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Modeling binge-like ethanol drinking by peri-adolescent and adult P rats

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ABSTRACT

Alcohol binge-drinking, especially among adolescents and young adults, is a serious public health concern. The present study examined ethanol binge-like drinking by peri-adolescent [postnatal days (PNDs 30–72)] and adult (PNDs 90–132) alcohol-preferring (P) rats with a drinking-in-the-dark-multiple-scheduled-access (DID-MSA) procedure used by our laboratory. Male and female P rats were provided concurrent access to 15% and 30% ethanol for three 1-h sessions across the dark cycle 5 days/week. For the 1st week, adolescent and adult female P rats consumed 3.4 and 1.6 g/kg of ethanol, respectively, during the 1st hour of access, whereas for male rats the values were 3.5 and 1.1 g/kg of ethanol, respectively. Adult intakes increased to ~2.0 g/kg/h and adolescent intakes decreased to ~2.5 g/kg/h across the 6 weeks of ethanol access. The daily ethanol intake of adult DID-MSA rats approximated or modestly exceeded that seen in continuous access (CA) rats or the selection criterion for P rats (≥5 g/kg/day). However, in general, the daily ethanol intake of DID-MSA peri-adolescent rats significantly exceeded that of their CA counterparts. BELs were assessed at 15-min intervals across the 3rd hour of access during the 4th week. Ethanol intake was 1.7 g/kg vs. 2.7 g/kg and BELs were 57 mg% vs. 100 mg% at 15- and 60-min, respectively. Intoxication induced by DID-MSA in female P rats was assessed during the 1st vs. 4th week of ethanol access. Level of impairment did not differ between the 2 weeks (106 vs. 97 s latency to fall, 120 s criterion) and was significant (vs. naïve controls) only during the 4th week. Overall, these findings support the use of the DID-MSA procedure in rats, and underscore the presence of age- and sex-dependent effects mediating ethanol binge-like drinking in P rats.

1. Introduction

Animal models have been successfully used to develop treatments for a number of medical and psychiatric disorders (e.g., Griffin, 2002; McKinney, 2001). An animal model has the advantage of allowing the experimenter to control characteristics of the animal's genetic background, environment and prior drug exposure. An animal model also permits the examination of neurobehavioral, neurochemical and neurophysiological correlates with the disorder being modeled, which, in turn, facilitates the development of pharmacological and/or behavioral treatments for this disorder. Despite reservations as to whether a valid animal model of alcoholism could be developed (Cicero, 1979), certain criteria for an animal model of alcoholism have been proposed (Cicero, 1979; Lester and Freed, 1973; McBride and Li, 1998). Briefly, these criteria are as follows: 1) the animal should orally self-administer ethanol; 2) the amount of ethanol consumed should result in pharmacologically relevant blood ethanol levels; 3) ethanol should be consumed for its post-ingestive pharmacological effects, and not strictly for its caloric value or taste; 4) ethanol should be positively reinforcing, in other words, the animals must be willing to work for ethanol; 5) chronic ethanol consumption should lead to the expression of metabolic and functional tolerance; 6) chronic consumption of ethanol leads to dependence, as indicated by withdrawal symptoms after access to ethanol is terminated; and 7) an animal model of alcoholism should also display characteristics associated with relapse.

A substantial literature (c.f., Bell et al., 2005, 2006b; McBride and Li, 1998; Murphy et al., 2002) indicates the P line of rat meets all of the existing adult criteria proposed for a valid animal model of alcoholism (Cicero, 1979; Lester and Freed, 1973; McBride and Li, 1998). Because ethanol binge-drinking is such a serious public health issue and is often directly associated with the development of alcohol abuse and dependence, its inclusion as a criterion for an animal model of alcoholism appears to be paramount. One model of binge-like drinking in rodents is the drinking-in-the-dark-multiple-scheduled-access (DID-MSA) procedure previously used in our laboratory (Bell et al., 2006a, 2006b, 2009; McBride et al., 2010). To the best of our knowledge, the only other rat (Sprague–Dawley) study using a DID procedure was recently published by Kosten (2011). Moreover, a systematic characterization of the DID-MSA model in rats has not been published thus far.

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Because the validity and utility of an animal model depend upon how well it mimics the human condition (c.f., Leeman et al., 2010), the present study evaluated whether binge-like ethanol drinking could be reliably demonstrated in adolescent and adult P rats of both sexes using the DID-MSA procedure. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) National Advisory Council (2004) has adopted the following definition of binge drinking: "A 'binge' is a pattern of drinking that brings blood alcohol concentration (BAC) to 80 mg% or above. For the typical adult (human), this pattern corresponds to consuming 5 or more drinks (male), or 4 or more drinks (female), in about 2 h." Therefore, proposed benchmark criteria for binge-like drinking in rodents are the presence of blood ethanol levels (BELs) greater than 80 mg% during, and following, a short (e.g., 1-h) ethanol access session and the induction of intoxication by this type of drinking (c.f., Crabbe et al., 2009; Rhodes et al., 2005). To date, no research has been published directly comparing the DID-MSA protocol of binge-like drinking in adult and adolescent P rats of both sexes. Nor has a comparison of daily ethanol intake levels seen in P rats, of both sexes, with DID-MSA vs. continuous access been made.

