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Similar withdrawal severity in adolescents and adults in a rat model of alcohol dependence

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Abstract

Alcohol use during adolescence leads to increased risk of developing an alcohol use disorder (AUD) during adulthood. Converging evidence suggests that this period of enhanced vulnerability for developing an AUD may be due to the adolescent's unique sensitivity and response to alcohol. Adolescent rats have been shown to be less sensitive to alcohol intoxication and withdrawal susceptibility; however age differences in ethanol pharmacokinetics may underlie these effects. Therefore, this study investigated alcohol intoxication behavior and withdrawal severity using a modified Majchrowicz model of alcohol dependence that has been shown to result in similar blood ethanol concentrations (BECs) despite age differences. Adolescent (postnatal day, PND, 35) and adult rats (PND 70+) received ethanol according to this four-day binge paradigm and were observed for withdrawal behavior for 17h. As expected, adolescents showed decreased sensitivity to alcohol-induced CNS depression as evidenced by significantly lower intoxication scores. Thus, adolescents received significantly more ethanol each day (12.3 ± 0.1 g/kg/day) relative to adults (9.2 ± 0.2 g/kg/day). Despite greater ethanol dosing in adolescent rats, both adolescent and adult groups had comparable peak BECs (344.5 ± 10.15 mg/dl and 338.5 ± 7.8 mg/dl respectively). Strikingly, withdrawal severity was similar quantitatively and qualitatively between adolescent and adult rats. Further, this is the first time that withdrawal behavior has been reported for adolescent rats using this model of alcohol dependence. A second experiment confirmed the similarity in BECs at various time points across the binge. These results demonstrate that after consideration of ethanol pharmacokinetics between adults and adolescents by using a model that produces similar BECs, withdrawal severity is nearly identical. This study, in combination with previous reports on ethanol withdrawal in adolescents and adults, suggests only a BEC-dependent effect of ethanol on withdrawal severity regardless of age.

Keywords

ethanol; alcoholism; alcohol use disorder; pharmacokinetics; binge drinking; seizure

The consequences of excessive alcohol intake in the alcohol use disorders (AUDs) cost society hundreds of billions of dollars each year, such that understanding their development is a critical public health concern. Initiation of alcohol consumption commonly begins in adolescence.

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Although adolescents tend to drink less frequently than adults, they drink quantities of alcohol similar to adults (Deas et al., 2000; White et al., 2006). By the 12th grade, most adolescents (70–80%) have tried alcohol and 25–30% reported “heavy drinking” or “having been drunk” in the last month (Johnston et al., 2007). Of those that reported using alcohol, 60–70% drink in a binge pattern, defined as five or more drinks per occasion (Miller et al., 2007; SAMHSA, 2008; Zeigler et al., 2005). Unfortunately, binge drinking is hypothesized to be a key link in the downward spiral from alcohol experimentation to addiction (Crews, 1999; Hunt, 1993; Koob and Le Moal, 1997). Given the high rates of binge drinking in adolescents, perhaps it is not surprising that nearly six percent of 12–17 year olds meet diagnostic criteria for an AUD (Harford et al., 2005), a value which then approaches adult rates in older adolescents (Martin and Winters, 1998).

Converging epidemiological and neurobiological evidence suggests that this unique period in ontogeny is also a period of enhanced vulnerability for developing an AUD (Spear, 2004). Adolescents are approximately four times more likely to develop an AUD if their first drink was taken before 15 years of age compared to those who wait until 21 (Grant and Dawson, 1997). This evidence suggests that alcohol has unique effects on brain physiology and function during this developmental transition. Several investigators hypothesize that adolescents may be predisposed to excessive alcohol intake, characteristic of binge drinking, due to a unique response or sensitivity to alcohol effects (Miller and Spear, 2006; Spear, 2002). Although the adolescent response to the effects of alcohol can be greater for some aspects such as memory impairment (Markwiese et al., 1998), neurotoxicity (Crews et al., 2000; 2006), and social facilitation (Varlinskaya and Spear, 2002), adolescent rats are less sensitive to the hypothermic, sedative, and motor-impairing effects of alcohol (Little et al., 1996; White et al., 2002; for review see Doremus et al., 2003; Spear and Varlinskaya, 2005). The latter two, reduced sensitivity to the sedative and motor-impairing effects of alcohol, are thought to promote excessive drinking by serving as cues to regulate consumption (Miller and Spear, 2006; Spear, 2002; Spear and Varlinskaya, 2005). Accordingly, in humans, a decreased response to an alcohol challenge as an adolescent predicts future alcohol problems (Schuckit, 1994).

