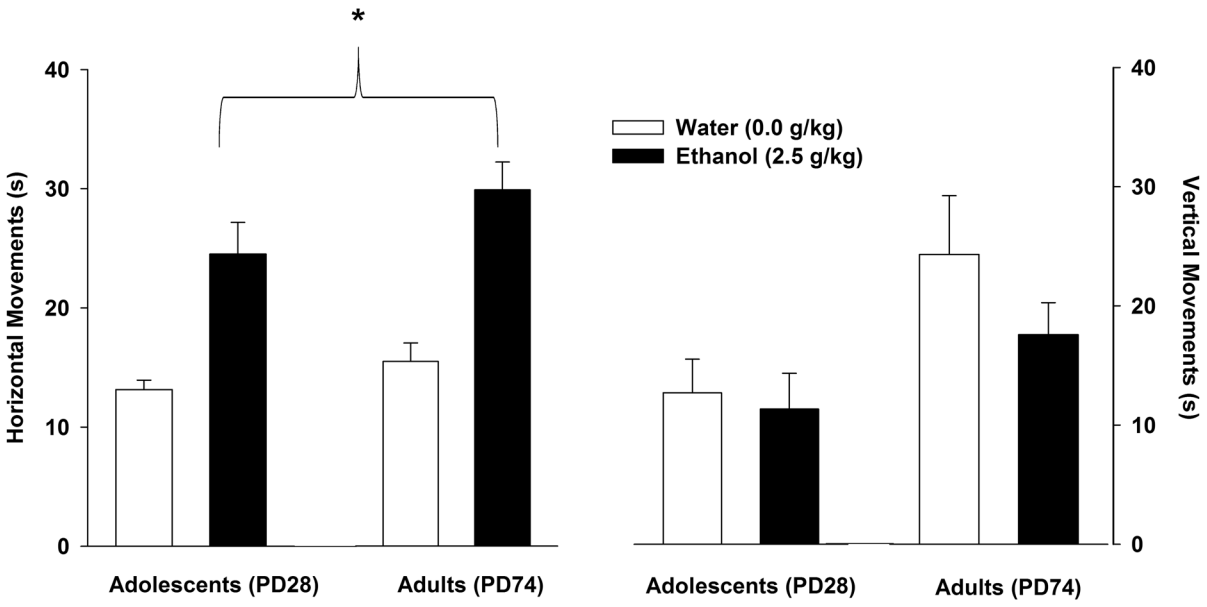

Figure 1.

Assessment of susceptibility to the motor stimulant effect of high-dose ethanol in adolescent (postnatal day 28, PD28) and adult rats (PD74). Left Panel: Horizontal (i.e., forward locomotion) activity (s) in animals given ethanol (2.5 g/kg, i.g.) or vehicle (tap water). Right panel: Wall climbing activity (s) in animals given ethanol (2.5 g/kg, i.g.) or vehicle (tap water). To facilitate data visualization, data have been collapsed across sex. The latter factor did not affect motor behavior nor significantly interacted with the remaining factors. Treatment with 2.5 g/kg ethanol induced a significant increase in horizontal movement, which was similar in adolescent and adult subjects. This significant main effect of ethanol is indicated by the asterisk. The vertical bars indicate SEM.

