The effects of repeated forced ethanol consumption during adolescence on reproductive behaviors in male rats

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Abstract

Adolescence is a sensitive period of brain development when changes in hormone levels may have long-lasting effects on synaptic connections and behavior. In humans, alcohol consumption frequently begins during this critical period, although the impact of early exposure has not been fully examined. The current study was designed to investigate short- and long-term effects of repeated forced ethanol consumption during adolescence on emerging reproductive behaviors. Twenty-six young male Long-Evans rats were assigned to ethanol (Young EtOH, n = 12) or water (Young Control, n = 14) groups at postnatal day (P) 32, receiving a modified binge protocol of 3 g/kg of solution via gavage twice per week from P32 to P80. For comparison, another cohort of rats received a similar treatment paradigm in adulthood from P75 – P133 (Adult EtOH, n = 8; Adult Control, n = 10). Reproductive behavior was assessed with tests for copulation, partner preference, and 50-kHz vocalizations during forced consumption (intoxication) and again after a 4–5 week period of abstinence. During forced consumption, the Young EtOH group showed significantly longer latencies on copulation tests than Young Controls, but these differences did not persist after abstinence. Different patterns were observed in Adult animals, who only showed significant, delayed impairments in the post-ejaculatory interval. Preference for sexually receptive females increased with sexual experience in both adolescent and adult rats, regardless of treatment during the forced consumption phase. However, after abstinence, the Young EtOH group showed a significantly reduced partner preference compared to the Young Control group, which may indicate long-term effects on sexual motivation. Additionally, during forced consumption the Young EtOH group tended to emit fewer ultrasonic vocalizations, perhaps reflecting impairments in sexual communication. Adult groups showed no differences in partner preference or vocalization tests at any time. Taken together, these findings indicate that repeated, intermittent ethanol exposure may have moderate effects on reproductive behavior that vary as a function of age. After abstinence, differences were only observed in the younger group, suggesting that the adolescent brain and behavior are more sensitive to ethanol exposure than the adult brain for sexual motivation and performance.

1. Introduction

Alcohol consumption in adolescents and young adults is extremely common, even when preventive laws and strict legal penalties exist to discourage it. In the United States, approximately 72% of 12th graders and 83% of college students report some experience with drinking (Johnston, O'Malley, Bachman, & Schulenberg, 2008). Heavy consumption is also common; roughly 45% of high school students indicate that they have consumed alcohol in a binge pattern (5 or more drinks in a row) in the previous 30 days (Miller, Naimi, Brewer, & Jones, 2007). Binge drinking is particularly problematic because it is associated with an increased risk of motor vehicle accidents, violent behaviors, suicide, and risky sexual behaviors, including multiple sex partners and unprotected sex (Calvert, Keenan Bucholz, & Steger-May 2010; Santelli, Robin, Brener, & Lowry, 2001; Stueve & O’Donnell, 2005).

Adolescence is also a critical period of development for both brain and behavior, when significant escalations in steroid hormones, and dramatic changes in cortical gray matter, synaptic connectivity, and myelination occur (Romeo, Richardson, & Sisk, 2002; Sisk & Foster, 2004; Sisk & Zehr, 2005). In normally developing males, adolescence is characterized by substantial increases
behavior tests for studying reproduction in rats are well established (Vagell, 2008). Some separate assays have been developed to measure sexual performance, as P30 to P50 (Tirelli, Laviola, et al., 2003) and sexual communication, partner preference, and ultrasonic (50 kHz) vocalizations in emerging male reproductive behaviors remain largely unexplored. The effects of binge drinking initiated in adolescence on emerging male reproductive behaviors remain largely unexplored. Animal models are especially useful in this regard because human studies have inherent limitations, including reliance on retrospective self-reports, expectancy effects, and special ethical considerations that occur when dealing with underage populations (Crowe & George, 1989; Frohmader, Pitches, Balfour, & Coolen, 2010; Witt, 1994). In rodents, the timing of adolescence is relatively short, occurring between weaning (around postnatal day [P] 21) and reproductive maturity (P59), with some defining it more narrowly as P40–50 (Tirelli, Laviola, & Adriani, 2009). Additionally, behavior tests for studying reproduction in rats are well established (Everitt, 1990; Meisel & Sachs, 1994; Vagell & McGinnis, 1998), and separate assays have been developed to measure sexual performance (Harding & Velotta, 2011; Meisel & Sachs, 1994; Vagell & McGinnis, 1998), sexual motivation (Everitt, 1990; Harding & Velotta, 2011; Pfaus, Kippin, & Coria-Avila, 2003) and sexual communication (Vagell & McGinnis, 1998; Harding & Velotta, 2011).

