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Adolescent rats are resistant to the development of ethanol-induced chronic tolerance and ethanol-induced conditioned aversion

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ABSTRACT

The analysis of chronic tolerance to ethanol in adult and adolescent rats has yielded mixed results. Tolerance to some effects of ethanol has been reported in adolescents, yet other studies found adults to exhibit greater tolerance than adolescents or comparable expression of the phenomena at both ages. Another unanswered question is how chronic ethanol exposure affects subsequent ethanol-mediated motivational learning at these ages. The present study examined the development of chronic tolerance to ethanol's hypothermic and motor stimulating effects, and subsequent acquisition of ethanol-mediated odor conditioning, in adolescent and adult male Wistar rats given every-other-day intragastric administrations of ethanol. Adolescent and adult rats exhibited lack of tolerance to the hypothermic effects of ethanol during an induction phase; whereas adults, but not adolescents, exhibited a trend towards a reduction in hypothermia at a challenge phase (Experiment 1). Adolescents, unlike adults, exhibited ethanol-induced motor activation after the first ethanol administration. Adults, but not adolescents, exhibited conditioned odor aversion by ethanol. Subsequent experiments conducted only in adolescents (Experiment 2, Experiment 3 and Experiment 4) manipulated the context, length and predictability of ethanol administration. These manipulations did not promote the expression of ethanol-induced tolerance. This study indicated that, when moderate ethanol doses are given every-other day for a relatively short period, adolescents are less likely than adults to develop chronic tolerance to ethanol-induced hypothermia. This resistance to tolerance development could limit long-term maintenance of ethanol intake. Adolescents, however, exhibited greater sensitivity than adults to the acute motor stimulating effects of ethanol and a blunted response to the aversive effects of ethanol. This pattern of response may put adolescents at risk for early initiation of ethanol intake.

1. Introduction

Repeated exposure to moderate and heavy ethanol exposure is widespread during adolescence. A study indicated that 85% of Argentinean male college students exhibited alcohol drinking during the last month, and 52% of the sample had 4 to 5 drinks per drinking occasion (Pilatti et al., 2014), which constitutes binge drinking (Courtney and Polich, 2009). It is thus not surprising that there is considerable interest in the effects of chronic ethanol exposure during adolescence; although the pre-clinical studies have often yielded mixed results, probably due to differences in methodology, route and length of ethanol dosing, among other factors (Swartzwelder et al., 2014). Protracted and continuous heavy ethanol exposure induces neuro-inflammation and neurotoxicity, and apparently to greater extent in adolescents, than in adults (Crews et al., 2000). On the other hand, moderate and intermittent ethanol exposure facilitates later ethanol consumption (Pascual et al., 2009), perhaps by facilitating the development of tolerance to the aversive and sedative effects of the drug. Tolerance is defined as decreased sensitivity to an effect of ethanol following exposure to the drug (Swartzwelder et al., 2014).

Age-related differences in sensitivity to ethanol-induced tolerance have been observed. Acute tolerance (i.e., a diminished response to ethanol's effects during the course of a single intoxication) to the sleep-inducing effect of ethanol was greater in infant and adolescent rats than in older counterparts (Silveri and Spear, 1998), and similar effects were found for ethanol-induced social impairment (Varlinskaya and Spear, 2006). On the other hand, rapid and chronic tolerance (a diminished response to ethanol's effects after a second ethanol administration or after repeated ethanol dosing across several days, respectively) to the sleep-inducing effect of ethanol are greater in adults than in adolescents (Silveri and Spear, 1999).

Tolerance can develop for some, but not for all effects of ethanol and seems to be related to the magnitude of the ethanol-induced disturbance. This is illustrated by a study in which infant rats were given ethanol every-other day from postnatal day (PD) 13 to 21

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(Hunt et al., 1993). Pups exhibited ethanol-induced hypnosis readily at PD 13 but ethanol-induced hypothermia only at PD19. On the challenge at PD21 chronically treated pups exhibited, when compared to animals only given ethanol on PD21, reduced ethanol-induced hypnosis, but a similar fall in core body temperature. Subsequently, Silveri and Spear (2001) compared acute, rapid and chronic tolerance to the disrupting effect of ethanol on a swim task, in infant, adolescent and young adults (PD16–22, PD28–34 and PD54–60, respectively), after having equated the initial level of ethanol-induced perturbation across age. Under this condition, minimal age-related differences were found in either type of tolerance.

Other studies on chronic tolerance to ethanol in adults and adolescents have yielded mixed results. Tolerance to the hypothermic and sedative effects of ethanol has been reported in adolescents (Swartzwelder et al., 1998), yet others found no change in ethanol-sedative effects following chronic adolescent ethanol exposure (Matthews et al., 2008); and other work indicated comparable expression of the phenomena (assessed via alterations in social behavior) at both ages (Varlinskaya and Spear, 2007). A more recent study (Broadwater et al., 2011) employed a relatively high ethanol dose (4 g/kg, every other day for 10 days) and found an attenuated response to ethanol-induced sedation at challenge in adult, but not in adolescent, rats. Similar outcome (i.e., adults but not adolescents exhibiting ethanol-induced chronic tolerance) was reported in mice (Linsenbardt et al., 2009), and Ristuccia and Spear (2005) found no change in ethanol-induced hypothermia in adolescents after a week of ethanol vapor exposure. Overall, it seems that adolescents are less prone, or need higher or lengthier ethanol dosing, to develop chronic tolerance than adult counterparts; yet it is clear that more information is needed to understand age-related differences in the expression of chronic ethanol tolerance.

