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VOLUNTARY BINGE CONSUMPTION OF ETHANOL IN A SWEETENED, CHOCOLATE-FLAVORED SOLUTION BY MALE AND FEMALE ADOLESCENT SPRAGUE-DAWLEY RATS

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Abstract

Background—The still maturing adolescent brain may be particularly vulnerable to lasting consequences of ethanol exposure. Yet, human adolescents are the age group most likely to engage in binge drinking (a pattern of drinking leading to blood ethanol concentrations (BECs) of 80 mg/dl or greater). Most studies to date assessing the long-term effects of adolescent ethanol exposure in outbred rodent populations have either used experimenter-administered ethanol to produce BECs in the binge range or assessed voluntary intake of ethanol at well below binge levels. Beginning with a modified schedule-induced polydipsia (SIP) procedure, this study examined the suitability of several approaches to induce voluntary binge-like consumption during adolescence in an outbred rat strain.

Methods—Adolescent male and female Sprague-Dawley rats were food deprived to 85% projected free-feeding weights beginning on postnatal day (P) 24, and were given 30 minutes of access to 10% ethanol in chocolate Boost® or Boost® alone daily from P28 to P41 (followed later by their daily allocation of food). Animals were tested within operant chambers (Exp.1a,1b,2) or home and novel cages (Exp.3). Animals received either scheduled delivery of banana pellets to examine schedule-induced polydipsia (Exp.1a,b) or massed pellet presentation (Exp. 2,3). Blood samples were collected via the lateral tail vein on P33 and P41.

Results—Intakes produced BECs frequently in the binge range (> 80mg/dl) and modeled binge-like consumption patterns, with high consumption days typically followed by 1–2 days of lower consumption; this variability was less evident with Boost® alone. Consumption was not schedule-induced, and was generally high across all studies, although consumption in males appeared to be particularly pronounced when animals were tested in the presence of their cage mate.

Conclusions—Binge-like patterns of ethanol consumption were produced using these procedures in adolescent Sprague-Dawley rats of both sexes, and may prove to be a useful model for work examining the short- and long-term consequences of high levels of voluntary ethanol intake in adolescence.

Abstract

Rates of binge drinking (intake producing blood ethanol concentrations of 80 mg/dl) is highest during adolescence in humans. This study assessed approaches to model voluntary binge-like

consumption in adolescent outbred rats. Using 30 minute daily, one-bottle sessions of 10% ethanol in Boost®, food-restricted animals of both sexes exhibited binge-like consumption patterns, with days of binge-level intake alternating with lower intake days within animals. This model has utility for examining contributors to and consequences of voluntary binge-like drinking in adolescence.

Keywords

Adolescent; Ethanol; Voluntary binge consumption; Sprague-Dawley; Schedule-induced polydipsia

1. Introduction

The onset of alcohol consumption in the United States often occurs during adolescence, and this demographic is the most likely to engage in binge drinking. The most recent National Survey on Drug Use and Health (NSDUH) found that one in nine adolescents aged 12 to 17 was a current alcohol consumer (10.8% of males and 12.3% of females), having drank alcohol within the past thirty days. In young adults aged 18 to 25, this proportion rose to three of five individuals surveyed (61.6% of males and 57.6% of females) (SAMHSA, 2014). Some of this consumption reaches high levels, classified as binge drinking, defined by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) as a pattern of drinking that induces blood alcohol concentration (BAC) levels of at least 0.08 g/dL. Among adults, these levels are typically attained by men consuming five drinks (or women, four drinks) in the span of approximately two hours (NIAAA, 2004). In the 2014 NSDUH survey, 60.6% of current underage alcohol users 10 to 20 years old reported consuming at least five drinks within the same occasion in the past month, with 1 in 7 engaging in five or more separate binge drinking episodes in that time frame. Among young adult alcohol drinkers ages 18 to 25, 37.7% were binge drinkers (43.6% of males and 31.8% of females), a percentage that dropped to 22.5% in adults aged 26 and older (30.7% of males and 15.1% of females) (SAMHSA, 2014). Within both age ranges, rates of binge drinking were greater in males (43.6% and 30.7%, respectively) than in females (31.8%; 15.1%).