The current study was designed to investigate the effects of repeated ethanol exposure during adolescence and young adulthood on male reproductive behaviors in rats by measuring copulation, partner preference, and ultrasonic (50 kHz) vocalizations in response to sexually specific stimuli 1) when under the influence of alcohol and 2) again after a period of abstinence. We used gavage to control the amount of ethanol exposure, modifying the binge paradigm used in earlier studies (Lauing et al., 2008), where a binge cycle was defined as a binge dose (3 g/kg) administered intraperitoneally (i.p.) for 3 consecutive days, followed by 4 days off, for a period of 4 weeks. In the present study, ethanol was administered via gavage to approximate drinking, and binge doses (3 g/kg) were given for 2 consecutive days with 5 days off after a period of 8 weeks to maximize exposure throughout the adolescent period. Ethyl alcohol (Fisher Scientific) or water was administered via gavage beginning at P32 (Young groups) or P75 (Adult groups) using a syringe and a ball-tipped 18 gauge × 3 inch stainless-steel feeding needle (Popper and Sons, Inc.). Behavioral studies began 30–60 min after gavage to correspond with maximum blood alcohol levels (BALs) in both adolescent and adult rats (Przybycien-Szymanska, Rao, & Pak, 2010; Walker & Ehlers, 2009). Although we did not measure blood alcohol levels directly, previous research suggests that the BAL would be approximately 180–200 mg% at this time point (Walker & Ehlers, 2009), exceeding the 80 mg% level in the National Institute on Alcohol Abuse and Alcoholism’s definition of binge drinking (National Institute on Alcohol Abuse and Alcoholism, Winter 2004, p. 3). Animals in the Control groups received similar volumes of distilled water via gavage. The total volume administered during gavage did not exceed 2 mL.

### 3. Procedures

#### 3.1. Drug administration

The dosing protocol was modified from the chronic binge model of Lauing et al. (2008), where a binge cycle was defined as a binge dose (3 g/kg) administered intraperitoneally (i.p.) for 3 consecutive days, followed by 4 days off, for a period of 4 weeks. In the present study, ethanol was administered via gavage to approximate drinking, and binge doses (3 g/kg) were given for 2 consecutive days with 5 days off after a period of 8 weeks to maximize exposure throughout the adolescent period. Ethyl alcohol (Fisher Scientific) or water was administered via gavage beginning at P32 (Young groups) or P75 (Adult groups) using a syringe and a ball-tipped 18 gauge × 3 inch stainless-steel feeding needle (Popper and Sons, Inc.). Behavioral studies began 30–60 min after gavage to correspond with maximum blood alcohol levels (BALs) in both adolescent and adult rats (Przybycien-Szymanska, Rao, & Pak, 2010; Walker & Ehlers, 2009). Although we did not measure blood alcohol levels directly, previous research suggests that the BAL would be approximately 180–200 mg% at this time point (Walker & Ehlers, 2009), exceeding the 80 mg% level in the National Institute on Alcohol Abuse and Alcoholism’s definition of binge drinking (National Institute on Alcohol Abuse and Alcoholism, Winter 2004, p. 3). Animals in the Control groups received similar volumes of distilled water via gavage. The total volume administered during gavage did not exceed 2 mL.

#### 3.2. Experimental design

Young and Adult male rats were handled upon arrival and were assigned to four groups: Young EtOH (ethanol; n = 14), Young Control (water; n = 12), Adult EtOH (n = 8), and Adult Control (n = 10). The first week of forced ethanol consumption began on P32 (Young EtOH group) or P75 (Adult EtOH group), and this was designated as Week 1. Tests for reproductive behaviors were conducted during Weeks 3–8 of gavage, and all rats were sexually naïve at the start of testing. The following parameters were
measured: sexual performance, using copulation tests; sexual motivation, using partner preference tests; and sexual communication, using 50-kHz vocalization tests. (Details about the tests follow.) All behaviors were scored live by two research assistants who were blind to experimental conditions. During the two days “on” ethanol, copulation tests were done on one day each week, while partner preference and vocalization tests were conducted on the other day in alternating weeks. After a completion of 8 binge cycles, rats were allowed 4 weeks of abstinence, and one test for each behavior was conducted to assess long-term effects (Post Tests). Please see Fig. 1 for a timeline of events.

