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Daily patterns of ethanol drinking in peri-adolescent and adult alcohol-preferring (P) rats

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Abstract

Alcohol abuse among adolescents continues to be a major health problem for our society. Our laboratory has used the peri-adolescent alcohol-preferring, P, rat as an animal model of adolescent alcohol abuse. Even though peri-adolescent P rats consume more alcohol (g/kg/day) than their adult counterparts, it is uncertain whether their drinking is sufficiently aggregated to result in measurable blood ethanol concentrations (BECs). The objectives of this study were to examine daily alcohol drinking patterns of adolescent and adult, male and female P rats, and to determine whether alcohol drinking episodes were sufficiently aggregated to result in meaningful BECs. Male and female P rats were given 30 days of 24 h free-choice access to alcohol (15%, *v*/*v*) and water, with ad lib access to food, starting at the beginning of adolescence (PND 30) or adulthood (PND 90). Water and alcohol drinking patterns were monitored 22 h/day with a "lickometer" set-up. The results indicated that (a) peri-adolescent P rats consumed more water and total fluids than adult P rats, (b) female P rats consumed more water and total fluids than male P rats, (c) there were differences in alcohol, and water, licking patterns between peri-adolescent and adult and female and male P rats, (d) individual licking patterns revealed that alcohol was consumed in bouts often exceeding the amount required to self-administer 1 g/kg of alcohol, and (e) BECs at the end of the dark cycle, on the 30th day of alcohol access, averaged 50 mg%, with alcohol intakes during the last 1 to 2 h averaging 1.2 g/kg. Overall, these findings indicate that alcohol drinking patterns differ across the age and sex of P rats. This suggests that the effectiveness of treatments for reducing excessive alcohol intake may vary depending upon the age and/or sex of the subjects being tested.

Keywords: Adolescence; Young adult; Juvenile; Alcohol-preferring rats; Alcohol-drinking

1. Introduction

Today's youth are initiating alcohol use earlier and experiencing more alcohol-related problems than ever before (Miller et al., 2001; Winters, 2001), and this is true for both men and women (Kandel et al., 1997; Nelson et al., 1998). Recent estimates indicate that 80% of high school seniors have consumed alcohol and half of these initiated drinking before the eighth grade (Johnston et al., 1999), with early onset of alcohol use serving as a strong predictor of future alcohol dependence (Grant and Dawson, 1997; Hawkins et al., 1997). Binge drinking during high school and college is becoming more prevalent and is also a strong predictor of future alcohol-related problems in both men and women (Presley et al., 1994; Wechsler et al., 2000). Pattern of drinking (binge drinking, which is characterized by periods of large volumes of ethanol intake per day separated by periods of abstinence, versus constant ethanol consumption, which is generally characterized by lower volumes of intake per day) and total volume consumed are important diagnostic criteria for the onset of alcoholism in adult individuals as well (e.g., Heather et al., 1993; Lancaster, 1994). Additionally, these criteria have been used to develop different typologies and/or drinking profiles for alcoholics (e.g., Babor et al., 1992; Cloninger, 1987; Conrod et al., 2000; Epstein et al., 1995; Prelipceanu and

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Mihailescu, 2005; Windle and Scheidt, 2004; Zucker, 1987). Furthermore, in some cases, the effectiveness of a particular treatment appears to depend upon where an individual ranks on the continuum of a typology (e.g., Cherpitel et al., 2004; Dundon et al., 2004; Epstein et al., 1995; Johnson et al., 2003). Therefore, both age-of-onset and pattern of drinking are factors that have predictive validity for a life-time diagnosis of alcoholism or alcohol abuse and, in some cases, the effectiveness of the treatment for the same. The importance of investigating patterns of ethanol intake is also supported by research with rodents. Recent research from our laboratory has indicated that different selectively bred, high alcohol-consuming lines of rats may display different "types" of drinking (e.g., binge-like versus more continuous-like) under free-choice, home-cage conditions (Bell et al., 2004), which has also been found when selectively bred, high alcohol-consuming rats were tested using operant procedures (Files et al., 1998; Samson et al., 1998). In addition, it appears that examination of ethanol intake patterns, across a 24 h period, may reveal the role of endogenous factors (e.g., hormones and/or neurotransmitters) that mediate ethanol self-administration behavior, which might not be evident from records of daily intake alone (c.f., Samson, 2000).

Given the prevalence of alcohol abuse during adolescence, a need for animal models of excessive alcohol drinking during adolescence has been indicated (Spear, 2000; Witt, 1994). Peri-adolescent pups of selectively bred high alcohol-consuming lines of rats (e.g., alcohol-preferring, P, rats) have been proposed as one animal model of adolescent alcohol abuse (c.f., McBride et al., 2005; McKinzie et al., 1999). This stems from the fact that the P line of rat successfully meets criteria (c.f., Bell et al., 2005; McBride and Li, 1998; Murphy et al., 2002) proposed for a valid animal model of alcoholism (Cicero, 1979; Lester and Freed, 1973). 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; and 6) chronic consumption of ethanol should lead to dependence, as indicated by withdrawal symptoms after access to ethanol is terminated (Cicero, 1979; Lester and Freed, 1973). The P line of rat was developed using bidirectional selection from a colony of Wistar rats at the Walter Reed Hospital (c.f., McBride and Li, 1998; Murphy et al., 2002). The general selection criteria for P versus alcohol-nonpreferring (NP) rats were that when given free-access to 10% ethanol and ad lib access to food and water, P progenitors would consume greater than 5 g of ethanol/kg of body weight/day and have an ethanol to water consumption ratio greater than 2:1, whereas NP progenitors would consume less than 1 g/kg/day and have an ethanol to water consumption ratio of less than 0.5:1 (c.f., McBride and Li, 1998; Murphy et al., 2002).