These data are troubling because adolescence is a period of notable neural transformation that may render the brain age-dependently vulnerable to long-term detrimental consequences

from alcohol and other drug use (Chambers et al., 2003; Crews et al., 2007). Indeed, a number of human studies have reported a variety of changes in neurocognitive function associated with adolescent ethanol use, including impaired cognitive functioning (particularly on attention, verbal learning, and memory tasks), irregular developmental trajectories of grey and white matter, poor white matter integrity, and attenuated activation of the frontal and parietal regions during working memory and inhibition tasks (Squeglia and Gray, 2016). Longitudinal studies are beginning to disentangle the extent to which these alterations may predate alcohol use (and hence serve as a risk factor for use) or are a consequence of that use (e.g., Squeglia et al., 2015). However, the accumulation of data within longitudinal designs in human studies is slow, and has been valuably complemented by studies of the consequences of adolescent ethanol exposure using laboratory animals.

Studies using rodent models of adolescence have shown robust and lasting consequences following repeated intermittent ethanol exposure during this time period. Such alterations include: disrupted cognitive flexibility (Vetreno et al., 2014; O'Tousa et al., 2013), impaired fear conditioning (Broadwater and Spear, 2013), attenuated sensitivity to ethanol-induced sedation (Silveri & Spear, 1998) and motor discoordination (White et al., 2002), and withdrawal-related anxiety (Van Skike et al., 2015), along with notable reductions in neurons expressing choline acetyltransferase (Vetreno et al., 2014) and lasting disruptions in hippocampal neurogenesis (Crews et al., 2006). Most of these studies have used experimenter-administered ethanol via intragastric intubation, vapor inhalation, or intraperitoneal injection routes that produce peak BECs well into the binge range (e.g., 150–200 mg/dl) that are comparable to BECs seen in field studies of human adolescents (e.g., Day et al., 2013).

There are, however, important distinctions to be made between these studies and binge use of ethanol by human adolescents. Binge drinkers often consume ethanol over a period of time (e.g., pre-partying; LaBrie & Pedersen, 2008) in contrast to instantaneously receiving a bolus containing the full amount as when ethanol is given via injection or intubation. When ethanol is orally consumed, it undergoes absorption through the gastrointestinal tract (unlike injections or vapor). Moreover, alcohol beverages consumed by human adolescents often contain pleasant flavorants that are caloric (e.g., Fortunato et al., 2014; Alberts et al., 2015); these adjuncts can influence ethanol clearance and are not present with injections, vapor administration, nor typically intubations. Many of these differences could be addressed through the use of models of voluntary ethanol consumption at binge levels in laboratory animals. Unfortunately, studies in rodents involving voluntary ethanol administration during adolescence are rare, and the intake levels are typically well below binge levels. These existing voluntary consumption models often involve lengthy access periods, such as 24 hour home cage voluntary access, making it difficult to determine when or whether pharmacologically relevant BECs are reached. Animals are often kept singly housed, which is a notable stressor for adolescents, with known long-term effects (see Buwalda et al., 2011, for review of stressors during adolescence, including isolate housing). This work with voluntary consumption during adolescence in outbred rats has, however, revealed a variety of lasting consequences, even at ethanol intake levels well below the binge range (e.g., Clark et al., 2012; Nasrallah et al., 2009, 2011; Schindler et al., 2014). Some voluntary consumption models have produced intakes into the binge range using animals selectively

bred for a high drinking phenotype (e.g., alcohol-preferring P rats, see Bell et al., 2006, 2011, 2014), thereby reducing the genetic diversity of the animals. It would be valuable to develop additional models that could produce binge-like consumption in adolescent rodents that are from outbred strains as well.

Here we report our first steps to create a model of adolescent binge ethanol intake that produces binge-like BEC levels in outbred Sprague-Dawley rats after a short daily limited access period. Our initial strategy was to assess adolescent ethanol intake using schedule-induced polydipsia (SIP) (Falk 1966, 1967), a procedure that has been used to induce excessive alcohol intake in food deprived adult rodents by providing continuous ethanol access during sessions where food access is provided only intermittently (see Ford, 2014, for review). For instance, using moderate food deprivation and scheduled delivery of small food pellets along with unlimited session access first to water and then increasing concentrations of ethanol over multiple weeks, Falk and colleagues ultimately produced BECs averaging 100 mg/dl in adult rats, with peak BECs in the 150–300 mg/dl range (Falk et al., 1972). Despite recent renewed interest in SIP as a model of excessive alcohol consumption (see Ford, 2014, Moreno and Flores, 2012, Ford et al., 2007, 2013), this means for induction of high alcohol consumption has received little, if any, exploration in adolescent animals. Although adolescence is a notably shorter period than the amount of time used to implement SIP by Falk and colleagues, our goal was to modify the SIP procedure by providing ethanol in a highly palatable vehicle solution in an attempt to induce high adolescent ethanol intakes. Although we found this modified SIP procedure to be effective for inducing notable ethanol intakes among adolescent rats in 30 minute daily sessions, we subsequently found that this effect was not schedule-induced and did not even require the presence of food pellets. Under these short daily intake conditions, the adolescents displayed binge-like patterns of intake over days, characterized by high consumption days being followed by several days of lower intake, with such self-imposed cycles of binges being more pronounced when animals were given access to the ethanol/Boost® solution than when given Boost® alone. Thus, these intake procedures permit the study of the lasting consequences of adolescent ethanol exposure following voluntary adolescent ethanol consumption in a binge-like pattern.

