Brief Report

Binge Ethanol Intoxication Heightens Subsequent Ethanol Intake in Adolescent, But Not Adult, Rats

María Carolina Fabio[1,2]
Michael E. Nizhnikov[3]
Norman E. Spear[3]
Ricardo Marcos Pautassi[1,2]

[1]*Instituto de Investigación Médica M. y M. Ferreyra, INIMEC-CONICET, Universidad Nacional de Córdoba, Córdoba, C.P. 5000, Argentina*
E-mail: rpautassi@gmail.com

[2]*Facultad de Psicología, Universidad Nacional de Córdoba, Córdoba, C.P. 5000, Argentina*

[3]*Center for Development and Behavioral Neuroscience, Binghamton University, Binghamton New York 13902-6000*

ABSTRACT: *A question still to be answered is whether ethanol initiation has a greater effect on ethanol consumption if it occurs during adolescence than in adulthood. This study assessed the effect of ethanol initiation during adolescence or adulthood on voluntary ethanol consumption when animals were still within the same age range. Adolescent or adult rats were given 5, 2, or 0 ethanol exposures. The animals were tested for ethanol consumption through two-bottle choice tests, before undergoing a 1-week deprivation. A two-bottle assessment was conducted after the deprivation. Adolescents, but not adults, given two ethanol administrations during initiation exhibited significantly higher ethanol intake during the pre-deprivation period. These adolescents also exhibited a threefold increase in ethanol intake after 7 days of drug withdrawal, when compared with controls. These findings suggest that very brief experience with binge ethanol intoxication in adolescence, but not in adulthood, impacts later predisposition to drink. © 2013 Wiley Periodicals, Inc.* Dev Psychobiol 56: 574–583, 2014.

Keywords: *adolescent; rat; ethanol initiation; ethanol intake; light-dark test*

INTRODUCTION

Ethanol use begins most frequently during adolescence, and earlier-onset ethanol initiation is associated with a greater probability of ethanol use disorders (DeWit, Adlaf, Offord, & Ogborne, 2000). Whether a causal relationship exists between these variables or whether they are manifestations of a third phenomenon is still unclear (Schramm-Sapyta, Kingsley, Rezvani, Swartzwelder, & Kuhn, 2009).

The prototypical pattern of ethanol self-administration in adolescence involves ethanol binge drinking, leading to blood ethanol concentrations $\geq$80 mg/dl. This binge drinking appears to facilitate the escalation of ethanol consumption (Hargreaves, Monds, Gunasekaran, Dawson, & McGregor, 2009).

Adolescent animal models have revealed differences between adolescents and adults in the initial response to ethanol (Silveri & Spear, 1998, 2001). Adolescents are more sensitive to ethanol-induced reinforcement and social facilitation (Pautassi, Myers, Spear, Molina, & Spear, 2008; Spear & Varlinskaya, 2005, 2010); but less sensitive than adults to the sedative and motor impairing, and acute hangover effects of ethanol (Doremus, Brunell, Rajendran, & Spear, 2003; Ristuccia & Spear, 2004; Silveri & Spear, 1998, 2001). This response pattern may represent a risk factor for the development of problematic ethanol intake.

Previous studies indicated that the adolescent consumption of ethanol can be further enhanced by early exposure to ethanol during the prenatal or early postnatal period (Abate, Pueta, Spear, & Molina, 2008). A scarcity of animal studies have assessed the effects of ethanol exposure during adolescence on later adolescent ethanol self-administration. An important question to test using animal models is whether adolescent ethanol initiation has a differential effect on ethanol intake than if initiation is delayed until adulthood. Hargreaves

Manuscript Received: 4 August 2012
Manuscript Accepted: 12 December 2012
Correspondence to: Ricardo Marcos Pautassi
Contract grant sponsor: NIAAA
Contract grant number: AA011960, AA013098, AA015992, AA017823, AA018164, PIP 2010-2012, SECYT-UNC, PICTPRH 246
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 22 January 2013
DOI 10.1002/dev.21101 • © 2013 Wiley Periodicals, Inc.

et al. (2009) found that intermittent access to ethanol-induced binge-like drinking in adolescent but not adult rats. Siegmund, Vengeliene, Singer, & Spanagel (2005) provided evidence that adolescent ethanol initiation enhanced stress-induced ethanol consumption. A subsequent study found that adult female rats that initiated ethanol self-administration during adolescence were more sensitive to stress-induced ethanol intake than controls that initiated ethanol intake in adulthood (Fullgrabe, Vengeliene, & Spanagel, 2007).

Overall, these studies are consistent with epidemiological data, in which the adolescent onset of ethanol exposure promoted ethanol consumption (but see Tambour, Brown, & Crabbe, 2008). One limitation of these long-term access studies is that they relied on the self-administration of ethanol as the method of initiation. With this method, level of intoxication is not controlled and the influence of age of ethanol initiation is confounded by baseline differences in drinking between adolescents and adults. Another limitation was the use of lengthy initiation phases that did not allow the endpoint section of intake tests to occur within the adolescent period.

One alternative is to expose animals to controlled intragastric or intraperitoneal administrations of ethanol, which allow greater control over dose. Adolescent rats repeatedly exposed to ethanol exhibited changes in neurotransmitter systems (Pascual, Boix, Felipo, & Guerri, 2009), that were associated with greater ethanol intake in adulthood. This study, however, lacked a comparison with adult animals exposed to ethanol in a similar fashion as adolescents.

The present study assessed the effects of brief ethanol initiation during adolescence or adulthood on the predisposition for voluntary ethanol consumption when the animals were still within the same age range. Animals were given five or two intermittent exposures to ethanol, or vehicle, and then were tested for ethanol intake. The intake protocol involved an initial phase of 4 days in which subjects were tested with a two-bottle procedure, followed by a 1-week ethanol deprivation phase and a post-deprivation assessment of ethanol intake. The hypothesis was that passive exposure to ethanol augments ethanol consumption in adolescent but not in adult rats. To examine the potential mechanisms that underlie this putative effect, anxiety was measured using the light/dark box test before self-administration and on the last day of the ethanol-deprivation period.