Another point that remains unclear is how chronic ethanol exposure affects subsequent ethanol-mediated motivational learning. Evidence suggests that adolescent rats may be more sensitive to the appetitive (Pautassi et al., 2008; Ristuccia and Spear, 2008) but less sensitive to the aversive effects of ethanol (Anderson et al., 2010) than adults. There is, however, little information on modulation of these age-related differences by chronic treatment likely to induce chronic tolerance, although early work suggested that chronic ethanol treatment may increase the net appetitive value of ethanol (Bozarth, 1990) by reducing the aversive consequences of ethanol (Cunningham et al., 2002). Also limited is the information on the relationship between chronic tolerance and ethanol intake. Broadwater et al. (2011) found greater consumption of ethanol in adolescent than in adults, which was unaffected by chronic ethanol exposure that resulted in the development of tolerance in adult, but not in adolescent, rats.

The present study examined chronic tolerance to ethanol, and the subsequent acquisition of ethanol-mediated conditioned odor aversion, in adolescent and adult Wistar rats. Tolerance was indexed via ethanol-induced hypothermia and ethanol-induced motor behavioral stimulation (Experiment 1), which indicates aversive and appetitive effects of ethanol, respectively. Ethanol-induced hypothermia regulates the acquisition of conditioned aversion by ethanol (Cunningham et al., 1992); and ethanol-induced behavioral stimulation is modulated by the same transmitter systems that modulate ethanol-induced appetitive conditioning. For instance, it was found that administration of naloxone (a general opioid antagonist) blocked conditioned place preference by ethanol and ethanol-induced motor activation in adolescent, Wistar rats (Pautassi et al., 2011). On the other hand, Cunningham et al. (1992) observed that exposure to high or low ambient temperature ameliorated or promoted, respectively, the expression of ethanol-induced conditioned taste aversion. After finding that adolescents did not exhibit signs of thermal tolerance after repeated ethanol exposure, we scrutinized mechanisms that may prevent adolescent animals from developing tolerance to ethanol's hypothermic effects. Experiment 2, Experiment 3 and Experiment 4 were conducted in adolescents only and manipulated context, length and predictability of ethanol administration.

2. Material and methods

2.1. Experimental designs

Experiment 1 was defined by a 2 (age: adolescence or adulthood) × 2 (treatment at experimental days 1, 3 and 5: ethanol or vehicle) × 2 (treatment at experimental day 7: ethanol or vehicle) × 2 [conditioned stimulus (CS) paired with ethanol during conditioning procedures: lemon or methyl salicylate]. Each of the 16 groups was composed by 8 subjects.

Experiment 2, Experiment 3 and Experiment 4 employed only adolescents. Experiment 2 used a 2 (treatment at experimental days 1, 3 and 5: ethanol paired or unpaired with exposure to the open field) × 2 (treatment at experimental day 7: ethanol or vehicle) factorial, with 9 subjects in each group.

A 2 (treatment at experimental days 1, 3, 5, 7, 9 and 11: ethanol paired or unpaired with exposure to the open field) × 2 (treatment at experimental day 13: ethanol or vehicle) × 2 (treatment at adulthood: ethanol or vehicle) factorial was employed in Experiment 3. Each group was composed by 11–12 subjects.

Experiment 4 had 3 groups, defined by the treatment at experimental days 1, 3, 5, 7, 9 and 11 [gradual (n = 9) or random (n = 9) ethanol administration, or vehicle (n = 7) administration].

2.2. General procedures

2.2.1. Subjects

A total of 307 Wistar male rats, representative of 77 L born and reared at the vivarium of the Psychology Department of the National University of Córdoba (Córdoba, Argentina) were employed (Experiment 1: 128 animals, 32 L; Experiment 2: 36 animals; 10 L; Experiment 3: 93 animals; 22 L; Experiment 4: 50 animals; 13 L). The rationale for using only males was that previous studies indicate that male rats are significantly more sensitive to ethanol-induced hypothermia than female rats (Taylor et al., 2009).

Births were examined daily and the day of parturition was considered PD0. Pups remained with their dam in maternity cages until weaning day at PD21. They were then housed in standard cages (45 × 30 × 20 cm, up to four animals per cage) with *ad libitum* access to water and food. Experimental procedures began at PD28 or PD70 (adolescence or adulthood, respectively). The colony was maintained on a 12 h. light/dark cycle (0800) at an ambient temperature of 22 ± 1 °C. Across experiments, no more than one subject per litter was assigned to the same experimental condition. This helped avoid litter effects (Zorrilla, 1997). During breeding and experimental procedures animals were treated according to the Guide for Care and Use of Laboratory Animals (National Research Council, 2011) and the guidelines indicated by the Institutional Ethics Committee.

2.2.2. Drug preparation and administration procedures

The ethanol doses of 0.0, 0.5, 1.0, 1.5, 2.0, 2.25, 2.5 and 3.0 g/kg were achieved by intragastrically (i.g.) administering 0.015 ml/kg of 0.0 (vehicle-treated control), 4.2, 8.4, 10.6, 16.8, 18.9, 21 and 25.2% ethanol solution (Porta Hnos., Cordoba, Argentina; vehicle: tap water). Intubations were executed through a section of PE 10 or PE 50 polyethylene tubing (Clay-Adams; length: 15 cm, internal width: 0.11 mm), for adolescents and adults, respectively, connected to a 5 cm^3 syringe mounted with a 27-1/2 gauge needle. The intragastric route was chosen to model the oral self-administration of ethanol normally observed in human adolescents and adults.

2.3. Specific procedures for Experiment 1

2.3.1. Measurement of ethanol-induced hypothermia and forward locomotion in an open field

In experimental days 1, 3 and 5, animals were given ethanol or vehicle (Experiment 1) administration. This pre-exposure or training