2. Methods and Results

2.1. Subjects

Adolescent male and female Sprague-Dawley rats ($n = 6 - 8$ animals per group), bred in our colony at Binghamton University, were used in these experiments. On the day following birth, litters were culled to 8–10 male and female pups, with sex ratios of 4/6 males/females or vice versa maintained whenever possible (but ratios of 3/5 and 4/5 also viewed as acceptable); ratios were not balanced across experiments. Litters were housed with their dams until weaning on postnatal day (P) 21. At weaning, all animals were pair-housed with a same-sex, same-age partner from a different litter to allow cage mates to be placed in the same intake condition without assigning more than one animal per sex to a test condition. Animals were maintained in a temperature controlled (20–22°C) vivarium on a 12-/12-h light/dark cycle (lights on at 7:00 a.m.), with ad libitum access to food (Purina Rat Chow, Lowell, MA) and tap water, unless otherwise noted. From the time of weaning, home cage

water access was provided through ball-bearing sipper tubes, the same tubes used for assessing ethanol intake. All animal maintenance and experimental procedures were conducted in accord with the guidelines established by the National Institutes of Health, using protocols approved by the Binghamton University Institutional Animal Care and Use Committee.

2.2. Food deprivation

In accord with previous studies of schedule-induced polydipsia (SIP), animals were food restricted. Beginning four days prior to the ethanol access period (P24) and continuing throughout the P28 to P41 access period, animals were food restricted to reach and then maintain 85% of their projected free-feeding body weights, using age/weight charts for each sex and standard procedures in our laboratory (see Anderson et al., 2013, for further information). Each day's food allocation was provided beginning 1–3 hours after the daily ethanol intake session, with body weights recorded each day and amounts of food given to each cage adjusted as necessary to ensure body weight trajectories within the target range.

2.3. Test solutions

Access solutions included 10% ethanol in chocolate Boost® (v/v) or chocolate Boost® alone. Prior unpublished data from our lab has shown that adolescent Sprague-Dawley rats readily consume this solution with or without the presence of ethanol in the solution. Water was not available to these otherwise non-water-deprived animals during the short thirty minute daily access periods. Preliminary data (see Results, Exp. 1b) showed that consumption of animals given water alone during the 30 minute access period was low, and would be expected to be even lower if animals were given simultaneous access to both water and the highly palatable solution.

2.4. Blood ethanol determination

On intake days 6 and 14, immediately after the completion of the drinking session, approximately 30µl of blood were collected from each animal via lateral tail vein incision. These days for sample collection were chosen based on unpublished pilot data from our lab showing that by intake day 6, ethanol consumption had generally reached notable levels, with intake tending to decline after day 14.

Immediately following collection, the blood was frozen at –80°C, and later assayed for blood ethanol concentrations (BECs) via headspace gas chromatography, using a Hewlett Packard (HP) 5890 series II Gas Chromatograph (GC) (Wilmington, DE) and standard procedures in our laboratory (e.g., see Ramirez and Spear, 2010). All animals had blood samples taken, regardless of whether their solution contained ethanol or not, to equate experience across animals, although samples from non-ethanol-drinking animals were not analyzed.

Experiment 1a – SIP with an Ethanol/Boost® Solution—This initial experiment explored intake of the Ethanol/Boost® solution under traditional SIP test procedures (Meisch and Thompson, 1972; Falk and Samson, 1975), whereby continuous solution access was provided in the operant chambers during fixed interval (FI) food delivery.

Methods: From P28-P41, adolescents (8 of each sex) were weighed and then placed into individual operant chambers (Med Associates, VT) for a daily 30 minute intake session between 1:00–3:00 p.m., for a total of 14 days. The chambers were sound- and light-attenuated, with a red house light remaining on in each chamber for the entire 30 minutes. Banana-flavored pellets (46mg/pellet) (Bio-Serv, Pleasant Prairie, WI) were dispensed into a trough on an FI 1 minute schedule, one pellet per delivery, with free access to a bottle containing ethanol in chocolate Boost® (10%, v/v). After the access period, animals were promptly removed from the chambers and returned to their home cages. The chambers were cleaned with 4% hydrogen peroxide and allowed to dry before initiating the next set of animals. No more than two squads of animals were tested in the operant chambers daily, with the test order counterbalanced across days. Pre- and post-session solution weights were determined and converted into daily g/kg ethanol intakes.