The adolescent predisposition to binge drink may also be due to a relative insensitivity to the negative consequences of excessive intake. Negative consequences include factors related to alcohol withdrawal such as anxiety, reduced social interaction, tremor, and seizure susceptibility. Although alcohol withdrawal symptoms are thought to correlate with severity of alcohol dependence and high levels of intake, by adult-based definitions, adolescents tend to experience withdrawal phenomena less often than in adults (Clark et al., 2002). Animal models also support a reduced sensitivity: adolescent rats show fewer signs of withdrawal across several spectrums including anxiety, seizure susceptibility, and overall withdrawal severity (Acheson et al., 1999; Chung et al., 2008; Doremus et al., 2003; Varlinskaya and Spear, 2004). However, a confounding factor in many of these studies is ethanol pharmacokinetic differences between adolescent and adult rodents. Thus, the lower blood alcohol levels reported in adolescent models may explain the reduced seizure susceptibility and severity (Clark et al., 2002). Interestingly, in the few studies where blood ethanol concentrations have been matched between adults and adolescents, typically through the use of vapor inhalation systems, few withdrawal behavior differences have been observed between age groups (e.g. Slawecki et al., 2006).

In summary, it is not clear whether adolescents are more or less susceptible to alcohol withdrawal phenomenon. Therefore, we sought to compare intoxication and withdrawal behavior in adolescent versus adult rats using a model of binge-like intoxication, the Majchrowicz model (1975). Though developed in adult rats, this model manipulates the CNS depression-blood ethanol concentration relationship to maintain sedation characteristic of high blood ethanol concentrations (BECs) in binge drinking alcoholics. Notably, this model has

been shown to result in similar blood ethanol concentrations between adolescent and adult rats (Crews et al., 2000).

Methods

Experiment 1

Subjects—Adolescent (n=58) or adult (n=71) male Sprague-Dawley rats (Charles River Laboratories; Portage, Michigan; Raleigh, NC) were subjected to a four day binge paradigm modified from Majchrowicz (1975) as reported previously (Morris et al., 2009; Nixon and Crews, 2004). Experimental procedures were performed with corresponding controls across several studies spanning from 2005 to 2009 and used in past and future neuroanatomical reports (e.g. Morris et al., 2009). All control rats behaved normally and did not demonstrate sedative or motor impairments and therefore are not included in any analyses. Adolescent rats were approximately postnatal day (PND) 28 upon arrival and acclimated to the vivarium for 5–7 days before experimentation began on PND 35 (average weight 116.3 ± 1.8 g). Adolescent rats received binge-like treatment from PND 35–39, which corresponds to mid adolescence (Spear and Brake, 1983). Adult animals were similarly allowed to acclimate to the vivarium for 5–7 days and were approximately PND 70–72 (average weight 325.2 ± 2.3 g) at the start of treatment. All rats were individually housed on a 12h light/dark cycle with ad libitum access to food and water during the acclimation period. All procedures performed were approved prior to the start of experimentation by the University of Kentucky Institutional Animal Care and Use Committee and strictly adhered to the Guidelines for the Care and Use of Laboratory Animals (NRC, 1996).

Binge-like Ethanol Treatment—Food was removed at the start of the binge, though water remained available ad libitum throughout the experiment. Rats were weighed daily and administered a nutritionally complete ethanol diet (25% w/v in Vanilla Ensure Plus®) every 8 hours for 4 days based on a behavioral intoxication score. Each rat received an initial, priming dose of 5 g/kg of ethanol via intragastric intubation (16-G needle for Adults; 18-G needle for Adolescents; Fisher Scientific, Waltham, MA) with subsequent doses based on the animal's intoxication behavior as scored from a scale modified from Majchrowicz (1975; Knapp and Crews, 1999; see also Figure 1). In brief, greater observed CNS depression (intoxication) resulted in the administration of a lower ethanol dose (e.g. a normally behaving rat receives 5 g/kg whereas a rat with no righting reflex receives only 1 g/kg). This model has been shown to produce both tolerance and dependence in both adolescent and adult rats and is a well-accepted model of alcoholic binge drinking (Crews et al., 2000; Morris et al., 2009).