In a recent review on adolescent brain and behavior development, Spear (2000) indicated that the boundaries (i.e., beginning and end) of the adolescent "window" of neurobehavioral development for rats may differ given the parameters (e.g., behavioral, neurochemical, etc.) examined. However, neurobehavioral discontinuities between post-weanling and adult rats suggest that adolescence spans postnatal days (PND) 28 through 42 (i.e., 28 through 42 days old; Spear, 2000; Spear and Brake, 1983). This developmental window corresponds with timing of the growth spurt (Kennedy, 1967; Spear, 2000), changes in NMDA receptor binding of the prefrontal cortex (Insel et al., 1990), timing of emergence from the protected nest in the wild (Galef, 1981) and maturation of genitalia in female (Döhler and Wuttke, 1975) and male (Clermont and Perry, 1957) rats. Spear (2000) suggests that this conservative window (PND 28 through 42) could be extended through PND 60 when assessing the effects of pharmacological treatment during the "entire" adolescent period on adult behaviors in male and female rats. Our laboratory has published several studies using this window of ethanol treatment (PND 30 through 60). In one study, it was found that peri-adolescent P rats consumed more ethanol per kg body weight than their adult counterparts (Bell et al., 2003), with similar results found for peri-adolescent high alcohol-drinking (HAD-1 and HAD-2) rats (Bell et al., 2004). Another study using female P rats indicated that, compared with naïve P rats, P rats with access to ethanol during peri-adolescence (PND 30 through 60) displayed (a) quicker acquisition of operant self-administration of ethanol, (b) retarded extinction, and (c) greater operant responding during relapse, when tested during adulthood (Rodd-Henricks et al., 2002a).

As indicated above, our laboratory has reported that peri-adolescent P rats acquire adult levels (>5.0 g/kg/day) of ethanol [15% volume/volume (vol./vol.)] intake by PND 39 and that by PND 60 male P rats consume ~ 9.0 g/kg/day and female P rats consume ~ 7.5 g/kg/day (Bell et al., 2003). However, the pattern of ethanol intake displayed by peri-adolescent P rats or the BECs achieved at multiple time points across the day were not determined. When studied under 23 h access (1 h per day was devoted to laboratory procedures) operant conditions, it appeared that most of the ethanol self-administered by adult male P rats was not associated with feeding (Files et al., 1992, 1994), with only ~30% considered prandial-associated, and this percentage decreased substantially under limited access conditions (Files et al., 1994). As far as we know, only one study has examined free-choice, home-cage access ethanol drinking patterns in P rats, and this study was limited to adult male rats (Murphy et al., 1986). These authors (Murphy et al., 1986) reported that adult (>PND 90) male P rats ($n=4$) consumed approximately 70% of their ethanol during the dark cycle. Two of the 4 animals displayed a large bout at the end of the dark cycle (>1.0 g/kg/h), with all 4 animals displaying a bout at the beginning of the dark cycle and bouts in the middle of the dark cycle (Murphy et al., 1986). A bout is defined as a "cluster" of drinking/licking behavior that is sufficiently aggregated such that the organism must stop ongoing behavior in order to carry out this "cluster" of

drinking/licking behavior. For example, a brief interruption of feeding behavior to obtain fluid in order to soften the food, with an immediate return to feeding behavior, would not be considered a bout, at least not in the context of the present experiment. However, a period of fluid consumption upon waking, generally substantial across species, to replenish fluids lost during sleep would be considered a "bout" of drinking behavior, within the context of the present experiment.

To date, there are no published reports of a detailed examination of drinking patterns that have taken into account age and sex of animal across weeks of free-choice, home-cage ethanol access with the P, or for that matter any, line of rats. Therefore, the present study examined the ethanol drinking pattern of adolescent (starting PND 30) and adult (starting>PND 90) P rats across the first 4 weeks of access. Because sex differences in ethanol intake for P rats were found in a previous study (Bell et al., 2003) and have been reported for other rat lines in the literature (e.g., Adams, 1995; Juárez and De Tomasi, 1999; Lancaster and Spiegel, 1992; Li and Lumeng, 1984), both male and female rats were tested. It is well known that the presence of a researcher, with or without the action of taking blood samples, disrupts free-choice ethanol self-administration behavior. Therefore, to preserve "naturalistic" self-administration behavior, as much as possible, the study was conducted with an unobtrusive measure, a "lickometer" set-up, such that when an animal licked either the water or ethanol bottle sipper tube it closed a circuit and a computer program recorded one unit of measure. We hypothesized that (a) P rats would consume more ethanol during the dark-period of the day than the light-period of the day, (b) P rats would consume ethanol in bouts, with larger bouts occurring at the beginning and end of the dark-period, (c) the pattern of drinking displayed by the adolescent P rats would gradually change to resemble that displayed by the adult P rats, (d) female P rats would consume more fluids than male P rats, and (e) appreciable BEC levels would be detected in adolescent and adult P rats.

2. Materials and methods

2.1. Animals

Ethanol-naïve male and female P rats ($n=10-15$/age/sex), from the S50 to S53 generations, were obtained from the Indiana University Medical Center/Veterans Affairs Medical Center (Indianapolis, IN, USA) breeding colonies. Adolescent rats were between postnatal days (PND) 21 and 24 of age, when delivered. All rats were double-housed by sex and age. At least four litters were represented in each condition, to limit litter effects. The vivarium was maintained on a 12/12 h light/dark cycle (lights on at 0700 hours) with temperature (21 °C) and humidity (50%) controlled. Animals used in these procedures were maintained in facilities fully accredited by the Association for the 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 (Indianapolis, IN, USA) and are in accordance with the guidelines of the *Public Health Service Policy on Humane Care and Use of Laboratory Animals* (Office of Laboratory Animal Welfare, National Institutes of Health, 2002) and the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (Institute for Laboratory Animal Research, Division on Earth and Life Studies, National Research Council, 2003).

2.2. Procedures

On PND 28±1 day, for adolescents, or PND 88±1 day, for adults, the animals were single-housed in hanging wire-mesh cages with water and food freely available throughout the experiment. Two days later all rats were given access to 15% [volume/volume (vol./vol.)] ethanol as well. The ethanol and water bottles were standard glass bottles holding approximately 300 ml of fluid, with a stopper (no. 10) holding an angled (~135°) stainless steel sipper tube. Because the bottles were outside the cage and the sipper tube protruded into the metal cage, the sipper tubes were insulated with black shrink wrap except for 1.5 cm at the tip and another 1.5 cm as the sipper tube exited the bottle through the no. 10 stopper. Solutions were changed twice a week, and bottles were changed every 2 weeks. Starting on the first day of ethanol access, body weight, and water and ethanol bottle weights were obtained using a Sartorius Balance BP 6100 and Sartorius Interface V24/V28-RS232C(-S)/423 (Sartorius Instruments: McGaw Park, IL, USA) and recorded by a personal computer program (SoftwareWedge, Professional Edition v 5.0 for DOS, Sartorius Instruments: McGaw Park, IL, USA). Weights were measured at least 5 days per week. All weights were obtained during the light cycle (1100–1300 hours). Values for days when weights were not measured were taken as the average of the weights taken on the days preceding and following the missing data point. Number of licks on the water, or ethanol bottles, were obtained with a "Lickometer" (LabLinc V System) set-up from Coulbourn Instruments (Allentown, PA, USA). Essentially, for each bottle (water or ethanol), an electrical lead was attached to the sipper tube (outside of the stainless steel cage) and another electrical lead was attached to the cage rack (ground). Therefore, when the animal licked the sipper tube tip a circuit was closed. Closures of the circuit were summed and recorded every 6 min from 1300 through 1100 hours the next day by a personal computer program (DATAQ software, Coulbourn Instruments, Allentown, PA, USA).