Results: Across the fourteen intake sessions in Exp. 1a, intakes averaged ~ 3.5 g/kg over 30 minutes, with average BECs approximating binge-like levels of consumption (see Table 1). Mean g/kg ethanol intakes were not significantly different between adolescent males and females, although there was a trend for intakes to be higher in males, as can be seen in Table 1 ($p = 0.08$). Likewise, t-tests revealed no significant difference between males and females in BECs on either sample collection day. BECs were highly correlated with intake ($r = 0.879$, $p < 0.001$).

Daily consumption data for individual animals is shown in Figure 1. From inspection of this figure, it can be seen that intakes for each animal notably varied across days, with animals generally showing a cycle of one day of high intake followed by 1–2 recovery days of lower intake, followed by higher intake again. To assess variation within animals across days, a coefficient of variation (CV) was calculated for each animal as: the standard deviation of each animal's intake across the 14 days, divided by its mean over those days. The CV enables comparison of the degree of variability across all intake sessions among groups of animals varying in mean intakes, via assessing relative proportions of variability. The average CVs for males and females were similar (see insert to Fig. 1), with a t-test revealing no significant difference.

Experiment 1b – SIP with Boost® alone—This experiment was conducted to determine whether similar intake patterns, including variation in intake across days, would be seen in a SIP paradigm using a solution containing no ethanol.

Methods: Identical procedures were used in Experiment 2 as in Exp. 1, except that instead of an ethanol solution, the 8 adolescents of each sex in this study were only given access to the Boost® solution. A small set of 4 adolescents of each sex were also provided with tap water as the only solution available during the operant chamber sessions.

Results: Animals drinking Boost® without ethanol consumed, on average, over twice as much fluid in ml/kg as their ethanol-drinking counterparts did in Exp. 1a (see Table 2). A t-test comparing intake (ml/kg) between males and females revealed no significant effect of sex.

As can be seen in Fig. 2, daily Boost® consumption of individual animals was also somewhat variable over days, reminiscent of the ethanol data in Exp. 1a. Although 1a and 1b were conducted sequentially rather than in parallel, when a 2 (sex) × 2 (solution) exploratory ANOVA was used to compare CVs across the two studies, the analysis yielded a significant effect of solution ($F = 22.926$, $p < 0.001$), with variances significantly greater among Boost®/Ethanol animals than Boost® alone. Thus, the self-imposed variations in intake across days appear less marked in Boost® alone animals than in animals that received ethanol. Animals only given access to water did not consume notable amounts [males: 1.8 (± 0.2) ml; females: 0.9 (± 0.1) ml] and their data was not analyzed further.

Experiment 2 – Massed Presentation—The low levels of water intake under SIP test conditions in Exp. 1b suggested that the abbreviated SIP procedure we used may not have been sufficient to induce polydipsia (at least for water) within the 14 day testing period during adolescence. To determine whether the binge-like ethanol consumption patterns observed in Exp. 1a were schedule-induced, in this experiment all pellets were delivered to the animals at the onset of the test rather than on an FI delivery schedule.

Methods: Eight adolescents (all female) were used for this study. Given that there was no sex difference in ethanol/Boost® intake in Exp. 1a, only females were used here because of their availability at the time the study was conducted. All procedures were identical to Exp. 1a, except that, rather than receiving banana pellets on an FI schedule, the pellets (1400mg total) were provided via massed presentation to determine whether intakes during the SIP sessions in Exp. 1a were a function of the scheduled access to food per se. Each adolescent female was placed into the operant chamber with all banana pellets for the session already accessible in the trough, and consumption during the 30 minute daily access periods was determined as in Exp. 1a.