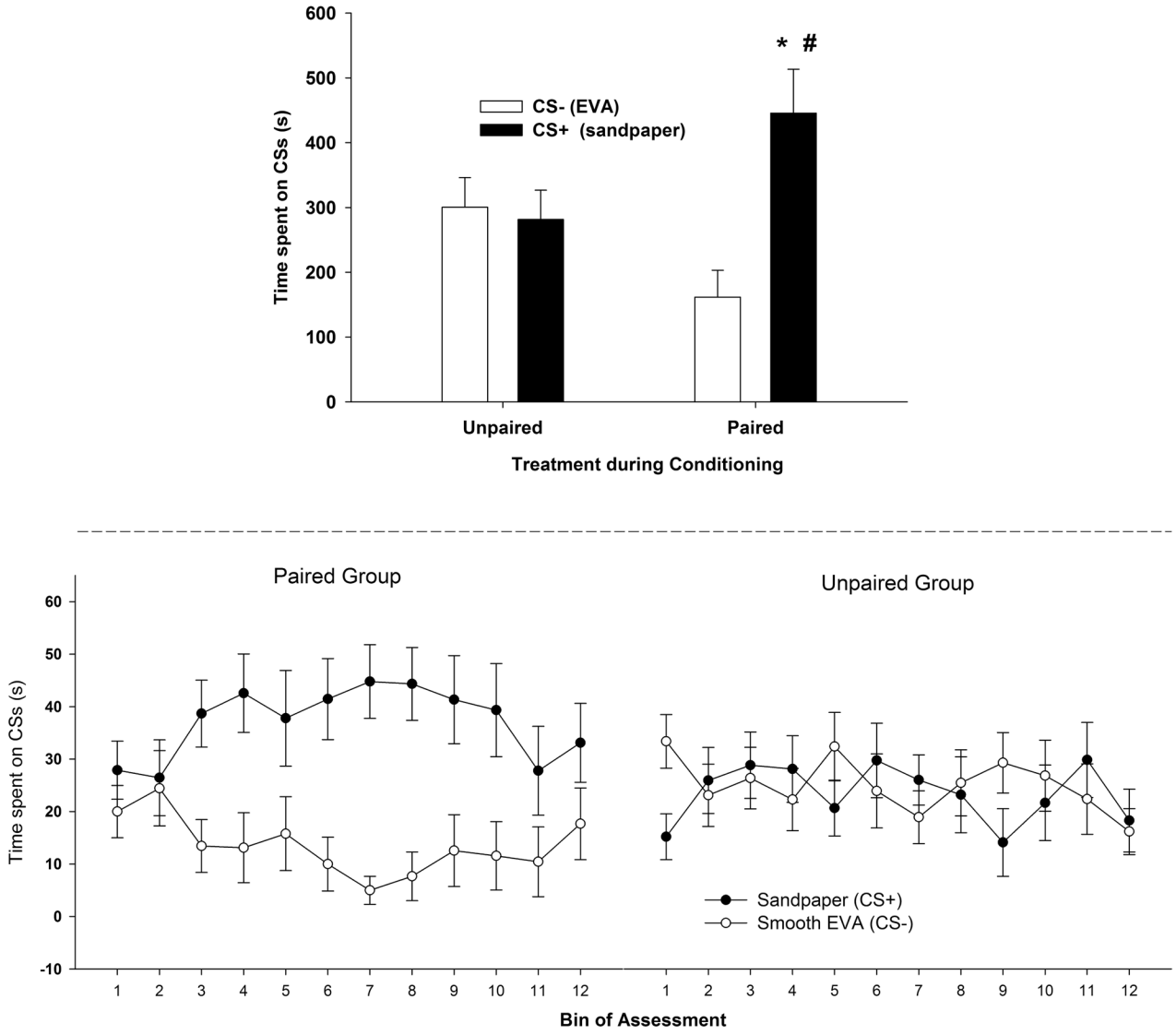

Figure 2.

Ethanol-induced conditioned texture preferences in adolescent rats (postnatal day 32, PD32). *Upper panel* depicts total time (s) spent on the sandpaper conditional stimulus (CS+) and the smooth surface (CS−, EVA) during the 12-minute test as a function of treatment during conditioning (sandpaper paired or unpaired with ethanol's effects). During conditioning (PDs 30–31), the Paired group received ethanol-sandpaper parings [US-CS+] whereas the Unpaired group was exposed to sandpaper (CS+) 90 minutes prior to the administration of ethanol (US) [CS+ - US: 90 min delay]. The statistical analysis indicated a significant interaction between texture and conditioning treatment. Paired rats spent significantly more time in sandpaper than in smooth at test and also exhibited greater sandpaper preference than unpaired adolescents. These significant differences are indicated by the asterisk (*) and the pound (#) sign, respectively. *Lower panel* depicts time spent on sandpaper and the smooth EVA (s) as a function of treatment during conditioning (paired or unpaired) and bin of assessment (1–12 min). 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. The vertical bars indicate SEM.