Adult female rodents generally consume more ethanol, in grams per kilogram of body weight, than their male counterparts (Adams, 1995; Juárez and De Tomasi, 1999; Lancaster and Spiegel, 1992; Li and Lumeng, 1984; Moore et al., 2010; Sherrill et al., 2011; Tambour et al., 2008; Truxell et al., 2007; Vetter-O'Hagen et al., 2009). This sex-specific effect, although modest, has also been found in peri-adolescent and post-weaning selectively bred rats (Bell et al., 2003, 2004; McKinzie et al., 1998a; 1998b). Thus, the present study examined the ethanol binge-like drinking behavior of both male and female P rats. Previous research indicates that concurrent access to multiple concentrations of ethanol increases ethanol intake, compared with access to a single ethanol concentration, in adult outbred rats (Holter et al., 1998; Wolffgramm and Heyne, 1995) as well as periadolescent (Bell et al., 2003, 2004) and adult (Rodd et al., 2009; Rodd-Henricks et al., 2001) selectively bred rats. Therefore, to maximize ethanol intake, the present studies used concurrent access to 15% and 30% ethanol.

Evidence indicates that adolescent rodents generally consume greater amounts of ethanol than their adult counterparts (Doremus et al., 2005; Moore et al., 2010; Spear, 2004b, 2007; Truxell et al., 2007; Vetter et al., 2007; Vetter-O'Hagen et al., 2009). Thus, the role of age (adolescence vs. adulthood) on ethanol binge-like drinking by P rats was also examined. Regarding age, the boundaries of the adolescent "window" of neurobehavioral development for rats may differ given the parameter(s) examined. Nevertheless, neurochemical and neurobehavioral differences between peri-adolescent and postweanling as well as adult rats support an adolescent developmental window of postnatal days (PNDs) 28 through 42 (Spear, 2000, 2007; Spear and Brake, 1983). However, Spear (2000, 2004a, 2007) has suggested that this conservative window (PNDs 28 through 42) could be extended to PND 60, when assessing the effects of pharmacological pretreatment during adolescence, on adult behaviors in male and female rats. This extended window permits the examination of the earliest adolescent changes in the female rat as well as the latest changes in the male rat. Therefore, different experiments within the present study assessed binge-like ethanol drinking in adolescence, by initiating ethanol access at PND 30, and adulthood, by initiating ethanol access at PND 90.

Given the existing literature and observations within our laboratory, we hypothesized that (a) the adolescent rats would consume more ethanol than their adult counterparts; (b) female rats would consume more ethanol than their male counterparts; (c) the intake of binge-like drinking animals would approximate that of the continuous access animals; (d) blood ethanol levels (BELs) achieved would reach and exceed 80 mg%; and (e) significant intoxication would be displayed during and after binge-like drinking.

2. Method

2.1. Animals

Subjects were adolescent or adult, ethanol-naïve, male and female selectively bred P rats (from the S66–S69 generations). The adolescent and adult rats were single-housed on a reverse 12 h/12 h dark-light cycle (light offset at 1000 h) on PND 28 and PND 88, respectively. Animals had ad libitum access to food and water. Animals used in these procedures were maintained in facilities fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Indiana University School of Medicine and are in accordance with the guidelines of the Institutional Animal Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

2.1.1. Experiment 1: drinking procedure

Male and female adolescent and adult P rats were given free-choice (water available ad libitum) access to ethanol (15% and 30% solutions available concurrently) access using a drinking-in-the-dark-multiple-scheduled-access (DID-MSA) or continuous access (24 h/day) procedure. The DID-MSA procedure involved three 1-h access sessions, across the dark cycle, with the first session initiated at lights out and each subsequent hour of access separated by 2 h of ethanol deprivation. Adolescent and adult rats experienced this DID-MSA procedure 5 days/week for 6 weeks beginning on PND 30 and PND 90, respectively. It is noteworthy that there appear to be species differences in the temporal methodology to maximize ethanol binge-like drinking in mice vs. rats. Early work on the DID procedure in mice indicated that maximal ethanol intake required initiating the procedure 2 to 4 h into the dark cycle (Rhodes et al., 2005), whereas, in P rats, maximal intake requires the first session to be initiated at the beginning of the dark cycle (Bell et al., 2006b).

2.2. Experiment 2: blood ethanol levels (BELs)

Peri-adolescent male P rats were used in this study. The drinking procedures were identical to those described in Experiment 1. BELs were determined at 15-min intervals across the 3rd hour of ethanol access on PND 45 or PND 47 (the 12th or 14th day of access). Separate squads of rats were used for each time-point for each PND.