Results: Massed presentation of banana pellets did not deter ethanol consumption in adolescent females. The average intake over all fourteen drinking sessions was 3.9 (± 0.17) g/kg ethanol, compared to the 3.2 (± 0.17) g/kg intake of SIP females in Exp. 1a. An exploratory t-test revealed this to be a significant difference ($p = 0.01$). Average intake on BEC assessment days was 5.4 (± 0.55) g/kg, resulting in average BECs of 75.7 (± 10.0) mg/dl. The mean CV was 0.46 (± 0.02) in these females, slightly but significantly less than the CV of 0.56 (± 0.04) seen in Exp. 1a females (exploratory t-test: $t = 2.17$, $p = 0.05$). Intakes on BEC assessment days were again highly correlated with BECs ($r = 0.895$, $p < 0.001$) From these data we conclude that the ethanol intakes observed using our operant SIP test procedure were not a function of schedule induction per se.

Experiment 3 – Home Cage vs. Novel Cage—Given the high levels of intake seen during massed presentation in the operant chambers in Exp. 2, this experiment assessed whether animals given massed presentation of banana pellets would consume similar amounts of ethanol in their home cage or a novel cage, rather than in the operant chambers.

Methods: The design of this study was a 2 sex × 2 context (home cage or novel cage) factorial, with 6–8 animals assigned to each group. Animals were food deprived as outlined in Exp. 1a. During the 14 day access period, home cage animals received 30 minute daily

access to the ethanol/Boost® solution (10%, v/v) in their home cage. For these intake sessions, home cages were placed in a dim room with a white noise generator running. Each pair of animals was then separated in their home cage by a wire mesh divider and left to habituate. Fifteen minutes later, each animal was provided with a small plastic container containing 1400 mg of banana pellets, and a bottle containing the ethanol/Boost® solution, using the same bottles and sipper tubes as used in the operant chambers. The ethanol solution and food dish were removed after 30 minutes of access, followed by removal of the mesh divider and return of the animals to the vivarium. Novel cage animals were treated identically, except that they were transferred to a new clean cage daily for the habituation and consumption periods. All animals were given ethanol solution given that the focus of this study was on development of a model of binge ethanol consumption, not on further exploration of consumption of Boost® alone.

Upon return to the vivarium, the initial cohort of animals (six adolescent females in the home cage condition) were assessed for intoxication daily using the Chandler-Crews Intoxication Scale (NADIA Consortium, see <https://www.med.unc.edu/alcohol/nadiaconsortium/standardized-methods>). Each female was observed for 30 seconds for motor behavior impairments, ranging from a score of 1, indicating no impairment, to 5, impairment severe enough to cause loss of reflexes such as the righting reflex and the eye-blink reflex. The remaining test animals (2 female and 6 male home cage drinkers, 6 male and 6 female novel cage drinkers) were assessed for level of intoxication using a daily negative geotaxis test (Ramirez and Spear, 2010), rather than the Chandler-Crews scale. For this test, within 5 minutes of the end of each consumption period, animals were placed upside down on a wire-mesh covered inclined plane (70° angle from horizontal) and the time between being placed on the plane and turning 180 degrees was recorded daily, up to a maximum of 30 seconds. If an animal fell off the apparatus and onto the 15.2cm thick pad of foam below, the maximum time of 30 seconds was assigned. These recordings were compared to a baseline recording collected on the day prior to the start of the consumption period, with the daily post-intake data converted to % change from baseline, calculated as $[(\text{Baseline Time} - \text{Post-intake Time}) / \text{Baseline Time}] \times 100$.

Blood samples were collected following the intake sessions on day 6 and day 14, as in Exp. 1a, to measure BECs.

Results: A 2 sex $\times$ 2 context ANOVA of mean intake revealed a significant effect of sex ($F = 26.929$, $p < 0.001$, with males averaging higher consumptions across all fourteen intake days, but with no effect of test context (see Table 3). A repeated measures ANOVA across the two blood collection days likewise revealed a significant effect of sex on BECs ($F = 16.849$, $p < 0.001$, higher BECs in males), but not context. BECs and intake on blood collection days were again highly correlated ($r = 0.916$, $p < 0.001$). An ANOVA of the CV data revealed significant effects of both sex ($F = 11.124$, $p < 0.005$) and context ($F = 5.816$, $p < 0.05$), with females and novel cage drinkers having more variable intake levels (i.e., higher CVs). The interaction of sex and context on CV was not significant.

The Chandler-Crews scale to assess visible markers of intoxication from 1 (normal activity) to 5 (extremely intoxicated) at the end of each drinking session yielded low ratings (1.3

± 0.07), although these ratings correlated significantly with intake: $r = 0.440$ ($p < 0.001$). These low ratings on the Chandler-Crews scale prompted the decision to employ the negative geotaxis task in the second cohort, as a potentially more accurate measure of degree of intoxication. However, intoxication assessed via % change in negative geotaxis was not significantly correlated with intake (g/kg): $r = 0.129$ ($p = 0.10$). Despite this, analysis of the negative geotaxis data revealed an average % increase from baseline of 87.63% post-consumption.