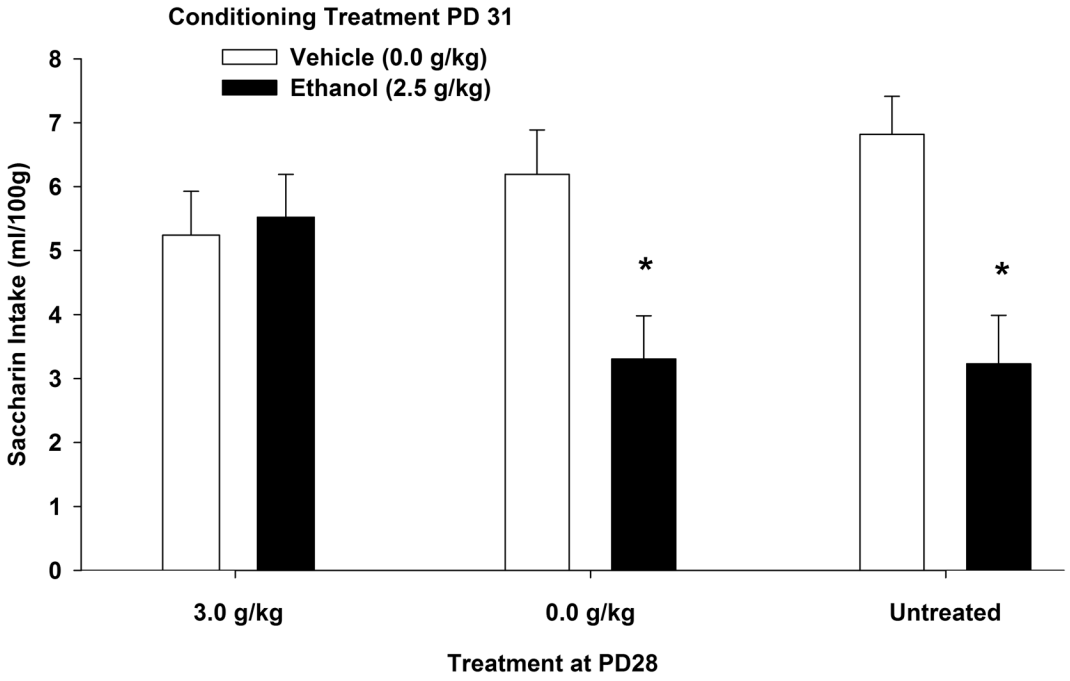

Figure 3.

Ethanol-induced conditioned taste aversion in adolescent rats. Saccharin intake (ml/100 g) at test in male and female adolescent rats as a function of ethanol treatment during postnatal day 28 (PD28) and conditioning (PD31). On PD28, the rats were treated with ethanol (3.0 g/kg, i.g.) vehicle (0.0 g/kg) or were left untreated (UT, animals received no intubation). During conditioning (PD31), saccharin intake was paired with ethanol administration (2.5 g/kg, i.g.) or vehicle (tap water, 0.0 g/kg). Two 60-min test sessions were conducted (PDs 33 and 34); the figure depicts average mean saccharin consumption across these tests. The statistical analysis indicated that animals given saccharin-ethanol pairings at training drank significantly less saccharin than vehicle-treated controls, but only if they had not been treated with ethanol on PD 28. These significant differences between ethanol and vehicle-treated subjects are indicated by the asterisk sign. Data were collapsed across the sex factor, which did not exert a significant main effect or significantly interact with the remaining variables. The vertical bars indicate SEM.

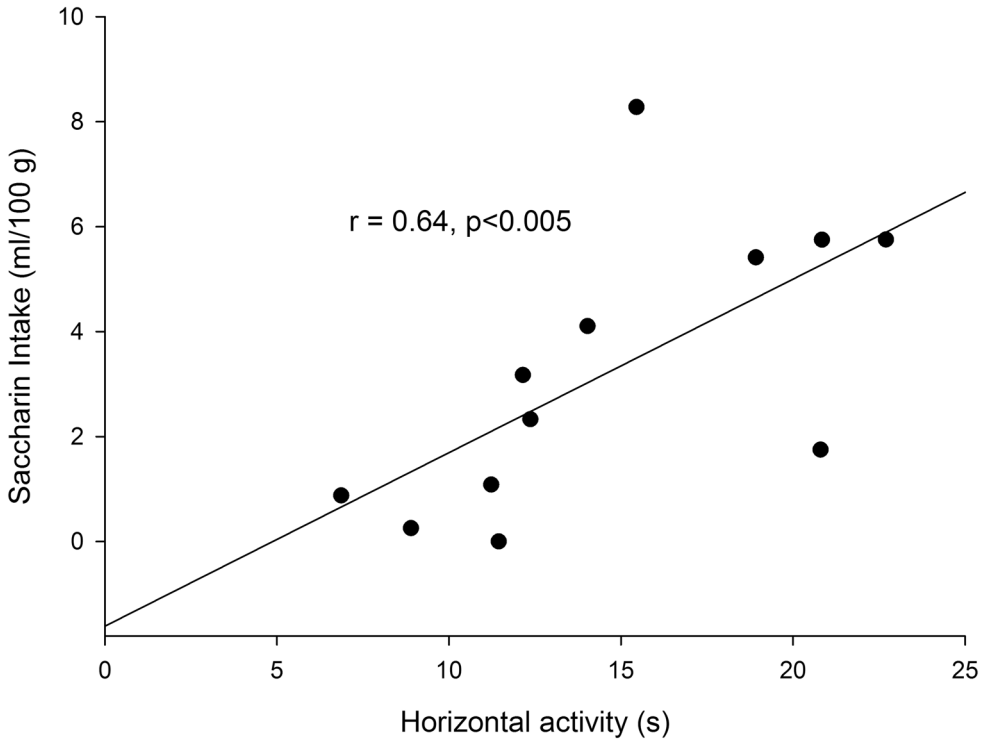

Figure 4.