3.3. Bodily measurements

Body weights were taken weekly during the consumption phase to determine ethanol doses and to ensure no differences in size between groups. After completion of behavioral tests, animals were overdosed with chloral hydrate (14% solution, 0.5 mL/100 g body weight, i.p.) and seminal vesicles were removed, expressed, and weighed as an indicator of testosterone levels (Armagan, Kim, Goldstein, & Traish, 2006). Testes weights were also recorded for comparison.

4. Behavioral tests

4.1. Copulation

On the days of testing for copulation, 4–6 hormonally primed stimulus females were used. Males were placed in glass arenas (25 × 20 × 18 cm) with sexually receptive females and were tested as described in previous studies (Harding & Velotta, 2011; Vagell & McGinnis, 1998). Measures of sexual performance, including the latency and frequency of mounts, intromissions, and ejaculations, were recorded. Stimulus females were rotated every 5–7 min to elicit interest from the males. The duration of the test varied based on the behaviors shown. Males were initially allowed up to 15 min to mount the female, and when there was no behavior within the allotted time, mount latency was assigned a value of 900 s. After the first mount, an animal was allowed 30 more minutes to ejaculate. When animals failed to ejaculate within 30 min, the test ended and the ejaculation latency was assigned a value of 1800 s. Otherwise, ejaculation latency was calculated as the time from the first mount until the subsequent ejaculation. The test ended immediately after the first post-ejaculatory behavior, and the post-ejaculatory interval (PEI: time from ejaculation until the next subsequent behavior) was recorded. Only animals that ejaculated were included in the calculation of PEIs (Agmo, 1997). There was a technical issue with data collection during Week 7 of treatment: several of the receptive females were unresponsive. Therefore, data are not shown for that time point, although all males received sexual experience.

4.2. Partner preference

Partner preference tests were conducted in a 3-chambered Plexiglas® apparatus (91 × 62 × 40 cm) lined with clean bedding as described previously (Harding & Velotta, 2011; Vagell & McGinnis, 1998). Chambers were all of equal size, allowing free passage of the male through small openings in the middle compartment. A sexually receptive female was placed behind a circular barrier constructed from chicken wire (diameter = 24 cm) in the right chamber. A non-receptive female was similarly contained in the left compartment, and the middle chamber remained empty throughout testing. Males were placed in the empty compartment, and the time spent in each chamber was monitored with a computer program for 10 min.

4.3. 50-kHz vocalizations

The frequency of ultrasonic (50 kHz) vocalizations was recorded during a 5-min test in a copulation arena lined with bedding collected from sexually receptive females. Vocalizations were quantified using a bat detector (QMC Model S 100 heterodyne receiver; Ultrasound Advice, London, UK) set to 50 kHz and connected to headphones as described before (Harding & Velotta, 2011).

5. Statistics

All data were entered into StatView 5.0 for analysis. During binge cycles, we used 2-way repeated-measures analysis of variance (ANOVA) tests to examine differences in copulation and partner preference tests, followed by Fishers PLSD post hoc tests when significant effects were seen. (In the absence of significant
interactions, planned comparisons were made between EtOH groups and age-matched controls receiving water at individual time points.) Between-group comparisons were made on Post Tests using t-tests. The data collected for ultrasonic vocalization tests were not normally distributed, violating the assumptions for an ANOVA, and therefore nonparametric statistics (Mann-Whitney U and Wilcoxon signed rank) were used to determine between-group and within-group differences.

6. Results

The major findings for copulation tests are depicted in Fig. 2 and Table 1. With regard to ejaculation latency (EL), a 2-way repeated-measures ANOVA revealed main effects of treatment (F [124] = 6.35, p = 0.019) and time (F[1,13] = 4.94, p = 0.004), with no significant interaction (F[3,72] = 0.91, p = 0.438) in the Young group. The Young EtOH group took longer to ejaculate during Week 5 (t[24] = 2.29, p = 0.036) and Week 6 (t[24] = 3.10, p = 0.005) compared to Young Controls. In contrast, in Adult animals, there was no effect of treatment on ejaculation latency (F[1,16] = 0.13, p = 0.725), with no interaction between treatment and time (F [3,48] = 1.29, p = 0.288). During the Post Test, no differences in EL measures were seen in the Young groups; however, a small difference between treatment groups was detected in the Adult animals (t[16] = 1.98, p = 0.065) that did not reach statistical significance.