3. Discussion

Using SIP procedures in moderately-food deprived Sprague-Dawley rats, we found that adolescent males and females provided with scheduled delivery of banana pellets willingly drank notable amounts of 10% ethanol in a palatable solution (Boost®) in short, 30 minute daily access sessions between the ages of P28 to P41. By collecting blood samples twice during the fourteen days of access, we established that this intake was often sufficient to raise BECs into the binge range ($> 80\text{mg/dl}$). Intake of individual animals was observed to occur in a binge-like pattern over days, typically characterized by a high consumption day followed by 1–2 days of drastically lower intake, cycling repeatedly. Such binge-like patterns were less evident in the animals undergoing identical testing procedures with Boost® alone. Surprisingly, when scheduled delivery of banana pellets was replaced with a massed presentation, voluntary consumptions of the ethanol/Boost® solution remained high, suggesting that the intakes were not schedule-dependent per se. Finally, when animals were given access to the ethanol solution under similar massed presentation conditions but in the presence of their cage mate in their home cage or a novel cage, intakes remained elevated, especially among males.

Whatever was driving this voluntary consumption, it does not appear to have been SIP per se. This conclusion is based on two findings. The SIP test procedures that were used led to high voluntary consumption in animals given Boost® with or without ethanol, but did not induce high consumption in animals given only water (Exp. 1b). This finding is inconsistent with prior literature, showing that SIP can be established with water only (Falk, 1961). Furthermore, in Experiment 2 where scheduled delivery was abolished, with animals receiving mass presentation of pellets at the onset of the intake session, high consumption levels were nevertheless maintained. It is likely that the lack of schedule dependency per se may have been a function of the limited number of test days permitted under these test conditions due to the time constraints of adolescence. Indeed, development of SIP in adult animals typically requires at least 10 intake sessions (Ford, 2014), with such tests often continuing over a period of many weeks in the operant chambers, during which the concentration of ethanol is gradually raised (Falk et al., 1972). When working with adolescent animals, increasing the number of testing days equates to testing progressively more mature animals, and hence any emerging SIP-induced increase in intake propensity could be countered by maturity-associated declines in ethanol intake. A 14 day (P28–41) intake period was chosen for these studies because preliminary work had revealed that consumptions declined notably after P41–P42, an ontogenetic time course highly similar to what we have observed in home cage consumption studies (Vetter-O'Hagen et al., 2009).

In Experiment 1b, animals given access to Boost® without ethanol consumed on average more than twice as much fluid (ml/kg) per intake session than their ethanol-drinking counterparts in Experiment 1a. This is likely to be due to the high palatability of chocolate Boost® for adolescent rats, which is diminished by the combination of the bitter/sweet taste properties of ethanol (Di Lorenzo et al., 1986). It is also likely that animals receiving ethanol may have modulated their intake in part due to pharmacological effects of ethanol (including post-clearance consequences). This seems particularly likely given the observed binge-like consumption patterns, with each high ethanol consumption day typically followed by several days of lower consumption. Intakes were also correlated with intoxication using the Chandler-Crews intoxication rating scale. The extent to which other palatable solutions would likewise precipitate high and relatively unvarying intake levels when administered alone, and induce “binge-like” ethanol intakes when used as a vehicle for ethanol is as yet unknown.

The CV enables the comparison of the variability of a measure over time between animals or groups of animals, and was used to demonstrate that the intakes over days of individual animals given access to Boost® with ethanol (Exp. 1a) were more variable than those drinking Boost® alone (Exp. 1b). These findings add credence to the notion that animals crossing the threshold for binge drinking (80 mg/dl), may require several days for recovery (acute withdrawal?) before consuming large amounts of ethanol again, whereas the animals consuming the Boost® solution alone are not subject to such constraints and can maintain more consistent intakes across the fourteen days of access. Although not used in the formal definition of binge use, binge-like consumption in adolescents typically follows a pattern of high levels of drinking followed by days of little consumption (e.g., binge drinking on weekends) (Reich et al, 2015). It is interesting that the intake of our animals showed a similar variable pattern under circumstances when free access was given daily, suggesting that the variability in binge pattern of use over day in human adolescents may not merely be opportunistic, but could reflect in part acute withdrawal and recovery. This pattern of self-administration may not be visible in binge drinking models that do not give daily solution access. Nevertheless, a cyclical pattern of intake may still be revealed: when using a home cage access model where adolescent CD rats were given 24 hours of free access to ethanol on Mondays, Wednesdays, and Fridays, adolescents showed enhanced intake on Mondays after a two day abstinence period, relative to their intake following 24 hours of abstinence on Wednesdays and Fridays, a pattern that was not evident in adults (Schramm-Sapota et al, 2014).