MATERIALS AND METHODS

Experimental Design

A 2 (age: adolescence or adulthood) × 3 (ethanol initiation treatment: 5-day exposure group, 2-day exposure group, and control group treated with vehicle) factorial design was employed, with 10–13 animals in each group. Fourteen animals (7 adolescent and 7 adults) were included in an untreated (UT) condition.

Subjects

Seventy-four (39 adolescents and 35 adults) outbred male Wistar rats were used. Adolescents and adults were 28 and 70 postnatal days (PD) old, respectively, at the beginning of procedures. Animals were born and reared in a temperature-controlled vivarium (21–22°C) at INIMEC-CONICET (Córdoba, Argentina). Births were examined daily, and day of parturition was considered PD0. Culling was conducted on PD1 and pups remained with their respective dams until PD 21. After weaning, animals were housed together until PD24 when they were paired-housed according to their experimental condition. Half of the males of a given litter were tested during adolescence, whereas the remaining half was tested during adulthood. The procedures complied with the National Institutes of Health Guide for Care and Use of Laboratory Animals (National Research Council, 1996) and were approved by the Institutional Animal Care and Use Committee.

Ethanol Administration (i.e., Initiation) Procedures

Ethanol initiation began on PD28 and PD70 for adolescents and adults, respectively. The animals were given intragastric administrations as described in Pautassi et al. (2008) every other day and received five intubations of 2.5 g/kg ethanol (5-day exposure group; ethanol on PD28, 30, 32, 34, and 36 [adolescents] or PD70, 72, 74, 76, and 78 [adults]), two intubations of 2.5 g/kg ethanol (2-day exposure group; ethanol on PD28 and 32 and vehicle on PD30, 34, and 36 [adolescents] or ethanol on PD70 and 74 and vehicle on PD72, 76, and 78 [adults]), or no intubations of ethanol (control group treated with vehicle on PD28-36 [adolescent] or PD70-78 [adults]). The ethanol dose was selected on the basis of studies from our laboratory (Acevedo, Molina, Nizhnikov, Spear, & Pautassi, 2010) that suggested its effectiveness in promoting ethanol intake in adolescents. This was achieved by administering .015 ml/kg of a 21% ethanol solution (96% proof ethanol, Porta Hnos., Córdoba, Argentina; vehicle: tap water).

Untreated animals experienced only standard housing during PDs 28-36 or PDs 70-78. These animals were then tested for sucrose intake on PDs 37 or 79, and for anxiety response on PDs 38 or 80 (adolescent and adult subjects, respectively). Untreated subjects were not tested for ethanol intake test. The purpose of including this group was to control for alterations in sucrose preference or anxiety resulting from the manipulations inherent to initiation procedures.

Homecage Ethanol Self-Administration Tests

On PD37 (adolescents) or PD79 (adults), the animals were individually housed and exposed for 24 hr to two bottles equipped with ball-point tubes, and filled with tap water or 1% w/v sucrose. The aim was to assess potential differences in sucrose preference after initiation procedures.

On PD39-42 or PD80-83 (self-administration phase for adolescents and adults, respectively) the animals had continuous access to two drinking bottles, one containing a 5.6% v/v ethanol solution sweetened with 1% w/v sucrose and another that contained water. Sweetened ethanol was chosen based on the finding that adolescent Wistar rats did not voluntarily consume unsweetened ethanol (5.6% v/v ethanol) but consumed slightly sweetened (sucrose 1% w/v) ethanol (5.6% v/v) overnight (Maldonado, Finkbeiner, & Kirstein, 2008). The available protocols to induce significant consumption of unsweetened ethanol in rats are quite lengthy and, if used in the present study, would not have allowed the post-deprivation intake test to occur within the adolescent period. We previously employed unsweetened ethanol to test adolescent self-administration (Ponce, Pautassi, Spear, & Molina, 2004, 2011), but these tests required extensive, 22-hr liquid deprivation, which is a significant stressor.

The position of the water and ethanol bottles was varied across sessions to avoid side-preference effects. On each test day, the bottles were positioned at 11:00 AM to 12:00 PM, and the animals were left undisturbed. On the following day (9:00 AM to 10:00 AM), the bottles were removed, weighed to the nearest .1 g and refilled. During weighing and refilling animals were allowed to interact with each other for 2 hr to minimize stress-induced isolation. Ethanol intake is expressed as grams of ethanol consumed per kilogram of body weight (g/kg). Spillage was accounted for by placing water and ethanol bottles in an empty holding cage overnight. The difference in weight was subtracted from each animal's intake score to account for spillage that may have occurred overnight. Food consumption was monitored by providing a premeasured amount of food and weighing the portion that remained each day.

After the 4-day self-administration phase, the animals underwent a 7-day ethanol recess phase, in which they had ad libitum access to water and food. On PD50 or 92 (post-deprivation assessment for adolescents and adults, respectively) animals were tested for 24 hr in ethanol self-administration, through the two-bottle procedure described for the pre-deprivation self-administration phase. The rationale for the week interval between the initial 4 days of self-administration and the last ethanol intake assessment was to fit the treatment and the test within the same adolescent period. We were also guided by a recent study in which animals experienced a short ethanol deprivation period between ethanol intake tests (Acevedo et al., 2010).

Light/Dark Box Test

A light/dark box (LDB) test was conducted on PD38 and 48 (adolescents) or PD80 and 90 (adults). The aim was to detect possible differences in anxiety-like behavior that could explain ethanol drinking patterns. The light/dark box exploits the conflict between the animal's tendency to explore a new environment and its fear of bright light (Cancela, Bregonzio, & Molina, 1995).

The apparatus ($42 \times 25 \times 25$ cm^3) consisted of two compartments made of high impact acrylic, one dark ($17.5 \times 25 \times 25$ cm^3) illuminated with a 25 W red bulb, and one white ($24.5 \times 25 \times 25$ cm^3) illuminated by a 60 W white bulb lamp. The compartments were separated by a divider with a 6.5×6.5 cm^2 opening at floor level. The test lasted 5 min and began by gently placing the animal in the center of the dark area, facing away from the white area. The test was videotaped and then analyzed by a researcher blind to the treatments. The latency to cross to the white compartment, number of transfers from one compartment to the other compartment, and time spent in the white compartment were measured using Etholog software (Ottoni, 2000).