2.3. Blood ethanol concentrations (BECs)

It was determined from the data, of the original 4 groups, that the largest 2 h bout of ethanol consumption occurred at the end of the dark cycle. For BEC analyses, 7 peri-adolescent male, 7 peri-adolescent female, and 5 adult male P rats were allowed to self-administer ethanol for 30 days under the same conditions as those described above. Review of the drinking, or lickometer, data from these animals did not reveal any significant differences from the previously obtained data. On

the morning of the 30th day of ethanol access, ethanol bottle weights were obtained for peri-adolescent female P rats at 0500 (2 h before light onset) and adult male P rats at 0600 (1 h before light onset). For the peri-adolescent male P rats, lickometer data from the 0600 to 0700 time period was used to represent level of ethanol intake. At light onset (0700), tail blood samples were obtained from the peri-adolescent male and female and adult male P rats by tail clip. Immediately after the tail blood sample was drawn, the rat was decapitated and a trunk blood sample was also obtained from each of these animals. Blood samples were collected in heparinized tubes and centrifuged in a Microfuge (Model B, Beckman: Palo Alto, CA, USA) for 45 s. The supernatant fractions were used for determining BECs. BECs were measured using an Analox Analyser (model GL5: Analox Instruments USA, Lunenburg, MA, USA). Each blood sample (tail and trunk blood, for each animal) was run in triplicate and the average of these 3 readings was taken as the BEC obtained for that animal and blood sampling site. Data from adult female P rats were not obtained due to lack of availability of these animals at the time of BEC testing.

2.3.1. Statistical analyses across weeks

From PND 33 through 60, for adolescents, and PND 93 through 120, for adults, weekly averages for body weight (g), ethanol (g of ethanol/kg body weight/day) and water (ml/kg/day) consumption, and ethanol (ml/day) to total fluid (ml/day) preference ratio were computed and evaluated for differences between the sexes and age groups across weeks using $2 \times 2 \times 4$ (sex by age by week) mixed ANOVAs, with week being the within-subjects variable and sex and age group being the between-subjects variables. Additionally, weekly averages for number of licks on the ethanol or water bottles were summed in 2 h blocks from 1300 through 1100 hours the next day and evaluated for differences between the sexes and age groups across 2 h blocks and weeks using $2 \times 2 \times 4 \times 11$ (sex by age by week by 2 h blocks) mixed ANOVAs, with week and 2 h blocks being the within-subjects variables and sex and age group being the between-subjects variables. Because the ethanol and water data were analyzed twice, once for intake in g or ml/kg/day (averaged over each week) and again for number of licks/2 h block/day (averaged over each week), alpha was set at 0.0125 (0.05/4) for these analyses.

2.3.2. Regression analyses of BECs

To confirm a significant association between number of licks on the ethanol bottle (0600 to 0700) and BECs obtained, for the peri-adolescent male P rats, a linear regression analysis was performed on the data. Similarly, to confirm a significant association between absolute level of ethanol intake (for peri-adolescent female: g/kg/0500 to 0700; and for adult male: g/kg/0600 to 0700) and BEC obtained, separate linear regression analyses were conducted. The tail and trunk BEC-values were taken as independent measures and were pooled together for the regression analyses. Alpha was set at 0.05 for the regression analyses.

3. Results

3.1. Ethanol intake, water intake, body weight and ethanol preference ratio across weeks

Regarding ethanol intake (g/kg/day), the omnibus $2 \times 2 \times 4$ (sex by age by week) mixed ANOVA revealed a significant 2-way interaction for sex by week: [$F(3,147)=9.63$, $p<0.001$]; and a significant main effect of week: [$F(3,147)=7.25$, $p<0.001$]; such that, overall, female rats drank slightly more ethanol (g/kg/day) than the male rats initially, but male rats drank more ethanol (g/kg/day) than female rats by the 4th week, with this effect being seen for the 2nd through 4th weeks in adult animals (Fig. 1, upper left).

Regarding water intake (ml/kg/day), the omnibus $2 \times 2 \times 4$ (sex by age by week) mixed ANOVA revealed significant main effects for sex: [$F(1,49)=40.16$, $p<0.001$], age: [$F(1,49)=254.39$, $p<0.001$] and week: [$F(3,147)=158.61$, $p<0.001$]; such that female rats drank more water (ml/kg/day) than male rats, and all but the adult female rats displayed a decline in water intake (ml/kg/day) across weeks, with the decline in water intake being more apparent in adolescent animals (Fig. 1, upper right).

Concerning body weight (g), the omnibus $2 \times 2 \times 4$ (sex by age by week) mixed ANOVA revealed a significant sex by age by week interaction: [$F(3,147)=11.65$, $p<0.001$]; and significant main effects for sex: [$F(1,49)=280.78$, $p<0.001$], age: [$F(1,49)=651.80$, $p<0.001$] and week: [$F(3,147)=3653.60$, $p<0.001$]; such that adult rats weighed more than adolescent rats, male rats weighed more than female rats and male rats gained weight faster than female rats, with this effect being more apparent in adolescent animals (Fig. 1, lower left).

As to ethanol preference ratio [(ml of ethanol/total ml of fluid) $\times$ 100], the omnibus $2 \times 2 \times 4$ (sex by age by week) mixed ANOVA revealed a significant sex by age by week interaction: [$F(3,147)=5.69$, $p=0.001$]; and significant main effects for sex: [$F(1,49)=19.02$, $p<0.001$], age: [$F(1,49)=70.15$, $p<0.001$] and week: [$F(3,147)=41.93$, $p<0.001$]; such that, in general, adult rats had greater preference ratios for ethanol across weeks than adolescent rats, and that preference ratios increased the greatest for adult male rats, which was significantly higher than the adult female P rat values for the 2nd through 4th weeks of ethanol access, with preference ratios increasing to a lesser degree in adolescent female and adolescent male rats (Fig. 1, lower right).