Post-ejaculatory interval (PEI) data are depicted in Table 1. Because only the males ejaculating were included in data analysis (Agmo, 1997), the numbers in parentheses indicate the number of animals ejaculating on each test. During forced consumption, a 2-way repeated-measures ANOVA revealed a significant effect of treatment in the Young group (F[1,11] = 17.08, p = 0.002), with no effect of time (F[1,3] = 2.15, p = 0.112) and no interaction between treatment and time (F[3,33] = 1.40, p = 0.260). During the Post Test, there was a difference between Young groups for PEI (t[22] = 2.04, p = 0.054), although it did not reach statistical significance. With regard to treatment effects, the Young EtOH group showed significantly reduced behavior after ejaculation (reflected in an increased PEI) during three tests (Week 3: t[14] = 4.37, p < 0.001; Week 4: t [19] = 4.00, p < 0.001; Week 5: t[19] = 2.48, p = 0.023), compared to Control rats.

In the Adult group, there was a marginal effect of treatment (F [1,8] = 3.76, p = 0.089) and time (F[1,3] = 2.36, p = 0.097) on PEI, with no significant interaction (F[3,24] = 0.97, p = 0.422). In looking at individual tests, the Adult EtOH group had increased PEIs compared to Controls during Week 5 (t[10] = 2.28, p < 0.05) and Week 6 (t[15] = 3.65, p < 0.003) of forced ethanol consumption.

6.1. Partner preference tests

Fig. 3 depicts the results of partner preference tests. Preference was calculated as the time (in seconds) spent with a sexually receptive female minus the time with the non-receptive female, such that a positive score reflected sexual motivation. A 2-way mixed ANOVA revealed a significant effect of time in both groups during the forced ethanol consumption portion of the study (Young groups: F[1,12] = 16.87, p < 0.0001; Adult groups: F[1,12] = 31.195, p < 0.001), but no significant effect of treatment (Young: F [124] = 0.97, p = 0.76; Adult: F[1,16] = 0.128, p = 0.725), and no interaction between time and treatment (Young: F[2,48] = 0.285, p = 0.75; Adult: F[2,32] = 0.240, p = 0.788). Post hoc tests showed that increased preference was particularly evident by Week 8 compared to Week 4 in the Young group (Fisher’s PLSD, p < 0.0001), and by Week 6 and Week 8 vs. Week 4 in the Adult groups (Fisher’s PLSD, p < 0.0001). During the Post Test (after abstinence), a significant effect for treatment was observed, with the Young EtOH group showing significantly less preference than the Young Control group (t[24] = −2.72, p = 0.012). No comparable difference between groups was seen during the Post Test in Adults (t[16] = 0.130, p = 0.90).

6.2. Vocalizations

The results of 50-KHz vocalization tests are depicted in Fig. 4. Overall, the Young EtOH group showed fewer vocalizations than the Young Control group during forced ethanol consumption (Mann-Whitney U, tied Z value = −2.234, tied p value = 0.026). Comparable differences were not seen in Adult groups (Mann-Whitney U, tied Z value = −1.605, tied p value = 0.109). Although no statistically significant differences for treatment were seen on individual tests, a trend was observed on Week 7 in the Young groups (Mann-Whitney U, tied Z value = −1.959, tied p value = 0.05). For within-group comparisons, there was a significant effect of time, with both Young and Adult rats showing significantly increased vocalizations by Weeks 5, 7, and the Post Test compared to Week 3 (Wilcoxon signed rank test, Young: tied Z value = −3.99, −4.42, and −4.46, respectively, all tied p values < 0.01; Old: Week 3 vs. 5: tied Z value = −2.66, tied p value = 0.0078; Week 3 vs. 7, tied Z value = −3.11, tied p value = 0.001; Week 3 vs. Post Test: tied Z value = −3.462, tied p value = 0.0005). No significant differences between treatment groups were seen during the Post Test for Young or Adult animals.