Data Analysis

The preliminary analysis indicated significant age-related differences in the initial consumption of ethanol, with adolescent subjects drinking almost twice as much ethanol as adults in self-administration Session 1 [$F_{1,65} = 5.17$, $p < .05$]. Adults subjects, on the other hand, exhibited greater overall locomotion in the light/dark box test [$F_{1,65} = 5.99$, $p < .01$]. Baseline differences between adolescents and adults have often been observed across several variables, including ethanol intake (Doremus, Brunell, Rajendran, & Spear, 2005), stress responsiveness (Varlinskaya & Spear, 2012), and preference for tactile cues (Pautassi et al., 2008). We followed the statistical approach of the latter studies and analyzed each dependent variable using separate analyses of variance (ANOVAs) for adolescents and adults. The loci of significant main effects or significant interactions were analyzed using Tukey post hoc tests. Planned comparisons were also conducted if justified by previous hypotheses.

Self-Administration During Pre-Deprivation and Post-Deprivation Sessions. For each age, ethanol intake (g/kg) during the 4-day pre-deprivation phase was analyzed using two-way mixed-factor ANOVAs. Ethanol initiation (5-day exposure, 2-day exposure, and the control group) was the between-group factor and session was the within-group measure. Similar ANOVAs were used to analyze water intake (ml/100 g) and food intake (g/kg) across the procedures. One-way ANOVAs (comparative factor: ethanol initiation condition) were performed for sucrose intake on the adaptation test day (g/kg, the UT group was also included in this ANOVA) and for ethanol intake (g/kg) in the post-deprivation assessment.

Light/Dark Box Tests on PD38 and 48 (Adolescents) or PD80 and 90 (Adults). The latency to cross to the white side, number of transfers from one compartment to the other compartment and time spent in the white compartment were analyzed for adolescents and adults using two-way ANOVAs (comparative factor between groups: ethanol initiation condition; within-group measure: day of testing). Planned comparisons were conducted between the UT and the vehicle-exposed group, for each of the variables measured on the first light–dark test.

We further analyzed ethanol intake measurements using a correlational approach. Pearson's *r* product–moment correlations were separately conducted for the adolescent and adult groups, and for each ethanol treatment at initiation. The specific question under analysis was to what extent initial self-administration of ethanol predicted later ethanol self-administration during the

initial 4-day phase and during the post-deprivation intake session. It has been suggested that the quality of the initial experience with ethanol significantly affects later ethanol drinking (Schramm-Sapyta et al., 2009). Moreover, it has been observed that patterns of ethanol intake are established early in adolescent rats, with subjects that drink heavily during the first exposure to ethanol keeping this behavior on subsequent sessions (Schramm-Sapyta et al., 2008). It is still unknown, however, if ethanol drinking behavior follows a similar trajectory in adult subjects, and if this pattern is altered in subjects given prior ethanol initiation.

RESULTS

Body Weight, Sucrose Consumption During Adaptation and Food Intake

The administration of ethanol during initiation did not significantly alter body weight during the 4-day pre-deprivation phase or during the post-deprivation assessment. As expected, the ANOVAs only indicated that body weight in the adolescents increased as a function of days during the pre-deprivation two-bottle intake assessment, $F_{3,99} = 361.4$, $p < .001$. Sucrose consumption (g/kg) at beginning of intake tests (PD37 or PD79) was not affected by ethanol initiation. Sucrose intake in animals given 5-day ethanol exposure, 2-day ethanol exposure, vehicle controls and in untreated controls was .54 ± .08, .52 ± .08, .45 ± .06, and .34 ± .10; and 2.12 ± .47, 2.58 ± .47, 2.11 ± .39, and 1.98 ± .54; for adolescents and adults, respectively.

Food intake during the 4 days in which subjects were tested with the two-bottle procedure increased in adolescents as a function of days [$F_{3,99} = 41.83$, $p < .001$] but remained stable in adults. These patterns were unaffected by ethanol initiation treatment. One-way ANOVAs indicated that food intake during the post-deprivation session was similar across initiated and non-initiated adolescents or adults.

Descriptive results for body weight (g) and food intake (g/kg) are depicted in Table 1.

Ethanol and Water Self-Administration During Ethanol Intake Tests

Adolescent Rats. The ANOVA for the 4-day phase in which subjects were tested with the two-bottle procedure revealed a significant main effect of session, $F_{3,99} = 5.19$, $p < .005$. The post hoc tests indicated greater ethanol consumption during Session 1 than in the subsequent sessions, which in turn did not differ

Table 1. Body Weight (g), Water Intake (ml/100 g of Body Weight), Food Intake (g/kg of Body Weight) and Ethanol Intake (ml/100 g of Body Weight); in Adolescent and Adult Rats During the 4 Days in Which Subjects Were Tested With a Two-Bottle Procedure (Pre-Deprivation Intake Sessions) and During the Post-Deprivation Intake Session