3.2. Ethanol and water consumption pattern (licks/2 h) across weeks

Regarding ethanol consumption pattern, the omnibus $2 \times 2 \times 4 \times 11$ (sex by age by week by time) mixed ANOVA revealed significant 2-way interactions for sex by age: [$F(1,50)=19.13$, $p<0.001$], sex by week: [$F(3,150)=4.23$, $p=0.007$], and sex by time: [$F(10,500)=18.20$, $p<0.001$]; and main effects for sex: [$F(1,50)=15.54$, $p<0.001$], age: [$F(1,50)=36.62$, $p<0.001$], week: [$F(3,150)=6.89$, $p<0.001$], and time: [$F(10,500)=110.93$, $p<0.001$], Fig. 2. As

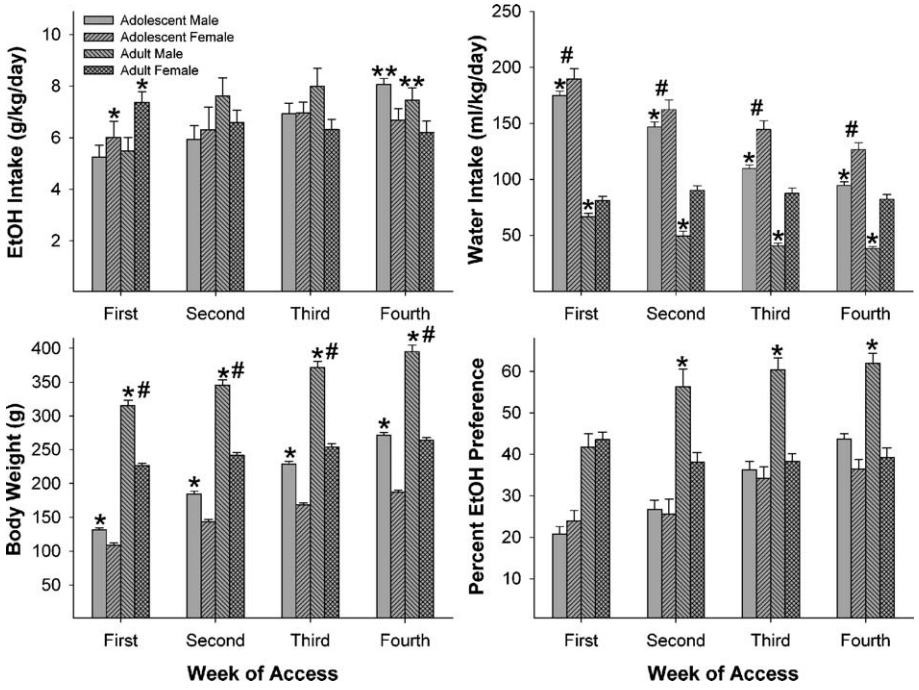


Fig. 1. Effects of sex of animal (data for male P rats are the 1st and 3rd bars, and data for female P rats are the 2nd and 4th bars), age of animal (data for adolescent P rats are the 1st and 2nd bars, and data for adult P rats are the 3rd and 4th bars), and week of access on ethanol intake (g/kg/day, mean ± S.E.M., upper left panel), water intake (ml/kg/day, upper right panel), body weight (g, lower left panel) and percent ethanol preference (%, lower right panel) across the 4 weeks of ethanol access ($n = 10–15$/age/sex). Female P rats drank more ethanol than male P rats during the 1st week of access (*), and this effect was reversed by the 4th week of access (**). Overall, adolescent P rats drank more water than adult P rats (#), and male P rats reduced their water intake to a substantially greater degree than female P rats across weeks of access (*). Adult P rats weighed more than adolescent P rats (#) and male P rats weighed more than female P rats (*). Even though both male and female adolescent P rats increased their preference ratio for ethanol, the only true (>50% of total fluid intake) preference for ethanol was displayed by the adult male P rats, which was significantly higher than the adult female P rat values (*).

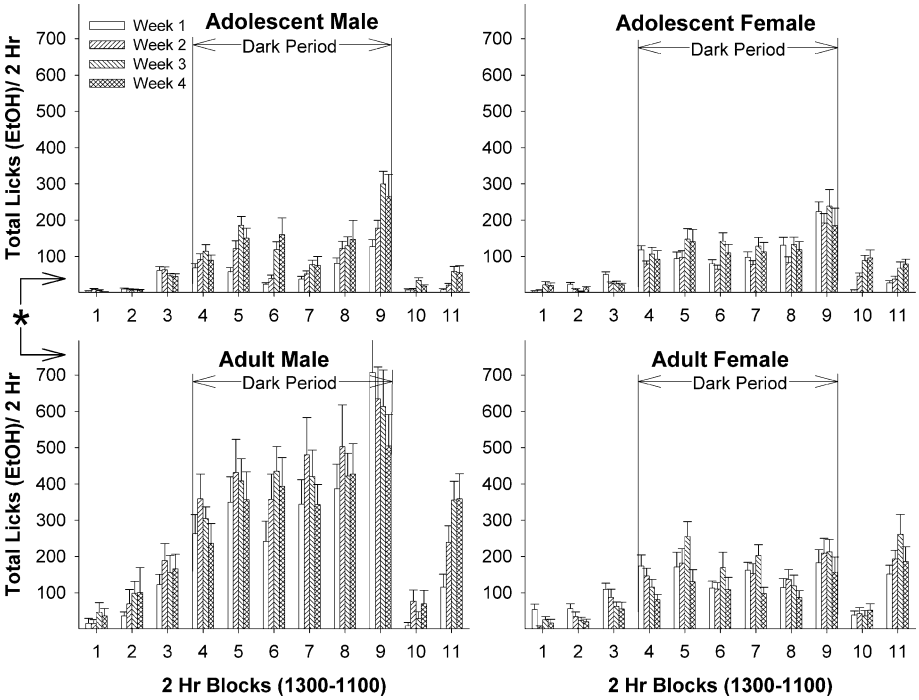


Fig. 2. Effects of sex of animal (male vs. female P rats), age of animal (adolescent vs. adult P rats), week of access and time of day on ethanol (15%, v/v) licking behavior (mean ± S.E.M.) across the 4 weeks of ethanol access ($n = 10–15$/age/sex). Adult animals licked the ethanol bottle sipper tube more often than adolescent animals, with most of the ethanol licking behavior of both adolescent and adult P rats taking place during the dark cycle. Additionally, increases in ethanol licking behavior across weeks were primarily displayed by the male P rats.