2.2.1. Blood ethanol level (BEL) analyses

Previous studies with Wistar rats (Bell et al., 2000) as well as P and NP rats (Bell et al., 2001; Lumeng et al., 1982) indicate that tail blood sampling does not provide accurate measures of changing trunk BELs when assessed less than 90 min post-ethanol exposure. Therefore, trunk bloods were collected which required that 2 squads be run. All four time-points were sampled within each squad tested. Blood samples were collected in heparinized tubes and centrifuged in a Microfuge (Model B, Beckman: Palo Alto, CA) for 45 s. The supernatant fractions were used to determine BELs. BEL was measured using an Analox Analyser (model GL5: Analox Instruments USA, Lunenburg, MA), per the manufacturer's instructions.

2.3. Experiment 3a: intoxication in adult animals

A separate group of adult female P rats was used to assess for intoxication, using an oscillating bar apparatus (Bell et al., 2000, 2001; Lê and Israel, 1994), following one hour of DID-MSA ethanol access. Female rats were used due to the fact that this sex was used in our previous publications with the oscillating bar apparatus (Bell et al.,

2000, 2001). In addition, because male rats are significantly larger than female rats and they require lower oscillation rates which introduce excessive variability into the data (unpublished observations), only female rats were used in the present study. The DID-MSA procedure was the same as that used in Experiment 1, such that adult female P rats initiated ethanol (15% and 30% available concurrently) on PND 90 and had three 1-h access sessions, each separated by 2 h of ethanol deprivation, beginning at lights out 5 days/week. A similarly-treated control group remained ethanol-naïve. Intoxication was assessed at two time-points. The first time-point followed the 1st hour of access on the Monday of the 1st week. The second time-point was, in the same animals, after the 1st hour of access on Wednesday of the same week. A second group of adult female P rats experienced the same protocol as the first group except that intoxication was evaluated on Monday and Wednesday of the 4th week of binge-like ethanol access. Note that an examination of the DID-MSA results indicated that adult P rats increased their ethanol intake per session across weeks. Therefore, separate groups of rats were used to reduce possible carry-over effects. Trunk blood was collected along with associated ethanol intake and BELs were evaluated, immediately following the intoxication test. Thus, trunk blood was harvested around 10 min after ethanol access was terminated.

2.4. Experiment 3b: intoxication in peri-adolescent animals

A follow-up examination of intoxication in peri-adolescent female P rats was conducted. The procedures were the same as those described for the adult female P rats in Experiment 3a, except only one test was conducted which took place 30-min into the 3rd hour of ethanol access on Wednesday of the 4th week. Peri-adolescent rodents display less ethanol-induced motor impairment relative to their adult counterparts (Linsenbardt et al., 2009; Ramirez and Spear, 2010; White et al., 2002), although this appears to be genetically line/strain dependent (Hefner and Holmes, 2007; Linsenbardt et al., 2009). Therefore, the test of motor impairment in peri-adolescent P rats was conducted after 30-min into the 3rd hour of ethanol access to capitalize on expected higher BELs. Trunk blood was collected and associated ethanol intake recorded for each animal and BELs were evaluated immediately following the intoxication test.

2.4.1. Intoxication test apparatus

A custom-built oscillating bar apparatus was used to assess for intoxication. The oscillating bar test apparatus has been described in detail elsewhere (Lê and Israel, 1994). Briefly, an oscillating rectangular wooden bar (3.8-cm wide, 1.9-cm thick and 89 cm long) was connected to a variable speed electric motor via a swing arm which allowed the bar to move in a 120° arc. A shock grid (0.5 mA scrambled shock) was located 42 cm below the oscillating bar to facilitate learning. The bar and grid were housed inside a Plexiglas enclosure.

2.4.2. Test procedures

Adult (Experiment 3a) or adolescent (Experiment 3b) female P rats were trained for 5 trials/day during the weekend before the 1st (Experiment 3a) or 4th (Experiment 3a and 3b) week of ethanol access. Adolescent rats were tested during later peri-adolescence, and not during early adolescence, because previous work in our laboratory has indicated that naïve and ethanol-treated juvenile and young adolescent rats will not stay on the oscillating bar despite the use of a shock grid to discourage jumping from the bar (unpublished observations). The criterion for staying on the oscillating bar was 120 s. On Saturday, the rats were given 5 trials to reach the 120 s criterion with the oscillation rate set at 20 osc./min. On Sunday, the oscillation rate was increased to 30 osc./min. Again, two tests were given in Experiment 3a, with the first test following the 1st hour of ethanol access on Monday, and the second test following the 1st hour of ethanol access on Wednesday of the same week. The subjects underwent two trials on each test day, with a rest period of approximately 30-s between each trial, to determine the average (of the two trials) latency to fall. For these test days, the oscillation rate was set at 40 osc./min.