	Adolescent Rats			Adult Rats		
	5-Day Group	2-Day Group	Control	5-Day Group	2-Day Group	Control
Body weight (g)						
Pre-deprivation intake session 1	185.92 ± 5.91	176.83 ± 4.10	182.09 ± 5.93	443.82 ± 13.10	433.75 ± 10.99	456.58 ± 9.00
Pre-deprivation intake session 2	196.62 ± 6.31	187.83 ± 4.70	195.64 ± 5.32	449.36 ± 13.12	439.25 ± 10.77	462.75 ± 9.27
Pre-deprivation intake session 3	205.08 ± 6.51	199.25 ± 5.10	206.72 ± 5.60	454.45 ± 13.54	440.33 ± 12.34	466.67 ± 9.55
Pre-deprivation intake session 4	214.54 ± 7.08	207.75 ± 5.15	214 ± 5.56	458.27 ± 13.56	419.42 ± 26.16	468.92 ± 9.39
Post-deprivation intake session	285.54 ± 10.98	278.83 ± 7.99	16.65 ± 4.59	482.64 ± 16.79	470.67 ± 11.45	496.33 ± 11.05
Food intake (g/kg)						
Pre-deprivation intake session 1	4.24 ± .26	3.83 ± .17	4.37 ± .20	13.19 ± .65	12.59 ± .68	13.41 ± .86
Pre-deprivation intake session 2	4.72 ± .37	4.20 ± .39	4.80 ± .41	12.86 ± .84	13.63 ± .81	14.42 ± .57
Pre-deprivation intake session 3	5.24 ± .33	5.02 ± .22	5.46 ± .25	13.36 ± 1.05	13.29 ± .78	14.15 ± .71
Pre-deprivation intake session 4	8.61 ± 3.55	5.08 ± .24	5.68 ± .40	12.57 ± 1.01	12.17 ± .95	13.91 ± .69
Post-deprivation intake session	7.86 ± .67	7.38 ± .53	8.29 ± .56	13.45 ± .70	13.74 ± .75	13.30 ± 1.39
Water intake (ml/100 g)						
Pre-deprivation intake session 1	9.70 ± 1.62	8.94 ± 1.82	12.99 ± 1.84	11.11 ± 1.31	9.10 ± 1.48	7.67 ± 1.02
Pre-deprivation intake session 2	9.28 ± 1.70	10.19 ± 1.86	12.20 ± 2.00	11.18 ± 1.82	10.23 ± 1.61	8.64 ± 1.32
Pre-deprivation intake session 3	11.91 ± 1.51	12.99 ± 1.90	14.39 ± 2.15	9.21 ± 2.53	9.25 ± 1.75	8.60 ± 1.39
Pre-deprivation intake session 4	11.8 ± 1.39	12.34 ± 1.55	12.65 ± 1.65	10.52 ± 1.73	12.19 ± 3.88	7.21 ± 1.55
Post-deprivation intake session	6.76 ± 1.36	6.68 ± 1.38	7.98 ± 1.58	10.63 ± 2.66	10.28 ± 2.24	7.63 ± 1.66
Ethanol intake (ml/100 g)						
Pre-deprivation intake session 1	7.12 ± 1.94	10.55 ± 2.42	5.43 ± 1.94	2.56 ± 1.02	5.38 ± 1.56	3.20 ± .88
Pre-deprivation intake session 2	7.44 ± 2.09	9.37 ± 2.39	4.93 ± 1.96	3.63 ± 1.31	4.02 ± 1.70	3.92 ± 1.61
Pre-deprivation intake session 3	5.15 ± 1.58	5.49 ± 2.00	3.04 ± 1.57	6.07 ± 1.85	3.54 ± 1.13	3.32 ± .78
Pre-deprivation intake session 4	8.69 ± 1.57	10.07 ± 1.94	5.78 ± 1.63	8.80 ± .24	10.47 ± 1.40	8.57 ± .18
Post-deprivation intake session	18.89 ± .62	19.40 ± .52	18.83 ± .52	7.59 ± 1.89	6.51 ± 1.83	7.88 ± 1.25

Results are presented as mean ± standard error of the means.

between each other. Guided by a priori hypothesis, planned comparisons were conducted between the 5-day or the 2-day ethanol treatment group and the control group, for each intake session. These analyses revealed significantly greater ethanol intake scores (g/kg) in adolescents given 2-day exposure than in control counterparts, during pre-deprivation intake sessions 1 and 2 ($F_{1,33} = 4.61$, $F_{1,33} = 5.24$, both p's $< .05$; for Sessions 1 and 2, respectively). These results are depicted in Figure 1.

