Behavioural Brain Research 225 (2011) 358–362

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Short communication

Different chronic ethanol exposure regimens in adolescent and adult male rats: Effects on tolerance to ethanol-induced motor impairment

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ARTICLE INFO

Article history:
Received 22 March 2011
Received in revised form 24 June 2011
Accepted 6 July 2011
Available online 22 July 2011

Keywords:
Chronic ethanol
Acute tolerance
Chronic tolerance
Adolescence
Motor impairment
Sprague-Dawley rats
Males

ABSTRACT

Findings are mixed regarding the expression of tolerance after repeated ethanol exposure, perhaps in part due to dose/frequency variations in exposure regimens. The present study compared age-related differences in tolerance development following 10 days of 1 g/kg twice daily, 2 g/kg once daily, or intermittent 4 g/kg ethanol exposure regimens. To measure expression of chronic tolerance and acute tolerance, ethanol-induced motor impairment was assessed on day 12, with functionally equivalent ethanol doses administered across age (2 g/kg – adolescents; 1.5 g/kg – adults). Subsequent challenge doses resulted in lower brain ethanol concentrations in both age groups as a function of the chronic ethanol regimens. Expected age-related differences emerged in acute tolerance expression in non-manipulated animals, with adolescents, but not adults showing acute tolerance. Regimens sufficient to induce alterations in ethanol metabolism did not result in chronic functional tolerance at either age, although chronic injections were sufficient to induce acute tolerance in adults.

Adolescence is a period of development in which not only initiation of alcohol use, but binge-level consumption is commonly reported, with 8.1% of 8th graders, 16% of 10th graders and 24.6% of 12th graders reported to have had 5 or more drinks in a row within the past two weeks according to a 2008 Monitoring the Future Survey [1]. Given the prevalence of alcohol use, research is critical to determine contributors to and potential adaptations of pervasive alcohol use during adolescence. One factor that may contribute to adolescents' propensity to consume binge amounts of ethanol is their relative insensitivity to many acute ethanol effects compared to adults, such as ethanol-induced sedation [2,3], motor impairment [4], and social impairment [5], all of which may serve as cues to terminate further consumption of ethanol. This decreased ethanol sensitivity typically observed in adolescent animals could be due at least in part to their greater ability to adapt to and counter the effects of ethanol within a single session of ethanol exposure – a form of ethanol adaptation known as acute tolerance (AT). Expression of AT is characterized by within session recovery from ethanol impairment that occurs more rapidly than the decline in blood or brain ethanol concentration (BEC and BrEC, respectively) (see [6,7] for review). Indeed, a number of previous studies have reported that adolescent rats exhibit more AT than adults to sedative [2,8,9] and social impairing [10] effects of ethanol.

Chronic tolerance (CT) (see [6] for review), characterized by a diminished response to a given dose of ethanol after repeated ethanol administrations, may be another factor that could contribute to elevated ethanol use during adolescence, possibly contributing to continued use and increasing the risk for development of future alcohol use disorders [11,12]. Unlike AT data, findings are more mixed regarding the acquisition of chronic tolerance (CT) in adolescent rodents, with some [13,14], but not all [15,16] studies indicating greater or equivalent CT acquisition in adolescents relative to adults. The mixed findings may be attributed to differences in experimental parameters across studies, such as length, dose and frequency of chronic exposure, species and genotype differences, as well as the task used to measure tolerance.

Although adolescents are predisposed to displaying greater AT to many ethanol effects upon initial exposure than adults, it remains to be determined whether similar age differences in AT are apparent following repeated exposures to ethanol, and whether age differences in these adaptations are influenced by the emergence of CT. Given that frequency/dose of ethanol exposure may influence tolerance expression, the current study examined AT and CT to ethanol-induced motor impairment in adolescent and adult rats after repeated exposure to one of three different ethanol regimens: 1 g/kg twice daily, 2 g/kg once daily or 4 g/kg every other day for 10 days.