2.4.3. Statistical analyses: drinking studies

Due to bodyweight differences, ethanol fluid intake was converted to g of ethanol/kg bodyweight/h (or day) of ethanol access. In addition, the ethanol intake for each hour was averaged across each 5-day block (i.e., Monday through Friday of each week). An omnibus $2\times2\times6\times3$ (sex by age by block by hour) mixed ANOVA was conducted on the ethanol intake data, such that sex and age were the between-subject factors and block and hour were the within-subject factors. A secondary analysis examined whether total ethanol intake per day by the binge-like drinking rats matched that of the rats given continuous access to ethanol. An omnibus $2\times2\times2\times6$ [ethanol condition (DID-MSA vs. Continuous) by sex by age by block] mixed ANOVA was conducted for this analysis, with ethanol condition, sex and age being the between-subject factors and block (average daily intake for each 5 day period) being the within-subject factor.

2.4.4. Statistical analyses: BEL study

The ethanol intake and associated BEL data were analyzed using regression analyses to determine the magnitude of the relationship between ethanol intake, at each 15-min time-point, and BELs achieved for the respective time-point.

2.4.5. Intoxication studies

For the intoxication data, the dependent variable was latency to fall (s), averaged across the 2 trials on each test day. This latency to fall data was subjected to a $2\times2\times2$ [ethanol condition (DID-MSA vs. naïve) by test week (1st vs. 4th) by test day (Monday vs. Wednesday)] mixed ANOVA with ethanol condition and test week serving as the between-subject factors and test day serving as the within-subject factor. Trunk blood, with ethanol intakes for the respective animals, was collected wherever possible and tests for differences in BELs and/or ethanol intake across test weeks, in Experiment 3a, were conducted.

Significant interactions or main effects were followed by appropriate simple effect or contrast analyses. Alpha was set at $p<0.05$ for all analyses, except where indicated (i.e., $p<0.025$ for some a priori multiple comparison analyses).

3. Results

3.1. Ethanol drinking behavior

The omnibus $2\times2\times6\times3$ (sex by age by block by hour) mixed ANOVA revealed the 4-way interaction [$F(10,430)=2.524$, $p=0.006$] was significant, with significant 3-way interactions for sex by age by block [$F(5,215)=11.375$, $p<0.001$] and sex by age by hour [$F(2,86)=6.067$, $p=0.003$], and significant main effects for sex [$F(1,43)=51.738$, $p<0.001$], age [$F(1,43)=344.571$, $p<0.001$] and block [$F(5,215)=9.067$, $p<0.001$].

The follow-up omnibus $2\times2\times2\times6$ [ethanol condition (DID-MSA vs. Continuous) by sex by age by block] mixed ANOVA of total daily ethanol intake, averaged across 5-day blocks, by DID-MSA versus continuous access rats revealed that ethanol access condition interacted with most of the factors (statistics in Table 1). In general, adolescent P rats consumed more ethanol than their adult counterparts and female P rats consumed more ethanol than their male counterparts (Fig. 1). Decomposing the interactions ($p's\leq0.025$) was conducted by examining each age separately. In general, whereas binge-like drinking adolescent P rats consumed significantly more ethanol than their continuous access counterparts, continuous-drinking adult P rats consumed significantly more ethanol than their binge-like drinking counterparts (Fig. 1, upper and lower panels, respectively).

Table 1
Significant effects from the 2×2×2×6 [Ethanol Condition (Binge vs. Continuous) by Sex (Male vs. Female) by Age (Peri-adolescent vs. Adult) by Block] mixed ANOVA conducted on the total ethanol consumed (g/kg) each day, averaged across 5-day blocks.

Factors	df's	F-value	p-value
Condition by block by sex by age	(5,425)	3.684	0.003
Condition by block by sex	(5,425)	4.374	0.001
Condition by block by age	(5,425)	8.853	<0.001
Condition by block	(5,425)	2.242	0.049
Condition by sex by age	(1,85)	4.078	0.047
Condition by age	(1,85)	77.004	<0.001
Block by sex by age	(5,425)	6.106	<0.001
Block by sex	(5,425)	21.224	<0.001
Block by age	(5,425)	83.428	<0.001
Sex by age	(1,85)	11.154	0.001
Block	(5,425)	7.529	<0.001
Sex	(1,85)	36.176	<0.001
Age	(1,85)	19.099	<0.001

3.2. Blood ethanol levels (BELs)

Because trunk blood was used, separate groups of adolescent male P rats were used to assess BELs and associated ethanol intakes attained during the 3rd hour of binge-like drinking on either PND 45 or PND 47. The regression analyses revealed a significant (p's<0.025) positive relationship between ethanol intake and BEL achieved at each of the 15-min time-points, see Table 2 for statistical results and values.