In Experiment 3, where the intake of animals was tested in the presence of their cage mates outside of the operant chamber, significant sex differences in intakes and BECs were apparent. Whereas in Exp. 1a there was only a trend for males to consume more of the ethanol-Boost® solution than females ($p = 0.08$), in Exp. 3 this difference reached significance. Although caution is always required when making comparisons across experiments, it nevertheless was interesting that males in Exp.3 consumed, on average, 1.2 g/kg more per session than males in Exp. 1a, with consumptions similar across experiments in females. The females in Exp. 3 also had particularly low BECs relative to Exp. 1a females; this difference, however, was likely due merely to the fact that Exp. 3 females consumed, on average, 0.8 g/kg less on the two blood collections days (2.7 ± 0.4 g/kg) than

did Exp. 1a females (3.5 ± 0.4), despite having consumed ethanol equivalently over the fourteen intake days overall. Given the notable differences in ethanol intake across days for individual animals, BEC assessment days with overall low intakes for a given group are not unexpected, though unfortunate. There was no main effect or interaction of drinking context in Exp.3, perhaps due to the novel cage not being sufficiently 'novel'. While the cages used were clean and contained new shavings every day, they were identical to the home cage, and hence were something that the animals had experienced previously when holding room cages were exchanged weekly. It should be noted that the sound- and light-attenuated operant chambers used in Exp.1 and 2 created social isolation during the access period, whereas Exp. 3 animals could see, hear, smell, and perhaps touch their cage mate across the wire mesh divider. These social circumstances of the novel/home cage tests may have contributed to the apparent elevated drinking of males in Exp.3, relative to their predecessors tested in operant chambers in Exp. 1a and 2. Prior work in our laboratory has shown that adolescent male (but not female) rats voluntarily consume ethanol for its socially facilitating and rewarding effects (Varlinskaya et al., 2015).

Just like any other, this model does come with several limitations. The most obvious of these is that animals were food restricted, albeit at a modest level (85% of projected free-feeding bodyweights) that allowed animals a normal growth trajectory throughout adolescence. Animals were food restricted for SIP testing in that such restriction has been shown to be necessary for the development and expression of SIP (see Ford, 2014). Such restriction was then maintained throughout the remainder of these experiments in an attempt to preserve high consumption levels while examining the effects of manipulating other variables on this intake. Food deprivation has been used extensively in prior research to encourage ethanol consumption, perhaps in part because it may counter intrinsic neophobia (e.g., Meisch and Thompson, 1971; Roehr and Samson, 1981). The stress of the restriction also likely contributes to elevated drinking, with blockade of restriction-induced increases in levels of the stress-related hormone, corticosterone, attenuating restriction-induced increases in ethanol intake (Hansen et al., 1995). Reminiscent of the literature with adult rats, in other work in our laboratory (Hosová & Spear, in progress) we have likewise observed that ethanol consumption seen in adolescent rats under these access conditions is notably enhanced by modest food restriction. A second limitation of the "binge-like" consumption model used here is that, due to the inherently variable nature of voluntary consumption, it can be difficult to precisely measure peak BECs, as it is not clear at which point exactly the animals drank. However, this issue is much attenuated by the short access period; a window of 30 minutes with blood collected immediately thereafter allows assessment of near peak BECs far better than a design that involves longer periods of solution access. Indeed, we found strong correlations between intakes and BECs across all experiments in this study. Nevertheless, it is possible that the BEC data we obtained could slightly under-represent peak BECs obtained under these conditions. Use of monitoring equipment to assess the precise timing of intake bouts could be used in future work to more precisely assess times between drinking and blood collection. A final potential limitation of this approach is also one of the strengths of this work. The high levels of voluntary ethanol intake and variability over days seen among the adolescent animals are important attributes of this model of binge-like patterns of intake. However, it is recognized that similarly high and variable intakes may

not be evident in other aged animals, perhaps limiting the assessment of age differences in the lasting consequences of the voluntary intake induced by using this model.