seen in Fig. 2, top vs. bottom panels, adult rats displayed more ethanol licking behavior than adolescent rats, with this effect being greater in male than female rats. Initially, adolescent female rats displayed greater ethanol licking behavior than adolescent male rats (Fig. 2 left vs. right panels), with this overall effect vanishing by the 3rd week (top), whereas adult male rats displayed significantly greater ethanol licking behavior than adult female rats throughout the 4 weeks (bottom). Additionally, there was a general increase in ethanol licking behavior across weeks for the adolescent rats and, to a lesser extent, adult male rats, with a modest decrease in ethanol licking behavior for the adult female rats during the 4th week of access (Fig. 2). The majority of the licking behavior took place during the dark cycle, with a gradual for adolescent rats (top) and a marked for adult rats (bottom) increase in licking behavior the 2 h before the ethanol bottle was removed for weighing and resetting the computer, at 1100 h each day (Fig. 2). The percentages of ethanol licks during the dark cycle for adolescent male (m) and female (f) and adult male (M) and female (F) rats for the 1st week were m—81%, f—87%, M—88%, and F—69%; and for the 4th week were m—88%, f—77%, M—76%, and F—67%, respectively.

As to water consumption pattern, the omnibus $2 \times 2 \times 4 \times 11$ (sex by age by week by time) mixed ANOVA revealed a significant 3-way interaction for sex by age by week: $[F(3,150)=11.70,\ p<0.001]$; significant 2-way interactions for sex by week: $[F(3,150)=5.86,\ p=0.001]$, and age by week: $[F(3,150)=3.90, p=0.010]$; and significant main effects for age: $[F(1,50)=18.31,\ p<0.001]$, week: $[F(3,150)=5.22, p=0.002]$, and time: $[F(10,500)=112.63, p<0.001]$, Fig. 3. In general, adult rats (Fig. 3, bottom) displayed more water licking behavior than adolescent rats (Fig. 3, top), with this effect being greater in male (Fig. 3, left) than female (Fig. 3, right) rats, at least initially. Initially, adolescent female rats (Fig. 3, top right) displayed greater water licking behavior than adolescent male rats (Fig. 3, top left), and adult male rats (Fig. 3, bottom left) displayed greater water licking activity than adult female rats (Fig. 3, bottom right). The significant interaction terms stemmed from the fact that across weeks, there was a general increase in water licking behavior by adolescent male (Fig. 3, top left) and adult female (Fig. 3, bottom right) rats, and a decrease in water licking behavior by adolescent female (Fig. 3, top right) and adult male (Fig. 3, bottom left) rats. Regarding time of day, the majority of the water licking behavior took place during the dark cycle. The percentages of water licks during the dark cycle for adolescent male (m) and female (f) and adult male (M) and female (F) rats for the 1st week were m—82%, f—89%, M—95%, and F—73%; and for the 4th week were m—91%, f—83%, M—95%, and F—74%, respectively.

3.3. Association between ethanol intake and BECs

Tail and trunk blood samples were taken at the end of 1 h (0600 to 0700) of measured ethanol drinking for the peri-adolescent and adult male P rats and at the end of 2 h (0500 to 0700) of measured ethanol drinking for peri-adolescent female

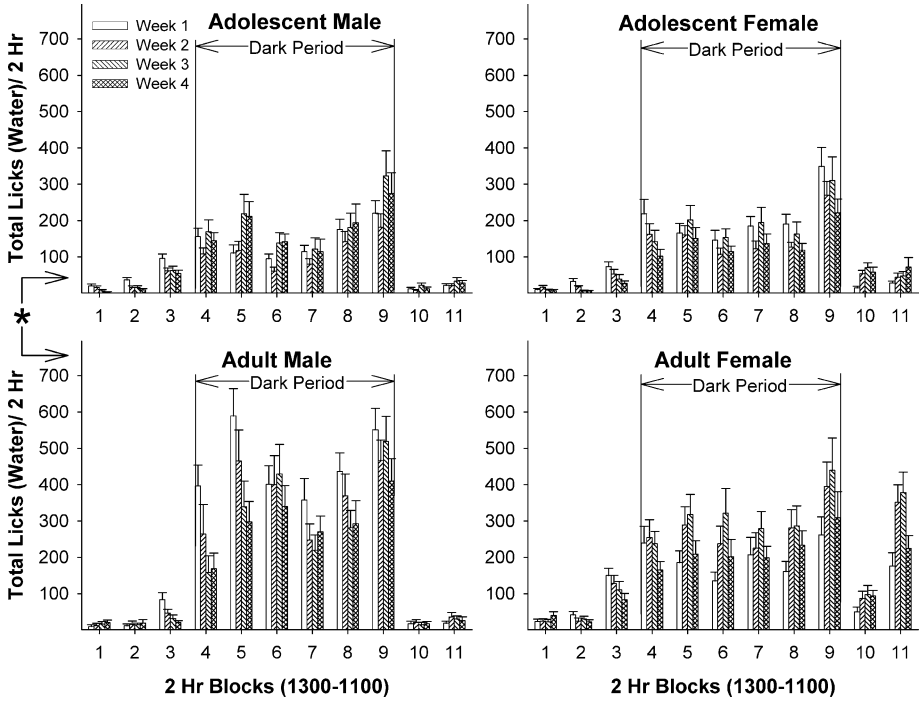


Fig. 3. Effects of sex of animal (male vs. female P rats), age of animal (adolescent vs. adult P rats), day of access and time of day on water licking behavior (mean ± S.E.M.) across the 4 weeks of ethanol access ($n=10–15$/age/sex). Overall, the data for water licking behavior were similar to the data for ethanol licking behavior (Fig. 2). Adult animals licked the water bottle sipper tube more often than adolescent animals, with both adolescent and adult P rats displaying most of their water licking behavior during the dark cycle. Adult male P rats displayed a decrease in water licking behavior during the dark cycle, whereas adult female P rats displayed an increase in water licking behavior during the dark cycle across weeks of ethanol access. The opposite effect was seen, to a lesser degree, in the adolescent animals.

P rats (Fig. 4). Linear regression analyses revealed a significant association between licking activity (0600 to 0700) on the ethanol bottle and BECs obtained for the peri-adolescent male P rats: (Fig. 4, top panel); a significant association between g/kg ethanol intake (0500 to 0700) and BECs obtained for the peri-adolescent female P rats: (Fig. 4, middle panel); and a significant association between g/kg ethanol intake (0600 to 0700) and BECs obtained for the adult male P rats: (Fig. 4, bottom panel). The mean tail vs. trunk BEC-values (mg%) did not differ significantly for each group, such that the values for the peri-adolescent male (m), peri-adolescent female (f) and adult male (M) P rats were m: 42 vs. 37; f: 58 vs. 53; and M: 60 vs. 51, respectively.