6.3. Bodily measurements

A 2-way repeated-measures ANOVA revealed a main effect of

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**Fig. 2. Copulation tests.** Ejaculation latency (EL): time from first mount until ejaculation; all data are presented as mean ± SEM. * – p < 0.05, * – p < 0.07 compared to Control group.
Table 1
Post-ejaculatory intervals during copulation tests in Young and Adult rats during and after forced ethanol or water consumption.

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
<th>Post test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young ETOH (n = 12)</td>
<td>481.6 ± 15.5* (7)</td>
<td>489.5 ± 16.7* (10)</td>
<td>437.6 ± 39.0* (9)</td>
<td>380.5 ± 24.9* (11)</td>
<td>376.5 ± 25.5* (10)</td>
</tr>
<tr>
<td>Young Control (n = 14)</td>
<td>387.0 ± 14.8 (9)</td>
<td>346.2 ± 30.5 (11)</td>
<td>332.2 ± 22.4 (12)</td>
<td>327.3 ± 19.1 (14)</td>
<td>308.9 ± 21.3 (14)</td>
</tr>
<tr>
<td>Adult ETOH (n = 8)</td>
<td>442.0 ± 63.7 (3)</td>
<td>402.7 ± 44.1 (6)</td>
<td>407.4 ± 56.7 (5)</td>
<td>438.4 ± 42.9* (7)</td>
<td>387.8 ± 45.8 (5)</td>
</tr>
<tr>
<td>Adult Control (n = 10)</td>
<td>355.4 ± 26.7 (7)</td>
<td>323.7 ± 22.4 (7)</td>
<td>289.0 ± 16.7 (7)</td>
<td>273.7 ± 23.2 (10)</td>
<td>325.9 ± 18.6 (10)</td>
</tr>
</tbody>
</table>

Data are reported as mean seconds ± SEM. The numbers in parentheses indicate the number of males ejaculating and included in the analysis. * = p < 0.05, # = p < 0.10 vs. age-matched Controls.

Discussion

Overall, our findings suggest that forced ethanol consumption in a binge-like pattern beginning in adolescence may affect reproductive behavior in male rats, with some deficits persisting later into adulthood after exposure has ended. In copulation tests for sexual performance, young male rats with forced ethanol consumption had longer latencies to ejaculate and to initiate behavior after ejaculation (reflected in the PEI) compared to controls. This effect was noticeably different from Adult animals that showed no effects on ejaculation latency during forced consumption of ethanol, and a delayed impairment on PEI. Of interest, there was some indication that alcohol had long-term effects on PEIs in Young animals and ejaculation latencies in older animals, although neither comparison reached statistical significance. Regardless of age, binge-like exposure did not impair the development of partner preference; however, repeated exposure to ethanol in adolescence was associated with reductions in this behavior after an abstinence period. Finally, with regard to 50-kHz vocalizations, we found that Young animals receiving ethanol vocalized less overall in the presence of sexual cues during forced consumption, whereas adult animals showed no differences in this behavior.

The results of copulation tests in young animals during forced consumption are largely consistent with findings that have been reported in adult mammals. In humans, acute alcohol consumption is associated with difficulty in attaining penile erections and achieving ejaculation, both in self-reports (Crowe & George, 1989; Masters & Johnson, 1966; Peugh & Belenko, 2001) and in laboratory conditions when these indices are measured directly (Briddell & Wilson, 1976; Malatesta et al., 1979; Rubin & Henson, 1976). Ethanol consumption in dogs (Teitelbaum & Gantt, 1958) and rats (Dewsbury, 1967) is also linked to delays and blocks in ejaculation, and similar effects have been observed when ethanol is administered via injections (for example, Ferraro & Kiefer, 2004; Pfaus & Pinel, 1989). Likewise, in this study, ejaculation latency was increased when younger animals received ethanol via gavage. Surprisingly, ejaculation latency was not affected with gavage treatment in the Adult group, suggesting that ejaculation is less susceptible to the effects of ethanol in adults vs. young animals.