Despite these limitations, this work provides an important first step in validating an adolescent model of voluntary binge-like ethanol consumption. First, we established that classic SIP procedures can be used to induce high consumption in male and female adolescents in a short, 30 minute access period. Next, we demonstrated that this model does not depend on scheduled delivery of the food reward. Finally, we investigated whether this consumption is driven by the novel, socially-isolating environment of the operant chamber, and found particularly high consumption levels by males in the presence of a cage mate, while not impacting that of the females. This model produces voluntary binge-like ethanol consumption in short, daily home cage access periods among socially housed adolescents from an outbred rat strain, thus providing a novel model for assessing potential lasting neurobehavioral consequences of binge-like patterns of ethanol use during adolescence.

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Figure 1.

a. Individual voluntary intakes of ethanol (g/kg) by males in thirty minute daily drinking sessions inside the operant chamber. Every point on a line represents the amount consumed by a single male on that postnatal day from P28-P41. Between-group analyses were conducted using mean intakes. “↑” reflects days of tail vein blood collection for blood ethanol concentration (BEC) assessment. ‘CV’: coefficient of variance (standard deviation / mean). The dotted black line reflects average daily intake by all males on each postnatal day.

b. Individual voluntary intakes of ethanol (g/kg) by females in thirty minute daily drinking sessions inside the operant chamber. Every point on a line represents the amount consumed by a single female on that postnatal day from P28-P41. Between-group analyses were conducted using mean intakes. “↑” reflects days of tail vein blood collection for blood ethanol concentration (BEC) assessment. ‘CV’: coefficient of variance (standard deviation / mean). The dotted black line reflects average daily intake by all females on each postnatal day.

Figure 2.

a. Individual voluntary intakes of Boost® (ml/kg) by males in thirty minute daily drinking sessions inside the operant chamber. Every point on a line represents the amount consumed by a single male on that postnatal day from P28-P41. Between-group analyses were conducted using mean intakes. “↑” reflects days of tail vein blood collection; blood was collected, but discarded without analysis. ‘CV’: coefficient of variance (standard deviation / mean). The dotted black line reflects average daily intake by all males on each postnatal day.

b. Individual voluntary intakes of Boost® (ml/kg) by females in thirty minute daily drinking sessions inside the operant chamber. Every point on a line represents the amount consumed by a single male on that postnatal day from P28-P41. Between-group analyses were conducted using mean intakes. “↑” reflects days of tail vein blood collection; blood was collected, but discarded without analysis. ‘CV’: coefficient of variance (standard deviation / mean). The dotted black line reflects average daily intake by all females on each postnatal day.

Table 1

Average blood ethanol concentrations (BECs) and intakes in Experiment 1a.

	Average BEC (mg/dl)	Average Ethanol Intake on BEC Assessment Days (g/kg)	Average Intake Overall (g/kg)
MALES	86.2 ($\pm$ 12.7)	4.1 ($\pm$ 0.41)	3.7 ($\pm$ 0.18)
FEMALES	74.8 ($\pm$ 10.1)	3.5 ($\pm$ 0.44)	3.2 ($\pm$ 0.17)

Data represented as mean ($\pm$ SEM).

Table 2

Average consumption (ml/kg) of Boost® with and without ethanol in Experiments 1a and 1b.

	Exp. 1a (Boost® + ethanol)	Exp. 1b (Boost®)
MALES	50.1 ($\pm$ 2.5)	126.4 ($\pm$ 6.2)
FEMALES	43.2 ($\pm$ 2.5)	112.3 ($\pm$ 6.6)

Data represented as mean ($\pm$ SEM).

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Average blood ethanol concentrations (BECs) and intakes of adolescents drinking in a home or novel cage in Exp. 3.

Table 3

	Test Context	Average BEC (mg/dl)	Average Intake on BEC Days (g/kg)	Correlation (r)	Average Intake Overall (g/kg)	Average CV
<i>MALES</i>	HOME CAGE	87.0 ± 13.6	5.4 ± 0.6	0.880 (p < 0.001)	4.7 ± 0.2	0.47 ± 0.05
	NOVEL CAGE	99.1 ± 24.5	4.2 ± 1.0	0.990 (p < 0.001)	4.6 ± 0.3	0.59 ± 0.11
<i>FEMALES</i>	HOME CAGE	38.6 ± 7.0	3.2 ± 0.4	0.896 (p < 0.001)	3.3 ± 0.2	0.62 ± 0.09
	NOVEL CAGE	25.8 ± 11.6	2.0 ± 0.8	0.957 (p < 0.001)	3.0 ± 0.3	0.92 ± 0.11

Data represented as mean (± SEM). 'Correlation' refers to the correlation between BECs and the average intake (g/kg) on blood collection days. 'CV': coefficient of variance (standard deviation / mean).