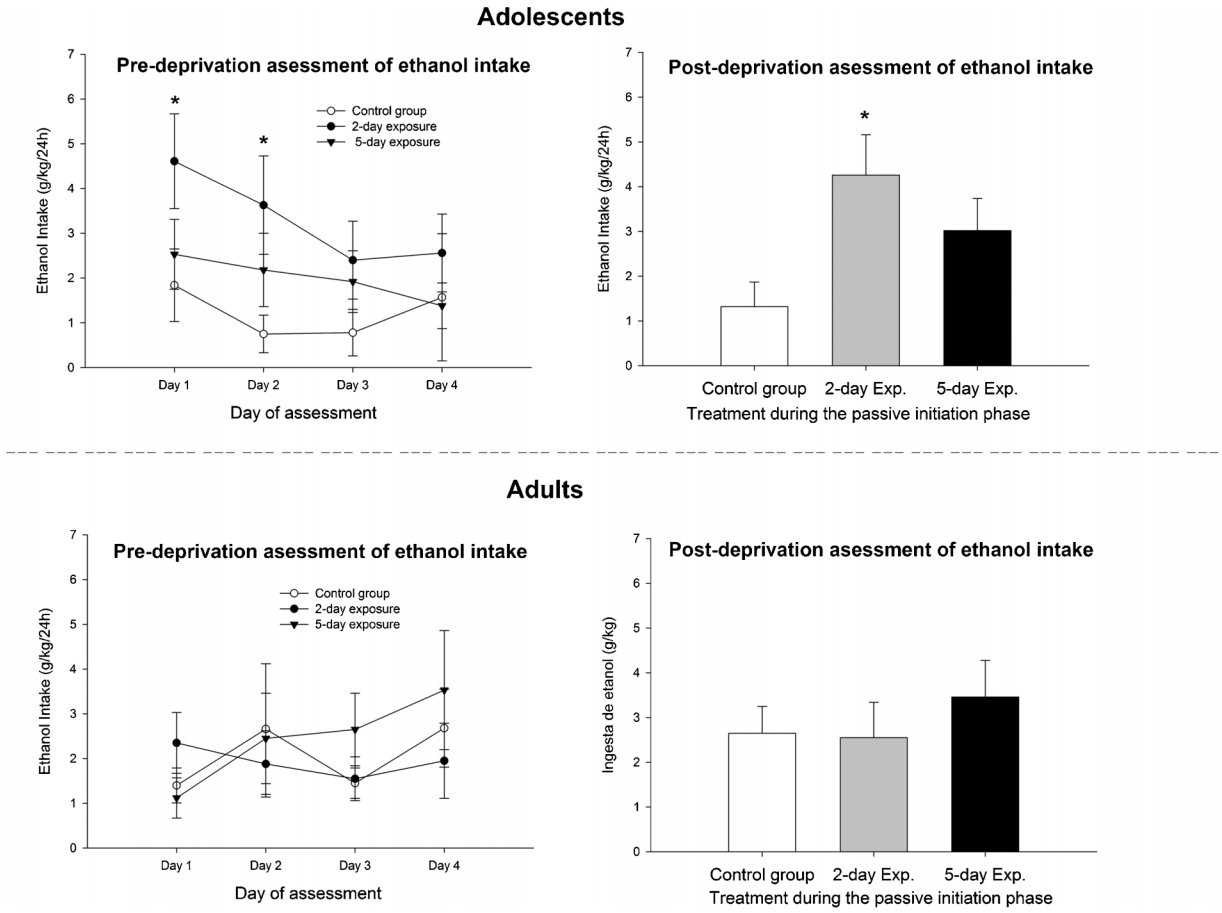

FIGURE 1 Ethanol intake (g/kg) in adolescent (upper panels) and adult (lower panels) male rats during the 4-day self-administration phase and during the post-deprivation assessment. Ethanol intake during the initial 4 days of self-administration is depicted as a function of day of assessment (Sessions 1, 2, 3, and 4) and ethanol treatment during initiation. Initiation occurred on postnatal days 28-36 (PD28-36) or PD70-78 for adolescents and adults, respectively. During initiation, the animals were given intragastric administrations every other day and received five 2.5 g/kg ethanol intubations (5-day exposure group; ethanol on PD28, 30, 32, 34, and 36 [adolescents] or PD70, 72, 74, 76, and 78 [adults]), two 2.5 g/kg ethanol intubations (2-day exposure group; ethanol on PD28 and 32 and vehicle on PD30, 24, and 36 (adolescents) or ethanol on PD70 and 74 and vehicle on PD72, 76, and 78 [adults]), or no ethanol intubations (control group treated with vehicle on PD28-36 [adolescents] or PD70-78 [adults]). The initial self-administration phase (i.e., pre-deprivation assessment of ethanol intake) lasted for 4 days, in which the animals were given continuous, 22 hr two-bottle choice between ethanol (5.6% v/v) and water in the homecage. After the last acquisition session, the animals had ad libitum access to only water and food for 7 days, and then a single 22-hr test of ethanol intake was conducted (two-bottle homecage choice test between 5.6% v/v ethanol and water; post-deprivation assessment). Adolescents but not adults given two ethanol exposures during initiation exhibited significantly greater ethanol intake than controls during pre-deprivation intake Sessions 1 and 2, and during the post-deprivation assessment. These significant effects of ethanol initiation are indicated by the asterisk. The vertical bars indicate SEM.

Ethanol initiation also appeared to heighten ethanol intake during the post-deprivation assessment (see Fig. 1). The one-way ANOVA revealed a significant main effect of treatment at initiation ($F_{2,33} = 3.73$, $p < .005$). Subsequent post hoc tests indicated significantly greater consumption of ethanol in adolescents given 2-day exposure than in the control group. Animals that received five intubations also exhibited greater ethanol intake than controls, but the difference did not achieve statistical significance.

The ANOVA for water intake during pre-deprivation intake sessions indicated only a significant main effect of session ($F_{3,99} = 4.41$, $p < .005$), and the post hoc tests revealed greater water intake on Days 3 and 4 of the pre-deprivation intake phase than on Days 1 and 2. Treatment at initiation did not modify water intake during pre-deprivation sessions or during the post-deprivation session. The average mean (±SEM) water intake and ethanol intake (ml/100 g) in adolescents given 5-day exposure, and 2-day exposure and controls is depicted in Table 1.

Adult Rats. Ethanol drinking in adult rats remained stable during the 4 days in which subjects were tested with a two-bottle procedure and was not affected by the ethanol initiation procedures. The ANOVAs indicated a lack of significant main effects or significant interactions. The planned comparisons between the 5-day or the 2-day ethanol treatment group and the control group did not achieved significance. Unlike adolescent subjects, post-deprivation ethanol intake in adult rats was insensitive to previous passive exposure to ethanol (see Fig. 1). The ANOVA revealed that ethanol-initiated and control (vehicle-treated) subjects exhibited similar levels of ethanol consumption.

The ANOVAs for water intake during the four pre-deprivation sessions and during the post-deprivation session indicated no significant main effects or significant interactions. Average mean and SEM water intake and ethanol intake (ml/100 g) across sessions is presented in Table 1.

Light/Dark Box Test

Adolescent Rats. Adolescents given 0, 2, or 5 ethanol exposures at initiation exhibited similar latency to cross to the white side, number of transfers from one compartment to the other, and similar time spent in the bright side of the light/dark box. This pattern was fairly similar during the first and second light/dark box tests. The ANOVA revealed a significant main effect of day of testing on the number of transfers, [$F_{1,33} = 4.27$, $p > .05$], indicating a reduction of overall locomotor activity in the second test session.

Adult Rats. Ethanol initiation did not exert significant main effects or significantly interact with the remaining variables in any of the measures. The number of transfers tended to decrease in the second test, as indicated by a borderline main effect of day of testing ($p = .056$).

Planned comparisons indicated that anxiety during the first light–dark test did not significantly differ between the untreated and vehicle controls, neither in adolescents nor in adults.

Correlation Between Measures of Ethanol Intake

Adolescent Rats. Ethanol intake was highly correlated during the four pre-deprivation intake sessions in all of the ethanol initiation conditions. Post-deprivation ethanol intake was predicted in the control group—but not in the 5- or 2-day exposure group—by ethanol intake during acquisition sessions 1–4 ($r = .75$, .89, .80, and .77; respectively) (Tab. 2).

Adult Rats. In control adults ethanol intake on pre-deprivation day 2 was predictive of ethanol intake on pre-deprivation day 3 and the latter predicted ethanol intake at pre-deprivation day 4. The animals given five ethanol exposures exhibited a significant association between ethanol intake on pre-deprivation day 1 and ethanol self-administration on pre-deprivation days 2 and 4. In this group, post-deprivation ethanol consumption was predicted by pre-deprivation day 3. In the 2-day ethanol initiation group ethanol intake on pre-deprivation day 1 predicted intake on pre-deprivation days 2 and 3, and post-deprivation ethanol intake was predicted by ethanol intake on pre-deprivation days 1, 2, and 3 ($r = .81$, .73, and .93, respectively).