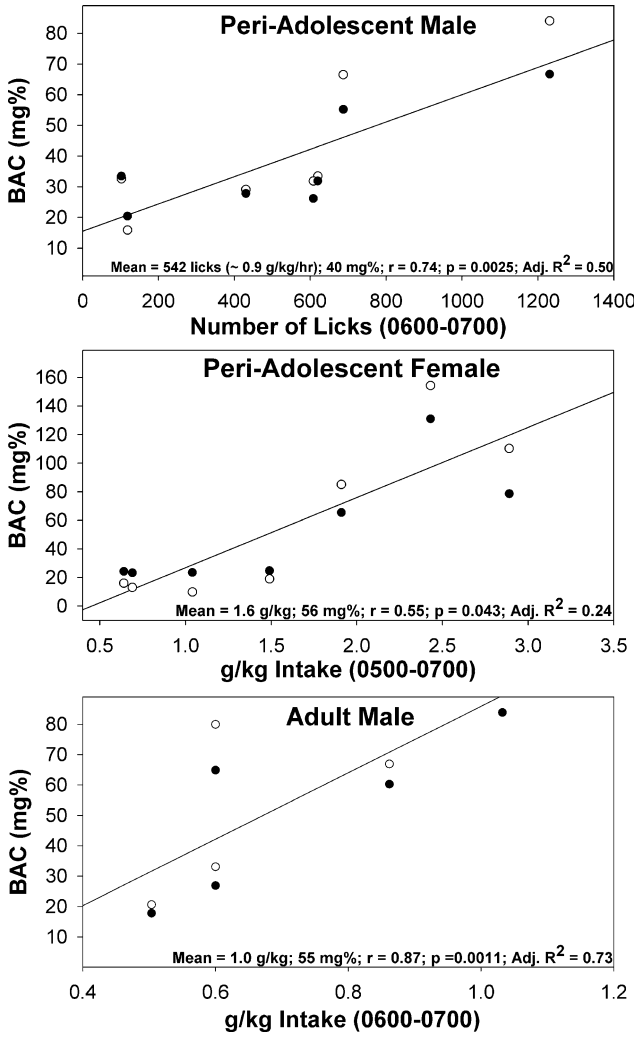


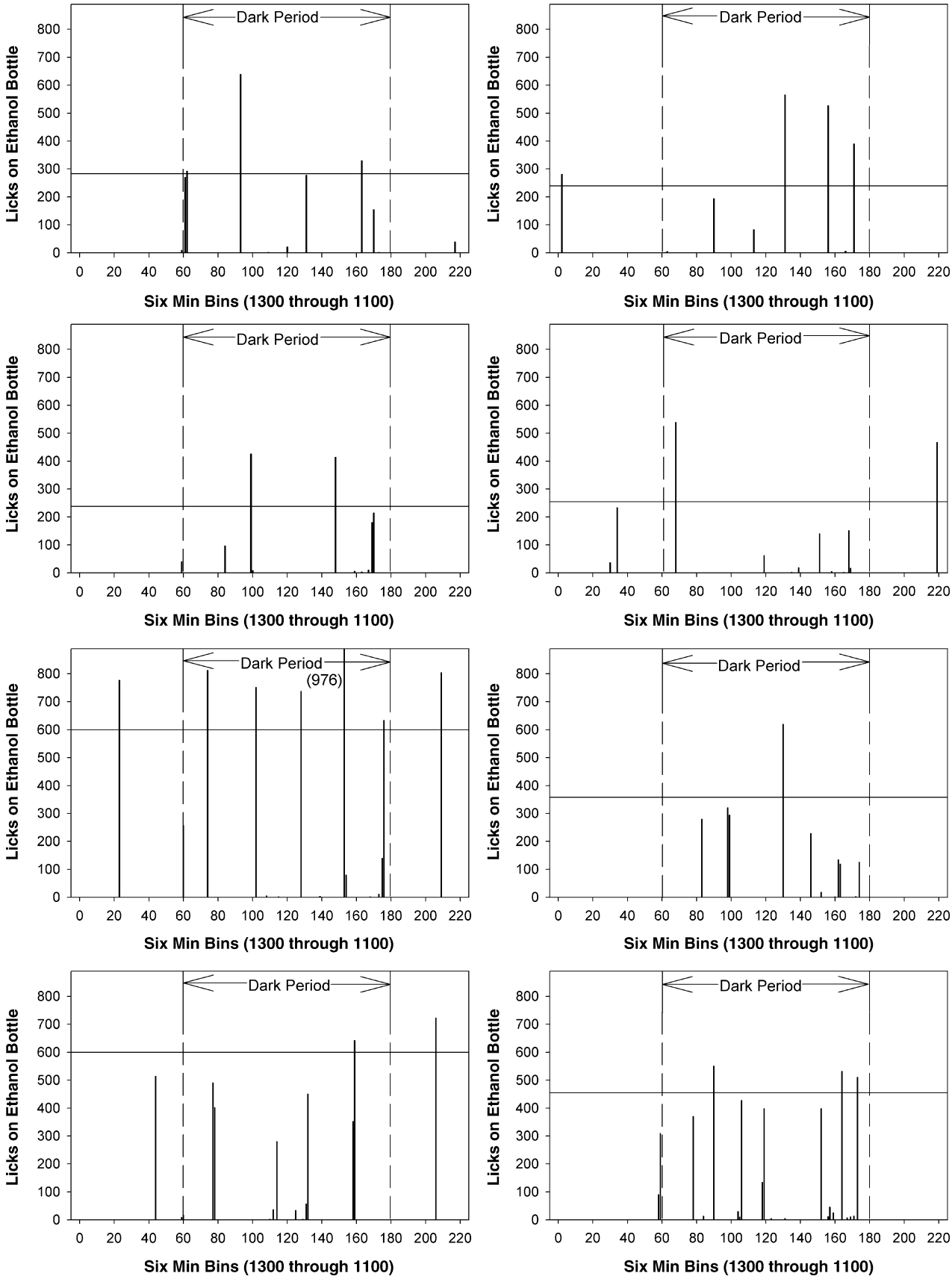
Fig. 4. Separate groups of rats were tested for blood ethanol concentration levels (BECs) at the end of the dark cycle on the last (30th) day of ethanol access, with open symbols indicating tail BECs and closed symbols indicating trunk BECs. For peri-adolescent male P rats ($n=7$; top panel), there was a significant association between number of ethanol (15%, v/v) licks, during the last hour of the dark cycle, and BECs. Additionally, there was a significant association between amount of ethanol consumed, during the last 2 h of the dark cycle, and BECs obtained from peri-adolescent female P rats ($n=7$; middle panel); and a significant association between amount of ethanol consumed, during the last hour of the dark cycle, and BECs obtained from adult male P rats ($n=5$; bottom panel).