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doi:10.1016/j.bbr.2011.07.013

A total of 200 juvenile/adolescent and adult male Sprague-Dawley rats bred and reared in our colony at Binghamton University were used in this experiment. On the day after birth, postnatal day (P) 1, litters were culled to 8–10 pups, with a sex ratio of 6 males and 4 females retained whenever possible. Pups were housed with their mother in a standard clear plastic tub with pine shavings until the time of weaning. Offspring were weaned at P21 and housed in same-sex littermate pairs; female offspring were used in other projects. All animals were maintained in a temperature-controlled vivarium on a 12:12-h light:dark cycle (lights on 07:00) with ad libitum access to food (Purina Rat Chow, Lowell, MA) and water. Animals used in this experiment were maintained and treated in accordance with guidelines for animal care established by the National Institutes of Health (1986), using protocols approved by the Binghamton University Institutional Animal Care and Use Committee.

Eight to eleven rats were assigned to each group defined by the 2 (age) × 5 (exposure condition: non-manipulated [NM], saline [SAL], 1 g/kg ethanol [E1], 2 g/kg ethanol [E2], or 4 g/kg intermittent ethanol [E4]) × 2 (test day injection-test interval: 10 vs. 60 min) factorial design of the study, with no more than one animal from a given litter placed into any one condition [17,18]. The E1 group was administered a 1 g/kg dose of ethanol twice daily (8–9 am and 3–4 pm), whereas animals in the E2 group received 2 g/kg ethanol every 24 h. A saline injection equivalent to a 4 g/kg ethanol injection volume was given to animals in the E4 group on day 1 and continuing every other day (i.e., the odd-numbered days) for the 10 day exposure period. Starting on day 2, E4 animals received a 4 g/kg dose of ethanol every 48 h (i.e., on the even-numbered days during the exposure period). Thus, the overall amount of ethanol administered to each animal was equated across groups while varying the dose of ethanol and schedule of administration. Chronic SAL animals were administered saline at an equivalent volume to a 4 g/kg ethanol dose every 24 h for 10 days. Saline (0.9% w/v) and ethanol (18.9% v/v in saline) were administered intraperitoneally (i.p.) at room temperature between 11:00 am and 12:00 pm unless otherwise specified (E1 group). Dose of ethanol was adjusted by volume rather than concentration to avoid concentration induced alterations in ethanol absorption [see 19]. NM animals were not handled during the 10 day exposure period.

The motor impairment test used was a slightly modified version of the procedure originally described by Ramirez and Spear [20], with three massed baseline tests given to each animal instead of one, and the best score (shortest latency) used as the baseline measure. Animals were tested for the negative geotaxis reflex (latency to rotate 180°) on a stationary inclined plane 48 h after the final exposure day. Prior to testing, each animal was weighed and placed individually into a clean holding cage until baseline trials commenced. Immediately following the third baseline trial, animals were injected i.p. with ethanol and then returned to their holding cage for the duration of the pre-assigned injection-test interval (10 or 60 min). Given that ontogenetic differences in initial sensitivity could affect expression of tolerance, impairment level was equated across age by using different ethanol challenge doses for adolescents (2 g/kg) and adults (1.5 g/kg) [based on preliminary data, in progress]. Animals were then given a test trial either 10 or 60 min following ethanol injection and sacrificed immediately thereafter via decapitation. Animals unable to complete the task post ethanol, including animals that fell off the apparatus, were assigned a latency score of 30 s given that animals have been previously found to be able to complete this task within that time frame [20], and thereby allowing data from all animals to be included in the regression analyses across the 10 and 60 min post-injection-test intervals for assessment of AT. Trunk blood and brains were collected, rapidly frozen and maintained at a temperature of −80 °C until analysis of blood and brain ethanol concentrations (BEC and BrEC, respectively) via gas chromatography (see [15] for details). BECs and BrECs were highly correlated at both ages (adolescent: $r = 0.84$, $p < 0.001$; adult: $r = 0.84$, $p < 0.001$) and at each injection-test interval (10 min: $r = 0.77$, $p < 0.001$; 60 min: $r = 0.79$, $p < 0.001$). Data analyses were focused on BrEC data given the behavioral task emphasis of this study.