On the third binge day, blood was extracted from the tail at 90 minutes after the 3:00 PM dose to assess peak BECs. This time point was chosen based on previous work that has shown that peak BEC is achieved 90 minutes following intragastric gavage in both adult and adolescent rats (Kelly et al., 1987; Nixon and Crews, 2002; Crews et al., 2006). Blood was centrifuged for 5 minutes at 1800 x g to separate serum and stored at -20°C until analysis. BEC was determined using a GM7 Alcohol Analyser (Analox, London, UK), which measures the rate of oxygen consumption in the conversion of ethanol to acetaldehyde based on an external standard (300 mg/dL). Serum was run in triplicate, averaged and presented in mg/dL.

Behavioral Observations of Withdrawal—Eight hours after the last dose of ethanol, food was replaced. Spontaneous withdrawal behavior was observed in 109 rats beginning ten hours after the final dose through T26 (26 hours after the last dose). This range corresponds to peak withdrawal activity as reported previously (Faingold, 2008; Majchrowicz, 1975; Penland et al., 2001). Rats were observed in their home cages and scored for spontaneous withdrawal behaviors in 30min intervals of every hour. Red headlamps were worn during the dark cycle

so as not to disturb circadian rhythms. Behaviors were scored based on the withdrawal scale of Penland et al. (2001) as modified from Majchrowicz (1975; see Figure 3 for detail). Each behavior observed was noted, then the corresponding ordinal scale scores assigned such that both a mean withdrawal severity score and peak withdrawal severity score could be determined. Mean withdrawal refers to the average of the highest score observed and recorded for each hour over the 17h period. A mean withdrawal score may include zeros for hours when rat's behavior appeared similar to normal rats. The peak withdrawal score, however, refers to the highest level of withdrawal symptoms observed during the 17h period.

Experiment 2

Subjects—Both adolescent (alcohol: n=8, control: n=3) and adult (alcohol: n=8, control: n=3) male Sprague-Dawley rats (Charles River Laboratories) were subjected to a four day binge paradigm as previously described in experiment 1. Adolescent rats were approximately postnatal day 30 upon arrival and acclimated to the vivarium for 5 days before experimentation began on postnatal day (PND) 35 (average weight 133.3 ± 3.2). Adult animals arrived at the same time as the adolescent rats and were allowed to acclimate to the vivarium for 5 days. Adult rats were approximately PND 70–72 (average weight 314.5 ± 2.2 g) at the start of treatment. As in experiment 1, all rats were individually housed on a 12h light/dark cycle with ad libitum access to food and water during the acclimation period.

Binge-like Ethanol Treatment—All experimental binge ethanol treatments are identical to that described previously in experiment 1. However, with the exception of the first day, tail blood was extracted twice daily to assess BEC prior to dosing and peak BEC following ethanol administration. The purpose of this experiment was to elucidate any possible differences in pharmacokinetics between the adolescent and adult rat groups that may affect BEC or intoxication behavior. A pre-dosing tail blood sample was collected one hour prior to the 3 p.m. ethanol administration. At this time, intoxication behavior was also assessed, as mentioned in the previous experiment. A post-dosing tail blood sample was collected 90 minutes after the 3 p.m. ethanol administration time point for the purpose of assessing peak BECs. Ethanol administration, blood collection methods, and BEC analyses were conducted exactly as described in experiment 1.

Statistical Analyses

Analyses were conducted in Graphpad Prism (Graphpad Software; La Jolla, CA) or SAS 9.2 (SAS, Cary, NC). All data are reported as mean $\pm$ standard error of the mean (SEM). All two-group comparisons, e.g. BECs (mg/dl), ethanol dose (g/kg), peak intoxication and peak withdrawal behaviors were analyzed by t-test. Behavioral scores across time, i.e. intoxication or withdrawal, were analyzed by repeated measures ANOVA with Bonferroni posthoc tests. The repeated measures ANOVA was chosen over the nonparametric Friedman's test because of the large, but unequal sample size. The ANOVA was more appropriate, offers more power, and allowed a closer assessment of interactions. The percentage of animals with convulsions was compared by Chi Square. Correlations were performed for a matrix of ethanol intoxication score, mean withdrawal, peak withdrawal, mean dose, and BEC. Pearson correlations were performed for continuous variables (e.g. dose of ethanol, BEC) whereas Spearman correlations were performed for noncontinuous variables (e.g. intoxication or withdrawal scores based on an ordinal scale). Correlations were compared via t-test for Spearman rank correlation and the slopes by pooling error of the slopes and also by t-test. In all analyses, differences were considered significant at the $p < 0.05$ level.