3.2.1. Level of intoxication in adult rats

The 2×2×2 [ethanol condition (DID-MSA vs. naïve) by test week by test day] mixed ANOVA on latency to fall revealed no effect of test day (interaction or main effect), so the Monday and Wednesday values were averaged and submitted to a 2×2 (ethanol condition by test week) ANOVA. The ANOVA revealed a significant main effect for ethanol condition [$F(1,58)=13.528$, $p=0.001$] but the test week effects were not significant ($p>0.28$). With alpha set at 0.025, the a priori multiple comparison Fisher's LSD t-tests (2-tailed) revealed that there was a significant effect of ethanol condition during the 4th [$t(42)=4.013$, $p<0.001$] but not the 1st ($p>0.065$) week of binge-like ethanol access (Fig. 2, upper left). Evaluation of ethanol intake during the 1st hour of DID-MSA access prior to the intoxication test and BELs present immediately following the intoxication test revealed significantly greater ethanol intake [$F(1,33)=4.716$, $p=0.037$] and BELs achieved [$F(1,33)=6.758$, $p=0.014$] during the 4th week of DID-

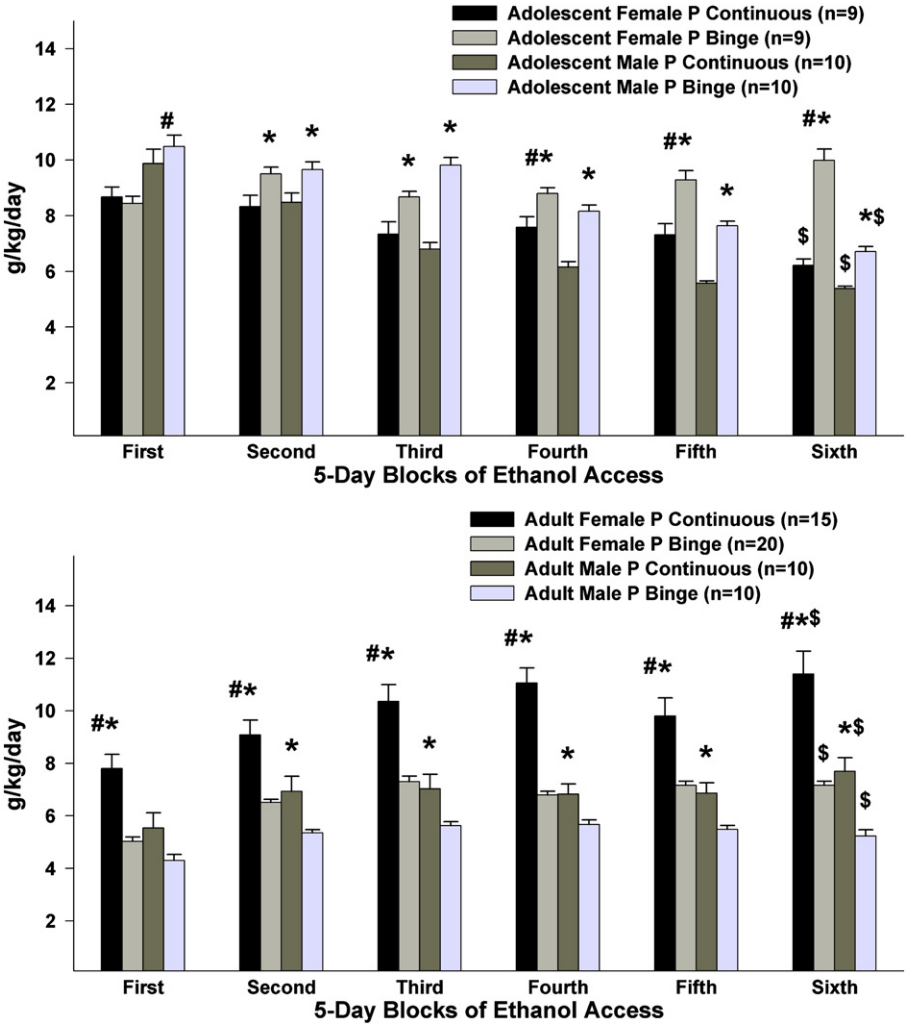

Fig. 1. Daily ethanol intake (mean ± SEM), averaged across 5-day blocks, by peri-adolescent female and male (top panel) and adult female and male (bottom panel) alcohol-preferring P rats given either continuous or binge-like (DID-MSA) concurrent access to 15% and 30% ethanol for 6 weeks. In general, peri-adolescent rats drank more ethanol than adult rats, female rats drank more ethanol than male rats, and adult female and male P rats increased, whereas peri-adolescent male P rats decreased, their ethanol intake across weeks. In addition, whereas peri-adolescent P rats given binge-like access generally consumed more ethanol than their continuous access counterparts, the pattern displayed by adult P rats was reversed with continuous access animals generally consuming more ethanol than their binge-like access counterparts. #, indicates a significant simple main effect of sex-of-animal for the respective 5-day block. *, indicates a significant simple main effect of ethanol condition for the respective sex-of-animal and 5-day block. $, indicates a significant change in ethanol intake between the 1st and 6th five-day blocks.