Association between spontaneous open-field behaviour and of ethanol-induced conditioned taste aversion. Saccharin intake (ml/100 g of body weight) during extinction test 1 (postnatal day 33, PD 33) as a function of horizontal motor activity in the open field at PD 28, in adolescent rats that remained untreated during the motor activity test (i.e., UT group) and were given parings of saccharin and ethanol (2.5 g/kg, i.g.) on PD31. A Pearson correlation coefficient ($r = .64$; $p < .05$) indicated that higher ambulation on the open field predicted higher consumption of the ethanol-paired CS and, therefore, a diminished expression of ethanol-induced conditioned taste aversion.

Table 1

Ethanol-induced locomotor activity (s) in adolescent and adult rats (Experiment 1)

Ethanol-induced LMA as a function of age, treatment at open field assessment and testing bin. On postnatal day 28 or 74 (PD28 or PD74: adolescents and adults, respectively) rats were given ethanol (2.5 g/kg) or vehicle (0.0 g/kg). Five minutes later animals were evaluated for locomotor activity during post-administration time 5–11 min (bins 1–7). Values are expressed as mean ± SEM.

	Adolescents		Adults	
Testing Bin	**dose: 0.0 g/kg**	**dose: 2.5 g/kg**	**dose: 0.0 g/kg**	**dose: 2.5 g/kg**
1	4.62 +/− 0.44	6.81 +/− 0.83	3.63 +/− 0.54	6.44 +/− 0.66
2	2.79 +/− 0.41	5.13 +/− 0.71	3.61 +/− 0.52	6.17 +/− 0.82
3	1.20 +/− 0.40	4.26 +/− 0.54	2.54 +/− 0.24	5.10 +/− 0.75
4	1.57 +/− 0.26	3.24 +/− 0.48	1.50 +/− 0.26	4.47 +/− 0.48
5	1.11 +/− 0.22	2.40 +/− 0.35	0.98 +/− 0.21	3.02 +/− 0.59
6	0.25 +/− 0.15	1.68 +/− 0.49	1.48 +/− 0.30	2.97 +/− 0.44
7	1.60 +/− 0.56	0.98 +/− 0.42	1.75 +/− 0.67	2.02 +/− 0.51

Table 2

Locomotor Activity (LMA) and Wall-Climbing scores in Adolescent Rats (Experiment 2a)

Locomotor Activity (LMA) and Wall-Climbing scores in male and female adolescent rats as a function of ethanol treatment (0.0 or 2.5 g/kg). Values are expressed as mean ± SEM.

	Locomotor Activity (LMA, s)		Wall-Climbing (s)	
Sex	**dose: 0.0 g/kg**	**dose: 2.5 g/kg**	**dose: 0.0 g/kg**	**dose: 2.5 g/kg**
Males	15.08 ± 2.60	25.04 ± 2.84	15.91 ± 4.11	8.77 ± 1.68
Females	18.04 ± 2.94	33.92 ± 2.64	13.42 ± 2.36	19.82 ± 5.00

Table 3
Saccharin intake (ml/100g) during conditioning (Experiment 3)

Saccharin intake (ml/100 g) during conditioning (postnatal day 31, PD31) in male and female adolescent rats as a function of ethanol treatment at PD28. On PD28, the rats were treated with vehicle (i.e., water, 0.0 g/kg), ethanol (3.0 g/kg) or remained untreated (UT). During conditioning (PD31), animals were given 30 min access to saccharin intake and animals were immediately administered ethanol (2.5 g/kg) or vehicle (tap water, 0.0 g/kg). Values are expressed as mean ± SEM.

	Treatment at postnatal day 28 (PD28)		
Sex	**0.0 g/kg ethanol**	**3.0 g/kg ethanol**	**untreated group**
Males	3.30 +/− 0.42	2.68 +/− 0.41	3.49 +/− 0.43
Females	2.88 +/− 0.38	3.52 +/− 0.44	2.65 +/− 0.40