3.4. Individual ethanol consumption pattern (licks/6 min)

The individual records for ethanol licking behavior indicated that, in general, male P rats increased their licking behavior between the 10th and 30th days of access, with a concomitant increase in the licking requirement to self-administer 1 g/kg/bout of ethanol. Female P rats displayed relatively stable licking behavior between the 10th and 30th days of access, although there appeared to be an aggregation of drinking behavior across time resulting in more discrete bouts on the 30th versus the 10th day of ethanol access (data not shown). For descriptive purposes, individual ethanol licking patterns for 8 adult male P rats on the 30th day of ethanol access are presented in Fig. 5. Three important features are present in these individual records (which were also present for the adolescent male and female and adult female P rats). First, the ethanol licking behavior of male P rats is a heterogeneous phenomenon, such that, despite a common genetically selected phenotype, different rats displayed different patterns of ethanol intake. Some rats limited their ethanol intake to the dark cycle, while others displayed bouts during the light cycle as well. Also, some rats displayed high lick per gram of ethanol intake ratios, whereas other rats displayed much lower lick per gram of ethanol intake ratios. Second, by the 30th day of ethanol access, there was an increase in the number of bouts which would result in the self-administration of 1 g/kg of ethanol, or higher, per bout. Third, by the 30th day of ethanol access, bouts which would result in the self-administration of 1 g/kg of ethanol, or higher, were often taking place in time spans of less than 12 min (i.e., the total time spent in consuming ethanol per day was often less than 1 h).

4. Discussion

This is the first published study that has examined 22 h, free-choice, home-cage ethanol and water consumption patterns of male and female, peri-adolescent and adult rats, with measurable blood ethanol concentrations (BECs) achieved. As hypothesized, these results indicate that adolescent, and adult, P rats display the majority of their drinking behavior during the dark cycle (Fig. 2). Even though rodents are, in general, nocturnal by nature (display most of their eating and drinking, along with other, behaviors during the dark period) the adolescent P rats displayed this behavior to a greater extent than the adult P rats. This age effect may be due, at least in part, to the fact that juvenile wild rats are just starting to explore the world outside of the nest at this developmental stage (i.e., adolescence: Galef, 1981), and the rats in the present study may, at some innate level, associate the dark cycle with the safety of the nest and the light cycle with the hazards of the world outside the nest. However, when water licking activity was examined, the relationship of adolescent animals displaying most of their licking activity during the dark cycle to a greater extent than adult animals only held true for female P rats; both adolescent and adult male P rats displayed most of their water consumption during the dark cycle (Fig. 3).


Dark Period
Licks on Ethanol Bottle
Six Min Bins (1300 through 1100)
Dark Period
(976)

There were also sex of animal effects on the amount of ethanol licking activity during the light cycle, with female rats displaying significantly more ethanol licking behavior than male P rats. Additionally, adult male P rats displayed significantly greater ethanol licking activity than adult female P rats, such that the adult male P rats displayed approximately three times the activity of the adult female P rats (Fig. 2). The primary reason for this difference appears to be related to the animals' size, such that male rats need to lick more often to obtain the same amount of ethanol (g/kg) as female rats. It is probable that the ratio of male versus female tongue surface area is much lower than the ratio of male versus female body mass. This is supported by the observation that the estimated number of licks required to consume 1 g/kg of ethanol (total number of licks per day divided by total intake in g/kg/day) increased dramatically across the 30 days of ethanol access in both adolescent and adult male P rats, whereas the increase was only marginal in adolescent and adult female P rats over the same time period (data not shown). However, this latter point suggests that body weight may not be the only factor increasing licking activity in male P rats, compared with female P rats. For instance, it appears that adolescent female P rats had sufficient weight gain across weeks (Fig. 1, lower left panel) to have elicited an increase in the licking requirement for 1 g/kg self-administration. However, they did not display this increase (data not shown). Regarding water intake, there was a similar positive relationship between body weight and licking activity for water (Fig. 3), which provides some support for a difference in body mass explanation for these sex differences. However, the extent of the support for this sex difference in body mass explanation is limited, because, rather than drinking the same amount of water, female P rats drank significantly more water (ml/kg) than male P rats throughout the experiment (Fig. 1, upper right panel).

Nevertheless, with the observed levels of ethanol intake in the present study (>6 g/kg/day) and the fact that the majority of the ethanol drinking took place in relatively discrete bouts during the dark cycle, the adolescent P rat probably achieved appreciable BECs at some time point(s) during the dark cycle. The regression analyses suggest that the lickometer data were, in general, representative of the drinking data, because significant associations were found between both lickometer data and BECs achieved and drinking data and BECs achieved (Fig. 4). The fact that appreciable BECs were achieved under free-choice self-administration conditions, when measured at the end of the dark cycle, suggests that the peri-adolescent and adult P rats were consuming ethanol, at least in part, for its pharmacological effects. The present study indicated mean BECs of 40 to 55 mg% were present at the end of the dark cycle. However, this single time point (end of the dark cycle) may not accurately reflect peak BECs achieved during free-choice ethanol drinking by P rats.

As in a previous study (Bell et al., 2003), the peri-adolescent male and female P rats did not display a strong preference for ethanol over water (Fig. 1). This may have stemmed from the fact that adolescent animals are, in general, hyperphagic (Spear, 2000) and by extension polydipsic (e.g., Nance, 1983). This polydipsia undoubtedly is due to the tremendous growth spurt seen during adolescence. This hypothesis is supported in part by the increase in preference for ethanol, with a parallel decrease in water intake, across weeks of access (Fig. 1). Additionally, the peri-adolescent and adult female P rats displayed similar ethanol preference ratios (~40%) during the 3rd and 4th weeks of access (Fig. 1). This may be due, at least in part, to the fact that a 15% ethanol solution was used in the present study, which "artificially" reduced the ethanol preference ratio. The P rat was selectively bred for ethanol preference over water using a 10% ethanol solution vs. water (c.f., Murphy et al., 2002).