Table 2
Significant results from the regression analyses of ethanol intake (mean ± SEM) and BELs (mean ± SEM) achieved by peri-adolescent male P rats (n = 10–12/time-point) across the 3rd hour of access on post-natal day (PND) 45 or PND 47.

Time	Intake (g/kg)	BEL (mg%)	F-statistic	p-value	r-value	R-squared
15 min	1.7 ± 0.2	57 ± 9.7	F(1,9) = 18.11	0.0028	0.83	0.69
30 min	2.2 ± 0.2	99 ± 10.9	F(1,10) = 7.73	0.0214	0.68	0.46
45 min	2.3 ± 0.2	76 ± 10.3	F(1,11) = 29.27	0.0003	0.86	0.75
60 min	2.7 ± 0.3	100 ± 13.9	F(1,11) = 7.53	0.0207	0.66	0.43

MSA drinking relative to the 1st week of DID-MSA drinking (Fig. 2, upper right and lower left panels, respectively).

3.2.2. Level of intoxication in peri-adolescent rats

The one-way ANOVA for the latency to fall data, from peri-adolescent female P rats tested 30-min into the 3rd bout of binge-like ethanol access on Wednesday of the 4th week revealed a significant main effect of ethanol condition [$F(1,15) = 52.577$, $p < 0.001$]. As seen in Fig. 2 (lower right panel), ethanol-consuming peri-adolescent female P rats had much lower latency to fall scores compared with their naïve counterparts. Average ethanol intake for the 30-min ethanol access period was 2.9 ± 0.3 g/kg (mean ± SEM) and average BELs were 88 ± 14 mg% (mean ± SEM). Note that these values are similar to ethanol intake levels and BELs achieved at 60-min by peri-adolescent male P rats (Table 2).

4. Discussion

Findings from the present study supported most of our hypotheses, such that (a) peri-adolescent P rats consumed more ethanol than their adult counterparts; (b) female P rats consumed more ethanol than their male counterparts; (c) the ethanol intake of adult binge-like (i.e., DID-MSA) drinking P rats approximated that of their continuous access counterparts, however, unexpectedly, DID-MSA ethanol intake of peri-adolescent P rats generally exceeded that of their continuous access counterparts; (d) BELs achieved during and after DID-MSA ethanol access approximated 100 mg%; and (e) significant intoxication was displayed by both chronic binge-like drinking peri-adolescent and adult female P rats.

The finding that peri-adolescent P rats consumed more ethanol than their adult counterparts (Fig. 1) matches the growing literature indicating this effect in rodents (Doremus et al., 2005; Maldonado et al., 2008; Moore et al., 2010; Spear, 2004b, 2007; Truxell et al., 2007; Vetter et al., 2007; Vetter-O'Hagen et al., 2009). Sex differences in ethanol intake, such that female P rats consumed more than male P rats, were robust only during post-adolescence, such that peri-adolescent female P rats (Fig. 1, upper panel), after the 3rd week of access, and adult female P rats (Fig. 1, lower panel) displayed greater ethanol intake than their male counterparts. Interestingly, Vetter-O'Hagen et al. (2009) reported a similar finding. These authors reported that whereas adolescent male Sprague–Dawley rats consumed more ethanol than their female counterparts, the reverse was true in adulthood. Moreover, the sex differences decreased across adolescence, although increasing the ethanol concentration from 6%