DISCUSSION

The present study analyzed whether brief and intermittent exposure to ethanol heightens later ethanol intake, particularly after a period of ethanol deprivation, and tested the age-specificity of this effect. Consistent with previous studies (e.g., Doremus et al., 2005), the initial self-administration of ethanol was greater in adolescents than in adults. The most important new finding was that adolescent rats exposed to binge ethanol intoxication subsequently exhibited greater ethanol intake than control subjects. Specifically, binge ethanol induced a significant increase in ethanol intake in adolescents during intake Sessions 1 and 2 of the initial self-administration phase. Moreover, when tested after 7 days of drug withdrawal, adolescents given two passive ethanol intubations exhibited a significant threefold increase in ethanol intake compared with age-matched controls. These effects were not found in adults and no changes in water intake were observed.

Table 2. Pearson Correlations Between Ethanol Intake (g/kg) During Acquisition Days 1–4 and During the Post-Deprivation Assessment, for Adolescent and Adult Rats Given 5 [5-Day Exposure Group], 2 [2-Day Exposure Group] or 0 [Control Group Treated With Vehicle] Administrations of 2.5 g/kg Ethanol During Ethanol Initiation Procedures

	Ethanol Intake at Acquisition Day 1	Ethanol Intake at Acquisition Day 2	Ethanol Intake at Acquisition Day 3	Ethanol Intake at Acquisition Day 4	Post-Deprivation Assessment of Ethanol Intake
Adolescent rats					
Ethanol intake at Acquisition day 1					
5-Day group	—	—	—	—	—
2-Day group	—	—	—	—	—
Control	—	—	—	—	—
Ethanol intake at Acquisition day 2					
5-Day group	.65	—	—	—	—
2-Day group	.87	—	—	—	—
Control	.82	—	—	—	—
Ethanol intake at Acquisition day 3					
5-Day group	.69	.68	—	—	—
2-Day group	.78	.95	—	—	—
Control	.65	.93	—	—	—
Ethanol intake at Acquisition day 4					
5-Day group	.64	.76	.90	—	—
2-Day group	.79	.94	.97	—	—
Control	.64	.91	.99	—	—
Post-deprivation assessment of ethanol intake					
5-Day group	.27	.47	.27	.46	—
2-Day group	.29	.27	.11	.21	—
Control	.75	.89	.80	.77	—
Adult rats					
Ethanol intake at Acquisition day 1					
5-Day group	—	—	—	—	—
2-Day group	—	—	—	—	—
Control	—	—	—	—	—
Ethanol intake at Acquisition day 2					
5-Day group	.76	—	—	—	—
2-Day group	.88	—	—	—	—
Control	.12	—	—	—	—
Ethanol intake at Acquisition day 3					
5-Day group	.53	.41	—	—	—
2-Day group	.75	.67	—	—	—
Control	.25	.71	—	—	—
Ethanol intake at Acquisition day 4					
5-Day group	.79	.97	.44	—	—
2-Day group	.06	.12	.33	—	—
Control	−.33	.10	.50	—	—
Post-deprivation assessment of ethanol intake					
5-Day group	.45	.57	.82	.57	—
2-Day group	.81	.73	.93	.10	—
Control	.06	−.29	.08	.10	—

Correlations significant at $p < .05$ are marked in bold. Mirrored correlations coefficients were deleted.

Rodd-Henricks et al. (2002a, 2002b) gave adolescent or adult ethanol-preferring rats free choice access to ethanol or standard housing. During adulthood, ethanol operant self-administration was greater in animals that had been initiated to ethanol during adolescence. This and others (e.g., Fullgrabe et al., 2007; Siegmund et al., 2005) are similar to the present study in that they examined the predisposition for ethanol intake after adolescent exposure. The previous studies, however, employed extensive initiation phases (e.g., >7 months, Tambour et al., 2008) that lasted past adolescence, not allowing the intake test to occur within the adolescent

period. Moreover, in these studies the initiation phase consisted of self-administration of ethanol, which implies habituation of neophobia, familiarization with the flavor of ethanol and usually results in baseline differences in drinking across sessions. The latter factor is particularly important when comparing adults and adolescents, given the propensity of adolescents to drink significantly more ethanol than adults (Doremus et al., 2005). In the present study, the choice of intragastric intubation as the method for ethanol initiation equated level of ethanol exposure across ages. The present study is also significant in that it specifically investigated whether early-onset drinking in an early stage of adolescence facilitates the escalation of ethanol consumption during adolescence.

The alcohol exposure procedure employed in the present study involved 5 or 2 binge exposures, followed by 4 days of self-administration to a moderate concentration of alcohol. Caution should be taken, therefore, when comparing the results of this study with others that employed protracted exposure to ethanol or repeated cycles of access to and withdrawal from ethanol (e.g., Bell et al., 2008; Spanagel, 2000). Those procedures are specifically aimed to model alcoholism and proven to induce tolerance, physical dependence, and withdrawal symptoms (Spanagel, 2000).

Another caveat of the present study is the use of sweetened ethanol. The enhanced predisposition for ethanol drinking in ethanol-initiated adolescents may have been attributable to early ethanol exposure altering the palatability of sucrose or inducing sucrose seeking due to its nutritional value. There were, however, no differences in sucrose intake across ethanol initiation conditions for adolescents or adults, when the sweet tastant was tested alone against water. Likewise, no differences were found in food intake or body weight that could have indicated altered nutritional status due to early ethanol exposure.

The most intriguing result of the study was the significant increase in ethanol self-administration observed in adolescents after just 2 days of ethanol exposure. These animals drank more ethanol than non-initiated counterparts during the first 2 days of self-administration as well as during the post-deprivation assessment. What is the potential mechanism underlying higher ethanol consumption in ethanol-initiated adolescents? Ethanol exposure during passive initiation or during the initial self-administration phase could conceivably alter anxiety-like behavior. The results of the light/dark box test, however, indicated similar patterns of exploration in ethanol-initiated and non-initiated animals.