Results

Experiment 1

This model of binge-like ethanol exposure produced similar patterns of alcohol dependence in both adolescent and adult rats. During the 4 days of binge-like treatment, intoxication behaviors were scored based on a modified Majchrowicz scale at each feeding, which was subsequently used to calculate the dose of ethanol administered (Knapp and Crews, 1999; see also Figure 1). Over the four-day alcohol administration period, both adolescent and adult rats displayed typical signs of intoxication including lethargy, loss of motor coordination, and loss of righting reflex, all of which have been correlated previously to BECs in adult rats (Majchrowicz, 1975). Repeated measures ANOVA revealed main effects of age and feeding time point for intoxication scores. Thus, mean intoxication scores, collapsed across the four days, were significantly lower for adolescents than adult rats (1.0 ± 0.1 vs. 2.1 ± 0.1 , $p < 0.0001$; Figure 1a inset). A significant interaction of age $\times$ time further revealed that across the binge, adolescent rats were less sensitive to the sedative effects of ethanol, as evident by lower intoxication scores [$F(11,1397) 14.91$, $p < 0.0001$; Figure 1a). Furthermore, the peak intoxication scores, i.e. the highest intoxication score achieved across the four days, were significantly lower in the adolescent group in comparison to the adults (2.2 ± 0.1 vs. 3.7 ± 0.1 , $p < 0.0001$; Figure 1b). Therefore, consistent with the lower intoxication scores, adolescents then received significantly higher doses of ethanol across the four days of binge-like treatment (Figure 2a), with an average dose of 12.3 ± 0.1 g/kg/d, while the adults received 9.2 ± 0.2 g/kg/d (Figure 2b; $p < 0.0001$).

BECs were measured from serum extracted from tail blood at 90 minutes after the first feeding of the third day of binge-like treatment, a time when BECs are expected to be at peak levels. Despite having lower intoxication scores and higher doses of ethanol, the adolescent and adult groups had comparable BECs of 344.5 ± 10.2 mg/dL and 338.5 ± 7.8 mg/dL respectively (Figure 2c; $p = n.s.$). In both the adolescent and adult groups, animals lost body weight over the four days of ethanol treatment. The percent weight loss on day 4 for adolescent and adult rats were $11.4 \pm 1.1\%$ and $13.9 \pm 0.5\%$ respectively. The adult animals lost significantly more weight than the adolescents ($p < 0.05$) over the binge time course. The data for intoxication and BEC suggest that the induction of dependence is different between adolescents and adults, as adolescents displayed fewer signs of intoxication and received higher doses of ethanol.

To investigate withdrawal behavior, both adolescent and adult rats were monitored and scored for typical symptoms of withdrawal using the ordinal scale defined in Penland et al. (2001), starting 10h after the last dose of ethanol. Withdrawal behaviors were rated at 30min intervals for 17h. Behaviors characteristic of withdrawal, such as splayed limbs, head and tail tremors, tail spasms and convulsions were observed in both the adolescent and adult alcohol groups (see Figure 3). The highest score attained during each hour was recorded as an index of severity. Repeated measures, two-way ANOVA revealed a main effect of hour and significant interaction of age $\times$ hour, but no main effect of age. Thus, overall, withdrawal severity scores averaged 1.4 ± 0.1 (adolescent, $n = 44$) and 1.5 ± 0.1 (adult, $n = 65$; Figure 3a inset) collapsed across the 17 hours, while the peak scores (highest score attained) were 3.3 ± 0.1 (adolescent) and 3.2 ± 0.1 (adult; Figure 3b), neither of which were significantly different between groups. However, the significant age $\times$ hour interaction [$F(16,1711) 2.04$, $p = 0.009$; Figure 3a] showed that adult animals initially had slightly greater withdrawal severity at the beginning of the observation period. A small number of adult animals began showing convulsions earlier than typically observed (e.g. Nixon & Crews, 2004; Nixon et al., 2008), which is likely driving this slight difference at a single time point. Withdrawal behavior is typically highly variable, but when large numbers of subjects are compared such as this, slight differences can be discerned. The most severe category, convulsions, was examined separately and showed that the percentage of rats observed having convulsions was similar: 16% of adolescents and 23% of adults.