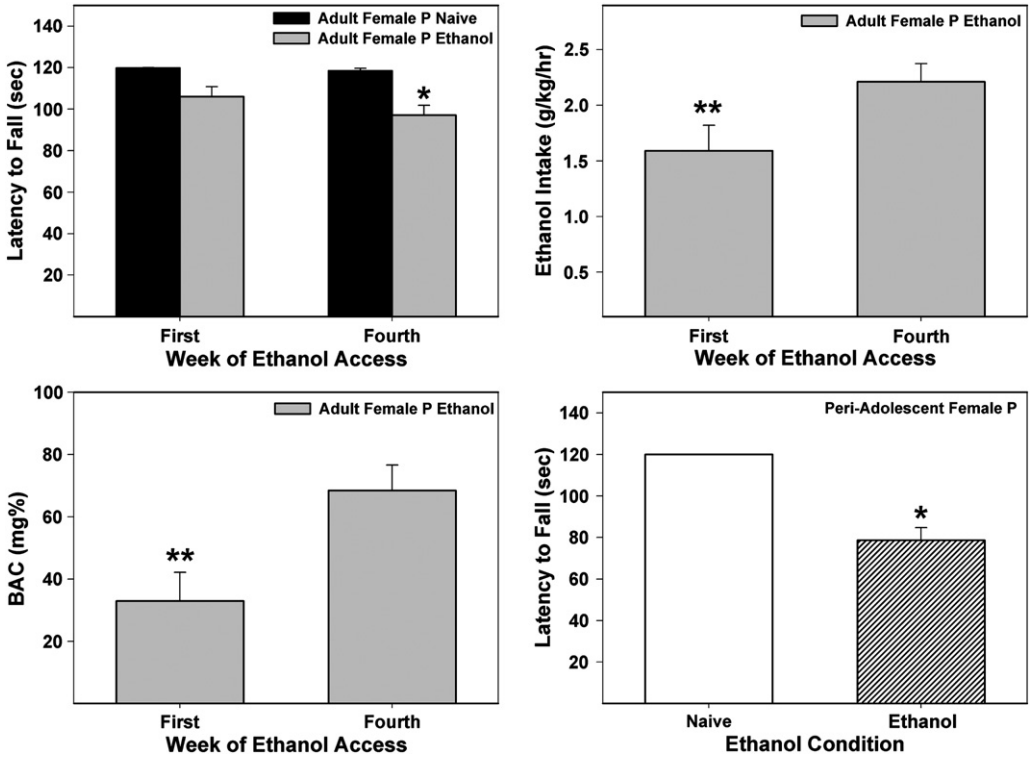

Fig. 2. Results from the studies examining intoxication in adult (Experiment 3a, upper left and right panels as well as the lower left panel) or peri-adolescent (Experiment 3b, lower right panel) female alcohol-preferring P rats experiencing binge-like ethanol access (DID-MSA, with 15% and 30% available concurrently). Intoxication was assessed using an oscillating bar task, with latency to fall (sec) as the dependent variable (120 sec criterion). Experiment 3a assessed latency to fall (upper left panel), ethanol intake (upper right panel) and BELs achieved (lower left panel) during the 1st (n = 11) vs. 4th (n = 24) weeks of binge-like ethanol access. In general, significant intoxication was present only during the 4th week of ethanol access, although the latency to fall did not differ between the rats tested during the 1st week and the rats tested during the 4th week of ethanol access. *, indicates a significantly lower latency (mean ± SEM) to fall by the ethanol-drinking compared with the ethanol-naïve rats, during the 4th week of ethanol access. **, indicates a significance difference between the values for ethanol intake for the hr before the test for intoxication (upper right panel) and BELs (mean ± SEM) measured immediately after the test for intoxication (lower left panel) between that seen during the 1st week of ethanol access and that seen during the 4th week of ethanol access. Note that, for the peri-adolescent female P rats tested during the 4th week of ethanol access (ethanol-drinking: n = 8, ethanol-naïve: n = 9), average ethanol intake prior to the test for intoxication was 2.9 ± 0.3 g/kg and average BELs achieved were 88 ± 14 mg% (mean ± SEM).

to 10% across days may have influenced these results (Vetter-O'Hagen et al., 2009). This effect of adolescent male Sprague–Dawley rats consuming more than their female counterparts has also been reported for both a plain or sweetened 30% ethanol solution licked off of the test cage floor, although this methodology suggests novelty may have influenced the results (Truxell et al., 2007). However, these authors reported very limited, if any, sex differences in ethanol intake during early adulthood (PND 60 to 64). It should be noted that a key methodological factor, in the present study, that could have influenced the delay in the expression of sex-differences and the stabilization of intake levels is the fact that the animals, at both ages, were transferred to a reverse dark–light cycle room two days before initiation of the DID-MSA procedures. Thus, adaptation to the changed circadian rhythm may have interfered with normal adolescent patterns of ethanol intake. Further research will be needed to assess this hypothesis.

Contrary to these and the present findings, a study examining sex and age differences in ethanol intake by Wistar rats on a limited access schedule found that female adolescent and adult Wistar rats consistently drank more ethanol than their male counterparts (Walker et al., 2008). Two caveats need to be stated regarding this Wistar study. First, the ethanol solution was sweetened, which may account for some of the differences in findings. Second, Walker et al. (2008) reported that the adolescent Wistar rats consumed significantly more sweetened ethanol during the light cycle vs. the dark cycle, whereas their adult counterparts displayed slightly more sweetened ethanol intake during the dark cycle. Thus, these and other methodological differences may account for the disparate findings. In a study examining drinking-in-the-dark (DID) ethanol intake of adolescent or adult C57BL/6J and DBA/2J mice, Moore et al. (2010) reported that, in general, female mice during both stages of development consumed more ethanol than their male counterparts. This suggests that these two inbred mouse strains do not display the sex by age interaction regarding ethanol intake reported in the present study and previously in Sprague–Dawley rats (Truxell et al., 2007; Vetter-O'Hagen et al., 2009). Also, despite major methodological differences, the Moore et al. (2010) study parallels findings reported by Walker et al. (2008).