Another possibility is that adolescent ethanol initiation facilitates subsequent ethanol intake by altering the normal pattern of development of specific transmitter systems. A previous study found that chronic intermittent ethanol exposure during adolescence altered basal level of dopamine in nucleus accumbens and induced a down-regulation of dopaminergic and glutamatergic receptors in prefrontral cortex (PFC; Pascual et al., 2009). Fabio, Nizhnikov, Spear, & Pautassi (2012) revealed that earlier ethanol initiation in the rat (during the last 4 days of gestation) also altered basal neural activity at PFC, when measured at adolescence. These effects were associated with heightened ethanol consumption.

Intriguingly, ethanol exposure on PD28 and PD32 increased later ethanol intake, but the 5-day exposure treatment apparently did not. It could be that ethanol initiation enhances subsequent ethanol intake when initiation occurs during restricted developmental timeframes, such as during early adolescence. Dopaminergic receptors, which modulate ethanol-induced appetitive learning, reach their peak at PD28, and then decline significantly (Tarazi & Baldessarini, 2000). The present results are also in agreement with Acevedo et al. (2010), in which ethanol exposure at PD28 but not at PD31, enhanced subsequent ethanol consumption. It is also possible that the 5-day exposure treatment lacked an effect on subsequent intake due to this treatment inducing tolerance to the appetitive effects of ethanol.

Yet another possibility is that animals exposed twice to ethanol on PD28 and PD32 acquired a conditioned preference for ethanol's orosensory cues due to perception of non-metabolic ethanol excretion (by urine or perspiration) in close contiguity with pharmacological properties of the drug (Molina, Chotro, & Spear, 1989). On the other hand, animals exposed to the 5-day exposure treatment may have developed a conditioned aversion, which competed with the preference acquired on the first days of exposure. In other words, the more protracted ethanol experience may have induced taste aversion to ethanol chemosensory properties and this memory affected subsequent ethanol intake.

The possibility that repeated administration of ethanol induced metabolic tolerance in adolescents, but not in adults, cannot be dismissed. A recent study (Van Skike, Novier Diaz-Granados, & Matthews, 2012) found metabolic tolerance in adolescent rats that had been exposed to ethanol vapor 16 hr a day for 4 days. In adolescent animals ethanol intake across the initial 4 days of self-administration was highly correlated; and in control adolescents there was a significant association between ethanol drinking before and after drug deprivation. It seems that patterns of ethanol intake were established early in adolescence and, for those given vehicle at initiation, maintained through the post-

deprivation test. Ethanol-initiated animals, on the other hand, did not exhibit a significant correlation between pre- and post-deprivation scores. It seems that, similar to the results found by Schramm-Sapyta et al. (2008), those control adolescents that consumed the most during the first exposure to ethanol kept drinking heavily during subsequent sessions and exhibited the highest drinking scores at the post-deprivation assessment. Passive exposure to ethanol disrupted this normal trajectory of ethanol intake otherwise seen in adolescents.

The present study is consistent with findings suggesting that onset of ethanol consumption exerts greater impact on predisposition to drink when it occurs at adolescence than when it is delayed until adulthood. The study shows that even brief binge ethanol intoxication during adolescence can increase drinking. Specifically, significantly greater ethanol drinking was observed in adolescents after just 2 days of alcohol exposure. In agreement with recent work (Schramm-Sapyta et al., 2008), adolescents also exhibited greater susceptibly to ethanol drinking relapse than adults.

NOTES

This work, a collaborative project between the Research Foundation of SUNY Binghamton and Instituto Ferreyra, was supported by NIAAA grants AA011960, AA013098, AA015992, AA017823 to NES, AA018164 to MN and grants PIP 2010-2012, SECYT-UNC and PICTPRH 246 to RMP.

REFERENCES

Abate, P., Pueta, M., Spear, N. E., & Molina, J. C. (2008). Fetal learning about ethanol and later ethanol responsiveness: evidence against "safe" amounts of prenatal exposure. Experimental Biology and Medicine (Maywood), 233, 139–154.

Acevedo, M. B., Molina, J. C., Nizhnikov, M. E., Spear, N. E., & Pautassi, R. M. (2010). High ethanol dose during early adolescence induces locomotor activation and increases subsequent ethanol intake during late adolescence. Developmental Psychobiology, 52, 424–440.

Bell, R. L., Rodd, Z. A., Schultz, J. A., Peper, C. L., Lumeng, L., Murphy, J. M., & McBride, W. J. (2008). Effects of short deprivation and re-exposure intervals on the ethanol drinking behavior of selectively bred high alcohol-consuming rats. Alcohol, 42, 407–416.

Cancela, L. M., Bregonzio, C., & Molina, V. A. (1995). Anxiolytic-like effect induced by chronic stress is reversed by naloxone pretreatment. Brain Research Bulletin, 36, 209–213.

DeWit, D. J., Adlaf, E. M., Offord, D. R., & Ogborne, A. C. (2000). Age at first alcohol use: a risk factor for the development of alcohol disorders. The American Journal of Psychiatry, 157, 745–750.

Doremus, T., Brunell, S., Varlinskaya, E., & Spear, L. (2003). Anxiogenic effects during withdrawal from acute ethanol in adolescent and adult rats. Pharmacology, Biochemistry and Behavior, 75, 411–418.

Doremus, T. L., Brunell, S. C., Rajendran, P., & Spear, L. P. (2005). Factors influencing elevated ethanol consumption in adolescent relative to adult rats. Alcoholism: Clinical and Experimental Research, 29, 1796–1808.

Fabio, M. C., Nizhnikov, M., Spear, N. E., & Pautassi, R. M., (2012). Adolescent rats exposed to ethanol during gestation exhibit altered c-fos activity in infralimbic cortex. Poster presented at the 2012 International Society for Biomedical Research on Alcoholism (ISBRA) World Congress. Sapporo, Japan, September 9–12.

Fullgrabe, M. W., Vengeliene, V., & Spanagel, R. (2007). Influence of age at drinking onset on the alcohol deprivation effect and stress-induced drinking in female rats. Pharmacology Biochemistry and Behavior, 86, 320–326.