Previous work examining adult male P rats under 23 h/day operant conditions revealed that the majority of ethanol self-administration occurred during the dark-cycle and was not associated with feeding periods (Files et al., 1992, 1994). Additionally, the primary mechanism of regulating ethanol intake by P rats was through changing the number of bouts rather than the size of the bouts (Files et al., 1993a, b). However, in the former study (Files et al., 1993a) a reduction in ethanol reinforcement schedule resulted in increases in bout size with no change in bout number per day. As indicated above, a bout is defined as a "cluster" of drinking/licking behavior that is sufficiently aggregated such that the organism must stop ongoing behavior in order to carry out this "cluster" of drinking/licking behavior. Furthermore, in the present experiment, for a period of drinking to have constituted a bout, no breaks in licking activity could have exceeded 30 s (the vast majority of these breaks were less than 18 s). Findings from the present study indicate that P rats under free-access, home-cage drinking conditions alter both bout size and number of bouts during the acquisition and maintenance of free-choice ethanol consumption. In a study using adult male Long–Evans hooded rats, an operant requirement, as little as fixed-ratio 1, decreased total ethanol intake per day, compared with home-cage drinking (Samson et al., 1992). It is probable that this effect is true for the P line of rat as well. For instance, Files et al. (1998) reported that adult male P rats consumed less than 4 g/kg/day under continuous access operant conditions. Therefore, a direct comparison between operant and home-cage self-administration of ethanol is tentative at best. Regarding the existing literature on 24 h (12 h dark / 12 h light) water intake in heterogeneous stock rats, it is clear that adult male Long–Evans hooded (Kutscher and Wright, 1977; Rosenwasser et al.,

Fig. 5. For descriptive purposes, individual ethanol licking patterns for 8 adult male P rats on the 30th day of ethanol access are presented. It is noteworthy that the ethanol licking behavior of male P rats is a heterogeneous phenomenon, with different rats displaying different patterns of drinking. By the 30th day of ethanol access, adult male P rats were self-administering an estimated 1 g/kg body weight of ethanol in bouts taking less than 12 min to complete, with the total time spent consuming ethanol per day being less than 1 h for many animals. The horizontal lines indicate the estimated number of licks required to self-administer 1 g/kg/bout of ethanol [total licks per day / total ethanol (g/kg) consumed per day].

1983), adult male Wistar (Greenwood et al., 1980; Mori et al., 1983; Spiteri, 1982) and adult male Sprague–Dawley (Zucker, 1971) rats consume most of their fluids in bouts at the beginning, middle and end of the dark cycle, with fewer bouts occurring during the light cycle.

Very few studies have examined daily patterns of free-choice ethanol self-administration in adult female rats. In one study, using Long–Evans hooded rats, it was found that there were subtle differences across estrous cycle phases, such that there were decreases in bout frequency (largest difference being 1.96 bouts per day) and increases in bout size (largest difference being 0.07 g/kg/bout) between estrus and first diestrus, as compared with proestrus (Ford et al., 2002). However, total ethanol intake and ethanol licking rate did not differ. One drawback of the present study was that estrous cycle phases were not monitored. A review of the individual drinking data from the female P rats did not reveal detectable changes between animals, across days. In addition, male rats were being run at the same time, on the opposite side of the hanging stainless steel cage rack, suggesting that the female rats had freely cycling phases and that group effects of estrous cycle phase would be difficult to detect due to averaging. Regarding adolescent rats, there have been no published reports on daily patterns of free-choice ethanol self-administration. Therefore, the primary comparisons, with the existing literature, are limited to the adult male P rat. As noted above, direct comparisons between operant and home-cage free-choice self-administration of ethanol must be considered with caution. From studies using adult male outbred rats, it was reported that, under free-choice conditions, the majority of the ethanol self-administered was done so in discrete bouts during the dark-cycle, which suggested the rats were self-administering ethanol, at least in part, for its pharmacological effects (Gill et al., 1986, 1996). Similarly, the present findings replicated the work of Murphy et al. (1986) indicating adult male P rats consume the majority of their ethanol in discrete bouts at the beginning, middle and end of the dark cycle (Figs. 2 and 5). Likewise, P rats displayed most of their water consumption during the dark cycle, with bouts at the beginning, middle and end, as well. An interesting observation from the present findings is the fact that adult female P rats displayed robust bouts of water consumption during the light cycle, whereas peri-adolescent male and female and adult male P rats did not display this phenomenon. It is unfortunate that the limited literature, on patterns of intake for female rats, does not provide sufficient information to indicate whether or not this effect holds true for heterogeneous stock rats as well.

In conclusion, the present findings revealed effects of age and sex of animal on preference ratio for ethanol, water intake and the circadian licking activity for both ethanol and water. In general, peri-adolescent P rats consumed more water and fluids (per kg body weight) than adult P rats and female P rats consumed more water and fluids (per kg body weight) than male P rats. Additionally, adult male P rats displayed substantially more licking behavior than peri-adolescent male and both peri-adolescent and adult female P rats, whereas there was only a modest difference between peri-adolescent and adult female P rats. Also, the individual ethanol licking records indicated that the ethanol licking behavior of adult male P rats differs across animals, despite their sharing a common genetically selected phenotype of ethanol preference over water. Both male and female, peri-adolescent and adult P rats consumed ethanol in discrete bouts with the number of licks displayed often exceeding that required to self-administer 1 g/kg of ethanol per bout. Furthermore, these bouts often occurred in time spans of less than 12 min, with total ethanol consumption time per day being less than 1 h. Given the above, it is expected that P rats regularly achieved significant BECs during free-choice drinking, with this conjecture supported by the detection of appreciable BECs at the end of the dark cycle on the 30th day of ethanol access. The fact that male and female P rats displayed different ethanol licking behavior in general and that peri-adolescent and adult male P rats displayed different ethanol licking behavior across time (weeks), suggest that an intervention to reduce ethanol intake may have differential efficacy between peri-adolescent and adult as well as between male and female P rats, as has been suggested for alcoholics in the clinical literature (e.g., Epstein et al., 1995; Kiefer et al., 2005; Meyerhoff et al., 2004; Rubin et al., 1996; Rubio et al., 2005). This would indicate that preclinical studies examining the effectiveness of pharmacological treatments for alcoholism and alcohol abuse should be interpreted in light of the possibility that age- and sex-of-animal-dependent differences may occur.

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