In order to examine whether different age-dependent relationships exist between ethanol intoxication measures and ethanol withdrawal measures, correlations were performed among these data. In both adolescents and adults, the mean withdrawal severity significantly correlated to mean intoxication score ($p < 0.05$; Figure 4a, b) and subsequently mean dose per day (data not shown). These mean withdrawal severity x mean intoxication correlations are significantly different between each age group ($t(1)=0.995$, $t(2)=0.9995$, $0.001 < p < 0.01$), but the slopes are similar ($t=0.95$, $p=n.s.$). BEC significantly correlated to mean withdrawal only in adults, despite the similar slope and scatter of the data (Figure 4c).

Experiment 2

To further examine potential pharmacokinetic differences between adolescents and adults in the Majchrowicz model, a separate experiment was performed where tail blood samples were collected twice a day during binge-like exposure: the time when intoxication behavior was assessed (“pre”) and 90min after ethanol was administered (“post;” Figure 5a). The same intoxication parameters that were assessed in experiment 1 were again assessed in experiment 2. As in experiment 1, a repeated measures, two-way ANOVA on intoxication scores revealed a significant main effect of age [$F(1,14)$ 19.53, $p < 0.001$], time [$F(11,154)$ 26.95, $p < 0.0001$] and interaction of age x time [$F(11,154)$ 4.03, $p < 0.0001$] across the binge. Similar to experiment 1, the average intoxication scores were significantly lower for adolescents than adult rats (1.0 ± 0.2 vs. 2.1 ± 0.4 , $p < 0.02$). Also similar to experiment 1, in spite of receiving higher doses of ethanol (11.8 ± 0.3 g/kg/day vs. 8.8 ± 0.6 g/kg/day), the grand mean BECs for all time points collapsed together were similar: 241.8 ± 19.2 mg/dl for adolescent rats and 285.6 ± 16.7 mg/dl for adult rats (Figure 5b inset). Analyzing BEC throughout the duration of ethanol administration by repeated measures ANOVA only revealed a significant age x time interaction [$F(6,78)$ 6.796, $p < 0.0001$; Figure 5b]. In both groups there was a steady increase in BEC when both pre and post measurements are considered together. When average pre and post BEC measurements are considered separately, adolescents had significantly lower BEC measures prior to dosing (“pre”), when intoxication behaviors were scored ($p < 0.05$; Figure 5c). The lower BEC measurements in adolescent rats correspond to an observed decrease in intoxication behavior at those time points. Blood samples were also collected from control animals on the same schedule as ethanol treated animals. The overall BEC for the adolescent and adult control groups were 27.2 ± 4.8 and 18.6 ± 2.1 mg/dL, respectively. These averages are considered to be background values by the manufacturers of Analox (Analox, London, UK).

Discussion

The roots of alcohol abuse and dependence begin in adolescence, as this is the time when alcohol experimentation begins and the first symptoms of an AUD may emerge. Several groups have suggested that the unique state or responsiveness of the adolescent brain to ethanol predisposes the individual toward excessive intake. Past studies however, could not rule out observed differences in BEC - i.e. ethanol pharmacokinetics - as a factor underlying the differences in sensitivity between adults and adolescents. Specifically, several groups report that adolescent rats have reduced sensitivity to seizure susceptibility during ethanol withdrawal (Acheson et al., 1999; Chung et al., 2008), though differences in ethanol pharmacokinetics, namely lower BECs in adolescent rats despite the same dose, could not be eliminated. In contrast to these previous works, the major finding of this study is that withdrawal severity is not different for adult versus adolescent rats when BECs are similar across a four-day binge-like exposure. By choosing a model that overcomes the pharmacokinetic differences between ages to result in similar BECs, we created a valid comparison between the two ages on ethanol withdrawal severity. Although the adolescent rats received 3 g/kg more alcohol per day than did the adults during the binge exposure period, BECs taken at the expected peak (“post”) were nearly identical between age groups (Figures 2c, 5). The similarity in BECs in this model was