Table 1
Acute tolerance assessment via slopes of regression.

	Impairment ratio				Radlow's AT method			
	Adolescent		Adult		Adolescent		Adult	
	Slope	*p* Value	Slope	*p* Value	Slope	*p* Value	Slope	*p* Value
NM	−0.42	0.04	−0.28	0.19	1.35	0.01	−0.30	0.48
Saline	−0.46	0.01	−0.82	<0.01	1.43	<0.01	1.87	0.01
E1	−0.66	<0.01	−0.58	0.02	1.94	<0.01	1.48	0.01
E2	−0.57	<0.01	−0.72	<0.01	1.67	<0.01	1.71	<0.01
E4	−0.74	<0.01	−0.77	<0.01	2.1	<0.01	1.85	<0.01

AT was indexed as a greater within session decline in motor impairment relative to brain ethanol concentration (BrEC) across time in each condition. For these determinations, impairment scores (each animal's own post ethanol latency–baseline latency) were divided by each animal's BrEC and then subjected to linear regression at each age and pre-exposure condition. Regressions yielding negative slopes significantly different from zero indexed AT [21]. In these analyses, significant negative slopes emerged in the regression of impairment ratios across the 10 and 60 min injection-test intervals for all age x condition groups except for NM adults (Table 1, left panel). Thus, evidence for AT emerged regardless of exposure condition in adolescents, whereas only adults that were exposed to some sort of chronic perturbation (i.e., chronic injections) exhibited AT.

Expression of AT was also confirmed using Radlow's method (see [7,10,22] for details). Using this method, BrEC and impairment scores were first transformed into percent maximum values for each age and condition. BrEC (% max) − impairment (% max) difference scores were then calculated for each animal and subjected to linear regression at each age and pre-exposure condition, with positive slopes significantly different from zero indexing AT. These regression analyses of Radlow's AT difference scores yielded identical findings, although in this case, significant positive, rather than negative slopes indexed AT (see Table 1, right panel).

A 2 (age) × 2 (time point: 10 and 60) × 5 (condition: NM, SAL, E1, E2, and E4) factorial ANOVA of baseline turn latencies revealed only a significant main effect of age [$F(1,175) = 15.21$, $p < 0.001$], with adolescents overall displaying shorter turn latencies (3.84 ± 0.16) than adults (4.98 ± 0.24). Thus, the chronic exposure regimens did not influence baseline motor performance in this task at either age. The chronic exposure regimens were effective, however, in influencing body weight gain. A 2 (age) × 5 (condition: E1, E2, E4, SAL and NM) factorial ANOVA of body weight on test day, day 12, revealed significant main effects of age [$F(1,282) = 5563.32$; $p < 0.001$] and condition [$F(4,282) = 24.97$; $p < 0.001$]. At test, adolescents of course weighed significantly less than adults. Regardless of age, animals in the E4 condition weighed significantly less than animals in all other exposure conditions and animals in the E2 condition weighed significantly less than their NM counterparts (Table 2).

Given the different ethanol challenge dose used at each age, BrEC data were analyzed separately at each age. The 2 (time point: 10 and 60 min) × 5 (condition: NM, SAL, E1, E2, and E4) factorial ANOVA of the adolescent BrEC data revealed a significant main effect of time point [$F(1,89) = 490.34$; $p < 0.001$] and a significant condition x time point interaction [$F(4,89) = 2.5$; $p < 0.05$]. As expected, BrECs decreased significantly from 10 to 60 min. At the 60 min time point, adolescents in the E2 and E4 conditions had signifi-