We had not hypothesized that the daily DID-MSA ethanol intake of peri-adolescent P rats would exceed, sometimes substantially, that of their continuous access counterparts (Fig. 1, upper panel). To the best of our knowledge, this is the first study demonstrating this effect in animals, and there appear to be very few clinical studies published that address this question directly. Regarding this, a substantial amount of research has examined the consequences of, and possible interventions for, binge drinking by children, adolescents and young adults (e.g., Adams and Effertz, 2010; Chamberlain and Solomon, 2008; Chersich and Rees, 2010; Dooldeniya et al., 2007; Henderson et al., 2007; Kelly-Weeder, 2008; Parker et al., 2009; Stolle et al., 2009; Wachtel and Staniford, 2009). However, very little research has sought to compare the consequences of binge drinking versus non-binge drinking in humans with notable exceptions from the laboratories of Stephens and Duka (2008), see also (Courtney and Polich, 2009). These authors noted profound differences in the effects of binge-like versus continuous ethanol consumption, which have been noted in adult rodent binge-like models as well (Bell et al., 2006a; see also Ward et al., 2009). Therefore, serious consideration should be given to using naïve as well as continuous access controls when evaluating rodent, and for that matter human, binge-like ethanol drinking and its consequences, especially as it pertains to ethanol-drinking during adolescence.

Our laboratory previously reported that the daily DID-MSA ethanol intake of adult P rats, using the same or similar procedures as those used in the present study, either approximated that of their continuous access counterparts or approximated the selection criteria (i.e., $\geq$5 g/kg/day) for daily ethanol intake by adult P rats (Bell et al., 2006a, 2006b, 2009; McBride et al., 2010). Therefore, the present (Fig. 1, lower panel) and previous findings indicate that adult P rats given DID-MSA ethanol access will consume between 1.5 and 2.5 g/kg per hour of access resulting in total daily intakes of approximately 5 or more g/kg/day. These levels of intake result in BELs approximating or exceeding 80 mg%. It is noteworthy that the animals in the present and our laboratory's previous studies (Bell et al., 2006a, 2009; McBride et al., 2010) had ad lib access to food and water. Regarding this, previous work from our laboratory indicates that food restriction limited to as little as an hour during the first hour of DID-MSA ethanol access results in BELs greater than 120 mg% (Bell et al., 2006b).

The present results that the DID-MSA protocol results in BELs approximating 100 mg% extends our similar findings in adult P rats (Bell et al., 2006b) to peri-adolescent P rats of both sexes (Table 2, and results section). The fact that BELs of the peri-adolescent male P rats peaked at 100 mg% at the 30- and 60-min time-points suggests that ethanol intake may have slowed from the 30-min to 45-min time-points followed by what appears to be a resumption of ethanol intake towards the end of the 1-h session. Changes in ethanol intake levels between each time-point provide some support for this hypothesis (Table 2), although separate squads of male rats were used for each time-point. Nevertheless, continued research examining BELs across 1-h access sessions will be needed to evaluate this hypothesis. In addition, the BELs [88 $\pm$ 14 mg% (mean $\pm$ SEM)] observed at 30-min in peri-adolescent female P rats, from the intoxication studies, are not significantly different (i.e., the SEM's intersect) than that observed in the male peri-adolescent P rats (Table 2).

Results from the present study indicate that not only are binge-associated BELs achieved by P rats experiencing a DID-MSA procedure, this procedure also results in significant intoxication. Intoxication appeared to be greater in the peri-adolescent, compared with adult, female P rats (Fig. 2, lower right and upper left panel, respectively); although, separate groups of animals, with associated differences in ages, were used in the two studies. The level of intoxication displayed by the peri-adolescent rats was unexpected given the fact that, in general, adolescent rats are less affected by the ataxic effects of ethanol than adult rats (Linsenbardt et al., 2009; Ramirez and Spear, 2010; White et al., 2002). Future research will be required to elucidate factors mediating this effect.

Overall, these findings indicate that the DID-MSA procedure induces binge-like drinking, to intoxication and BELs>80 mg%, in P rats paralleling results, using a DID procedure, found in mice (Boehm et al., 2008; Crabbe et al., 2009; Lyons et al., 2008; Moore and Boehm, 2009; Navarro et al., 2009; Rhodes et al., 2005). The present results also extend previous work indicating adolescent rodents consume more ethanol than their adult counterparts (Doremus et al., 2005; Moore et al., 2010; Spear, 2004b, 2007; Strong et al., 2010; Tambour et al., 2008; Truxell et al., 2007; Vetter et al., 2007; Vetter-O'Hagen et al., 2009) to include binge-like drinking by male and female P rats. Finally, and most importantly, the novel finding that binge-like drinking peri-adolescent P rats display greater daily ethanol intake than their continuous access counterparts is reported here for the first time. Therefore, the current findings have important implications for future decisions regarding the public health and clinical risks associated with binge alcohol-drinking, especially among youth and women. In addition, these findings support further investigations into sex- and age-specific differences in the neurobiology mediating ethanol binge-like drinking.

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