further supported in a second experiment, which examined BECs at multiple time points across the binge. A large number of subjects (rats) were examined in experiment 1 in order to discern the smallest of potential differences. However, the opposite was shown in that mean withdrawal and peak withdrawal severity are remarkably similar between age groups. Importantly, this is the first report on withdrawal behavior in adolescent rats using the Majchrowicz model of alcohol dependence. This model appropriately models the wide range of withdrawal severity seen in the human population (Hall and Zador, 1997). The full range of withdrawal behaviors described by Majchrowicz (1975) was observed in adolescents, which is of note since this scale was based on behaviors originally observed in adults. Qualitatively, adolescent withdrawal behaviors were similar to that of adults as well, including convulsions, the most severe behavior observed in this model. A minority of adult rats in this model develops spontaneous seizure-like convulsions during withdrawal and a similar percentage of adolescent rats were observed to have seizure-like convulsions. Indeed, the lack of withdrawal differences in this study where BECs were similar supports the lack of age difference in other aspects of withdrawal, such as withdrawal-induced anxiety after vapor inhalation of ethanol, a model that also matches BECs (Slawecki et al., 2006). Therefore, both qualitatively and quantitatively, these data suggest that when the concentration of ethanol is similar between adults and adolescents in a model of alcohol dependence, the resulting withdrawal is also similar.

Alcohol withdrawal seizures may reflect more severe alcohol dependence, higher levels of intake and/or longer durations of intake (Hall and Zador, 1997) and adolescent rats in this study received more ethanol than the adult rats. Therefore, to examine whether relationships varied for the different aspects of alcohol intoxication and withdrawal behavior between adults and adolescents, correlations were performed among model data gathered for each group. For both ages, mean intoxication score correlated with mean withdrawal (Figure 4a, b). Thus, the more intoxicated the animal over the four days, the higher its withdrawal severity on average. However, mean intoxication did not predict peak severity as there were no significant correlations between mean intoxication score and peak withdrawal score, defined as the highest score of withdrawal achieved during the 17 hours of observation. The large *n* associated with this study likely assisted in revealing these statistically significant but unremarkable correlations for BEC and various aspects of withdrawal. Further, it must be noted that it is difficult to attribute the blood level at a single time point to effects in withdrawal. As well, variation in the withdrawal data, especially in the peak withdrawal score, could be attributed to only 30-minute observations per hour or that multiple animals are being observed in their home cages at one time. Specifically, it is possible that if only a single seizure occurred, it was missed. Though in our experience, seizures do not just occur once. Further, this study follows the hourly observations of Majchrowicz (1975) and is more aggressive in its withdrawal observation than past work (e.g. Penland et al., 2001). The correlation between mean intoxication state and mean withdrawal severity, though unremarkable, support past studies where higher intake and/or higher BEC correlated to increased withdrawal in adolescent versus adult rats (Chung et al., 2008; Wills et al., 2008). Indeed, intake and severe withdrawal do not correlate particularly well in humans either; however, a high level of ethanol intake is a risk factor for significant withdrawal effects (Clark et al., 2002).

As mentioned above, despite similar BECs, adolescents demonstrate reduced behavioral intoxication and therefore receive approximately 3 g/kg more ethanol per day than adults. The Majchrowicz model of alcohol dependence is designed to maintain sedation for four days based on titrating the dose of ethanol according to an established relationship between BEC and the various behavioral signs of CNS depression in adult rats (Majchrowicz, 1975). Specifically, an intoxication scale (the modified form used in this study shown in Figure 1) utilizes the degree of CNS depression observed to assign a numerical score with an associated ethanol dose. A less intoxicated animal will receive a lower score and therefore higher dose of ethanol and vice versa (Majchrowicz, 1975). Considering that adolescents are less sensitive to the

sedative and motor-impairing effects of ethanol compared to adult animals (Little et al., 1996; White et al., 2002), it was not surprising that adolescent rats received lower intoxication scores when compared to adult animals (Figure 1). The Majchrowicz intoxication scale was developed in adult rats and is based on a relationship established for BEC and particular signs of CNS depression, specifically levels of motor impairments and sedation. As adolescents display better motor coordination and have shorter sleeping times than adults administered a similar dose of ethanol (Little et al., 1996; White et al., 2002), the CNS depression-BEC relationship could be different for adolescent rats. However, despite the different relationship, these data and specifically experiment 2 show that this model can be used to overcome pharmacokinetic differences to result in similar BEC.