Hargreaves, G. A., Monds, L., Gunasekaran, N., Dawson, B., & McGregor, I. S. (2009). Intermittent access to beer promotes binge-like drinking in adolescent but not adult Wistar rats. Alcohol, 43, 305–314.

Maldonado, A. M., Finkbeiner, L. M., & Kirstein, C. L. (2008). Social interaction and partner familiarity differentially alter voluntary ethanol intake in adolescent male and female rats. Alcohol, 42, 641–648.

Molina, J. C., Chotro, G., & Spear, N. E. (1989). Early (preweanling) recognition of alcohol's orosensory cues resulting from acute ethanol intoxication. Behavioral and Neural Biology, 51, 307–325.

National Institute of Health. (1996). Institute of Laboratory Animal Resources, Commission on Life Sciences. National Research Council. Guide for the Care and Use of Laboratory Animals. Washington, DC: National Academy Press.

Ottoni, E. B. (2000). EthoLog 2.2: a tool for the transcription and timing of behavior observation sessions. Behavior Research Methods, Instruments, & Computers, 32, 446–449.

Pascual, M., Boix, J., Felipo, V., & Guerri, C. (2009). Repeated alcohol administration during adolescence causes changes in the mesolimbic dopaminergic and glutamatergic systems and promotes alcohol intake in the adult rat. Journal of Neurochemistry, 108, 920–931.

Pautassi, R. M., Myers, M., Spear, L. P., Molina, J. C., & Spear, N. E. (2008). Adolescent but not adult rats exhibit ethanol-mediated appetitive second-order conditioning. Alcoholism: Clinical and Experimental Research, 32, 2016–2027.

Ponce, L. F., Pautassi, R. M., Spear, N. E., & Molina, J. C. (2004). Nursing from an ethanol-intoxicated dam induces short- and long-term disruptions in motor performance and enhances later self-administration of the drug. Alcoholism: Clinical and Experimental Research, 28, 1039–1050.

Ponce, L. F., Pautassi, R. M., Spear, N. E., & Molina, J. C. (2011). Maternal care alterations induced by repeated ethanol leads to heightened consumption of the drug and

motor impairment during adolescence: a dose-response analysis. Physiology and Behavior, 103, 477–486.

Ristuccia, R. C., & Spear, L. P. (2004). Adolescent ethanol sensitivity: hypothermia and acute tolerance. Annals of the New York Academy of Sciences, 1021, 445–447.

Rodd-Henricks, Z. A., Bell, R. L., Kuc, K. A., Murphy, J. M., McBride, W. J., Lumeng, L., & Li, T.-K. (2002a). Effects of ethanol exposure on subsequent acquisition and extinction of ethanol self-administration and expression of alcohol-seeking behavior in adult alcohol-preferring (P) rats: I. Periadolescent exposure. Alcoholism: Clinical and Experimental Research, 26, 1632–1641.

Rodd-Henricks, Z. A., Bell, R. L., Kuc, K. A., Murphy, J. M., McBride, W. J., Lumeng, L., & Li, T.-K. (2002b). Effects of ethanol exposure on subsequent acquisition and extinction of ethanol self-administration and expression of alcohol-seeking behavior in adult alcohol-preferring (P) rats: II. Adult exposure. Alcoholism: Clinical and Experimental Research, 26, 1642–1652.

Schramm-Sapyta, N. L., Kingsley, M. A., Rezvani, A. H., Propst, K., Swartzwelder, H. S., & Kuhn, C. M. (2008). Early ethanol consumption predicts relapse-like behavior in adolescent male rats. Alcoholism: Clinical and Experimental Research, 32, 754–762.

Schramm-Sapyta, N. L., Walker, Q. D., Caster, J. M., Levin, E. D., & Kuhn, C. M. (2009). Are adolescents more vulnerable to drug addiction than adults? Evidence from animal models. Psychopharmacology, 206, 1–21.

Spanagel, R. (2000). Recent animal models of alcoholism. Alcohol Research & Health, 24, 124–131.

Siegmund, S., Vengeliene, V., Singer, M. V., & Spanagel, R. (2005). Influence of age at drinking onset on long-term ethanol self-administration with deprivation and stress phases. Alcoholism: Clinical and Experimental Research, 29, 1139–1145.

Silveri, M., & Spear, L. (2001). Acute, rapid, and chronic tolerance during ontogeny: Observations when equating ethanol perturbation across age. Alcoholism: Clinical and Experimental Research, 25, 1301–1308.

Silveri, M. M., & Spear, L. P. (1998). Decreased sensitivity to the hypnotic effects of ethanol early in ontogeny. Alcoholism: Clinical and Experimental Research, 22, 670–676.

Spear, L. P., & Varlinskaya, E. I. (2005). Adolescence. Alcohol sensitivity, tolerance, and intake. Recent Developments in Alcoholism, 17, 143–159.

Spear, L. P., & Varlinskaya, E. I. (2010). Sensitivity to ethanol and other hedonic stimuli in an animal model of adolescence: implications for prevention science? Developmental Psychobiology, 52, 236–243.

Tambour, S., Brown, L. L., & Crabbe, J. C. (2008). Gender and age at drinking onset affect voluntary alcohol consumption but neither the alcohol deprivation effect nor the response to stress in mice. Alcoholism: Clinical and Experimental Research, 32, 2100–2106.

Tarazi, F. I., & Baldessarini, R. J. (2000). Comparative postnatal development of dopamine D(1), D(2) and D(4) receptors in rat forebrain. International Journal of Developmental Neuroscience, 18, 29–37.

Van Skike, C. E., Novier, A., Diaz-Granados, J. L., & Matthews, D. V. (2012). The effect of chronic intermittent ethanol exposure on spatial memory in adolescent rats: The dissociation of metabolic and cognitive tolerance. Brain Research, 1453, 34–39.

Varlinskaya, E. I., & Spear, L. P. (2012). Increases in anxiety-like behavior induced by acute stress are reversed by ethanol in adolescent but not adult rats. Pharmacology, Biochemistry and Behavior, 100, 440–450.