In experiment 1, the observation of similar BECs despite the significantly higher doses, strongly suggested a difference in ethanol pharmacokinetics between these two age groups. This suggestion was confirmed in experiment 2 where BEC was analyzed at several time points throughout the binge. Although no main effect of age was observed, a significant interaction of age by time revealed that BECs prior to alcohol administration were lower in adolescent rats versus adults (Figure 5c “pre”). This result coupled with the pattern of BEC across the binge suggests that adolescent rats may metabolize alcohol at a faster rate than adult rats. This observation is supported by past work showing faster elimination rates or clearance in adolescent versus adult rats (Brasser and Spear, 2002; Little et al., 1996; Varlinskaya and Spear, 2004). Although a large body of work has attempted to investigate the contribution of ethanol pharmacokinetics to altered sensitivity (e.g. Silveri and Spear, 2000), pharmacokinetic differences, or lack thereof, may depend on route and dose of ethanol administration (Walker and Ehlers, 2009). An intragastric gavage method of alcohol administration was used to model the oral route of consumption in human AUDs. Previous reports using this technique and an acute 5 g/kg dose (the maximum used in the present study) produce a BEC peak of 233 mg/dl in adult rats (Nixon and Crews, 2002) but only 131 mg/dl in adolescent rats (Crews et al., 2006). These findings are consistent with a direct comparison in a different strain of rats that showed that adolescent rats generally had lower peak BECs despite the same dose as adults (Walker and Ehlers, 2009). Altogether, these studies support that both initial absorption (e.g. Chung et al., 2008; Crews et al., 2006; Walker and Ehlers, 2009) as well as metabolism (e.g. Brasser and Spear, 2002; Varlinskaya and Spear, 2004) contribute to pharmacokinetic differences between adolescents and adults in this study. Further, lower BEC measurements in adolescent rats in this study corresponded to lower intoxication scores, which would support that reduced adolescent intoxication behavior in this model is due to age-dependent differences in ethanol pharmacokinetics. The role of pharmacodynamic differences in age-related, ethanol-induced sedation, however, cannot be ruled out by this study. It is well accepted that the diminished sedative effect of ethanol in adolescents may be due to its differential influence on γ -aminobutyric acid (GABA), the primary inhibitory neurotransmitter. Electrophysiological studies have shown that adolescent neurons are less sensitive to the ethanol-induced potentiation of GABA_A receptor-mediated inhibitory post synaptic currents; which may underlie the delayed onset of sedation described for adolescent animals (Li et al., 2003). Because some of our behavioral measures may be sensitive to these age-dependent pharmacodynamic differences, both pharmacokinetic and pharmacodynamic differences should be considered to have potential roles in the reduced intoxication behavior observed for adolescents in this study.

Another factor that could influence the reduced intoxication behavior observed in adolescent rats is differential acquisition of ethanol tolerance, whether metabolic or behavioral, between adult and adolescent rats (e.g. Swartzwelder et al., 1998). However, the pattern of intoxication scores versus BEC in experiment 2 (data not shown) does not necessarily support a differential development of tolerance for the behavioral measures used in this model (motor impairment/sedation), though this study was not designed to investigate tolerance. The acquisition of

tolerance is influenced by dose, route, pattern and duration of ethanol exposure as well as the measure examined (Ristuccia and Spear, 2005; Silveri and Spear, 1998; Silveri and Spear 2001; Silvers et al., 2003; Matthews et al., 2008). Remarkably, adults and adolescents do not differ in their development of tolerance when they are dosed to a similar level of motor impairment (meaning, different doses needed to result in the same behavior; Silveri and Spear, 2001). As that approach was quite similar to the approach of the Majchrowicz model to maintain sedation, it would predict that tolerance is not strikingly different between adults and adolescents within this model.

Although no differences in withdrawal severity were observed between adult and adolescent rats matched for BEC, the reduced sensitivity to ethanol intoxication observed may be the more critical issue when it comes to understanding adolescent vulnerability to developing an AUD as reduced sensitivity to ethanol intoxication is much more predictive of a future disorder (Schuckit, 1994). Comparatively reduced sedation following ethanol intoxication has been suggested to lead to higher levels of ethanol intake in adolescents (Chung et al., 2008; Spear and Varlinskaya, 2005; Wills et al., 2008). Further, altered sensitivity to withdrawal in either direction (greater or lesser) has been argued to contribute to alcohol seeking and relapse (e.g. Acheson et al., 1999; Koob and Le Moal, 1997; Slawecki et al., 2006). Alcohol withdrawal is merely the expression of dependence, thus the bigger question may be how adolescents develop dependence. Some argue that their low sensitivity to alcohol intoxication coupled with fewer years/duration of intake suggest that adolescents are not alcohol dependent. Certainly, more dependence-type symptoms than abuse symptoms are reported by adolescents, but sometimes not enough in number to warrant an official diagnosis of alcohol dependence (see Clark et al., 2002; Martin and Winters, 1998 for review). These data confirm that adolescent rats exhibit alcohol dependence. Regardless of definitions, excessive drinking has significant consequences on brain structure, function and physiology that lead to dependence. Adolescent insensitivity to alcohol intoxication, which results in few physiological clues upon which to self regulate intake, coupled with increased sensitivity to positive effects of intake (social facilitation, anxiolysis and rewarding properties of alcohol) create a perfect combination of factors that promote excessive drinking. This interaction of factors may underlie the greater likelihood of an adolescent drinker to have later alcohol problems. Thus, understanding both the dose- and concentration-dependent effects of alcohol are critical to understanding how AUDs develop.

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Figure 1.

(a) Behavioral intoxication scores for each session are shown across the four days of binge-like ethanol administration for both adolescents ($n=58$) and adults ($n=71$). Behavioral intoxication scores are zero, for normal rats, immediately before the first dose as indicated by the arrow. Bonferroni posthoc tests confirmed that adolescents were less behaviorally intoxicated at all feeding time points after ethanol administration. As well, on average, adults displayed significantly higher levels of typical intoxication behavior (inset in graph a). (b) Adults also displayed significantly higher peak intoxication behaviors (highest score achieved over 17 hours) in comparison to adolescents. (c) Behaviors used to assess intoxication levels during ethanol administration are shown. All data points represent the mean $\pm$ SEM, $*p<0.05$.

Figure 2.

(a) Because the doses of ethanol administered are determined by intoxication behavior, adolescent rats received significantly higher doses of ethanol than adults over the four-day administration period. (b) The grand mean dose of ethanol per day was also significantly higher for adolescents. While adolescents received significantly higher doses of ethanol over the binge-like administration period, there was no significant difference in BEC between the adolescent and adult rats when measured on the first feeding of day 3 of the binge (c). All data points represent the mean $\pm$ SEM, * $p < 0.05$.

Figure 3. Withdrawal behavior emerges approximately 10 to 13 h after the last dose of ethanol. Thus, withdrawal severity was examined for 17 hours from 10–26 hours after the last dose of alcohol. This range allowed for time on either side of the peak withdrawal behavior time point of 17–21 hours (Penland et al., 2001) in case adolescents had a different time course of withdrawal. (a) Bonferroni posthoc tests revealed that although adults had slightly higher withdrawal scores at the 2nd hour of observation only in general, withdrawal severity was similar between adolescents and adults (inset, no main effect of age). (b) Peak withdrawal behavior (highest score achieved) was not significantly different between adolescents and adults. (c) The scale

used to assess withdrawal behavior is shown. All data points represent the mean $\pm$ SEM, * $p < 0.05$.

Figure 4.

(a) In both adolescent and adult rats, withdrawal behaviors were found to significantly, but only modestly, correlate to mean intoxication scores and BEC (as indicated by Spearman coefficients). (b) Mean intoxication behavior correlated to mean withdrawal behavior in both adolescents and adults. (c) BEC correlated to mean withdrawal in adults, but not adolescents. BEC did, however, correlate to peak withdrawal in adolescents (not shown graphically).

Figure 5.

The timeline for experiment 2 is shown in (a). Specifically, ethanol is administered every eight hours starting at 3:00p.m. Tail bloods for BEC determination were drawn just after behavioral scores were obtained on Days 2–4 of the binge (grey arrows: “pre”) and at 90 minutes following the 3:00pm dose each day (black arrows: “post”). (b) BECs across the binge show no main effect of age (inset shows the grand mean BEC collapsed across all “pre” and “post” measures), thus BECs are statistically similar overall between adult and adolescent rats. (c) However, a significant interaction of age by time indicates that adolescent rats have lower BECs at the pre dose time point. All data points represent the mean $\pm$ SEM, * $p < 0.05$.