The findings in young animals are also consistent with research examining chronic exposure during late adolescence. When ethanol is given continuously (in food) throughout late adolescence and into adulthood (P55–P105), fewer males ejaculate (22% compared to 50% of isocaloric controls), and those that achieve ejaculation have significantly longer post-ejaculatory intervals (Oliva et al., 2006). Although we did not report differences in the percentage of males ejaculating, we did see increases in the PEIs during copulation tests after forced consumption in both Young and Adult animals. The differences in PEIs also appear to vary as a function of age, with Young animals showing an increased refractory period early on, and Adult animals showing increased PEIs after repeated ethanol exposure. Whether increases in PEIs occur because of enhanced satiation or reduced motivation is a question for future studies. The findings presented here suggest 1) that the developing brain is more susceptible to the suppressive effects of alcohol and 2) that alcohol exposure during adolescence can influence sexual behavior, when forced consumption mimics a binge pattern, a common method of drinking in teens and young adults (Johnston et al., 2008; Miller et al., 2007; Wechsler & Nelson, 2001).

The effects of binge-like forced consumption on sexual performance, however, may be short-lived, since statistically significant differences in behaviors were not observed after abstinence (Post Tests) for Young or Adult groups.

Repeated forced ethanol administration did not prevent the
expression of partner preference in either age group, suggesting no effect of ethanol at this dosage on sexual motivation. All groups showed increased preference for the sexually receptive female as they gained sexual experience (see Fig. 3), spending significantly more time with the sexually receptive female by the third test (after 8 weeks of ethanol or water) compared to the first test (after 4 weeks of treatment). This suggests that alcohol exposure during adolescence or adulthood does not prevent the development of this behavior, despite coinciding problems with sexual performance. However, after abstinence, the Young EtOH showed suppressed partner preference, indicating a possible long-term effect on the selection of a sexual partner. In fact, Young EtOH animals spent significantly more time with the non-receptive female than Young Controls, suggesting an inability to differentiate cues indicating sexual receptivity. There are two possible reasons for this diminished response. First, exposure to ethanol during development may affect sexual motivation later in life, as the Young EtOH group showed significantly less preference during the Post Test compared to the Young Control group. Alternatively, the presence of alcohol during the development of sexual behavior may have produced state-dependent learning, such that the EtOH group is unable to differentiate between receptive and non-receptive females in the absence of the drug. In either case, these results indicate that differences in the structure of reward pathways or sensory areas in the brain may exist. Future studies could test whether partner preference is restored when ethanol is administered to the EtOH group, i.e., in the state in which it was learned (after forced alcohol consumption).

Studies examining the effects of alcohol on sexual motivation have been confined to adult animals, and are fairly limited and inconsistent (Frohmader et al., 2010). In one study (Pfaus & Pinel, 1989), rats were initially trained to differentiate between receptive and non-receptive females in a bi-level chamber. After training, animals that received ethanol were more likely to initiate mating with non-receptive females, suggesting disinhibition (Pfaus & Pinel, 1989), and perhaps enhanced (or misdirected) sexual motivation. Moreover, Ferraro and Kiefer (2004) reported a significant increase in activity (level changing) before the arrival of a sexually receptive female in rats receiving ethanol compared to controls. In contrast, another study found that rats receiving comparable amounts of ethanol were less likely to press a bar for access to a sexually receptive female, suggesting suppressive effects of ethanol on sexual motivation (Scott, Ettenberg, & Olster, 1994). Surprisingly, in the present study, adult rats showed high levels of preference for receptive females after forced consumption of ethanol, and no differences in behavior were seen after abstinence. Because different effects were found for adolescents and adults, the findings suggest that the adolescent brain may be more vulnerable to long-term effects of ethanol exposure.

Finally, we elected to measure ultrasonic (50 kHz) vocalizations in the presence of estrous female bedding to evaluate differences in sexual communication (Harding & Velotta, 2011). Male and female rats vocalize at a frequency of 50 kHz to indicate positive social events (Portfors, 2007), and to solicit sexual partners and coordinate mating (Barfield & Thomas, 1986). One recent study has shown that spontaneously emitted 50-kHz vocalizations may be inhibited by chronic exposure to alcohol (Thakore et al., 2016). High-alcohol drinking (HAD-1) male rats were subjected to a Drinking in the Dark paradigm for 8 weeks beginning at P56 (late adolescence), and the number of 22-kHz and 50-kHz vocalizations was monitored during consumption. The study reports that the frequency of 50-kHz vocalizations was suppressed in alcohol-experienced vs. alcohol-naïve rats in this strain. Likewise, our research suggests that males receiving binge-like patterns of forced consumption earlier in adolescence (beginning at P32) show reductions in 50-kHz vocalizations in response to sexual cues. Although all groups showed increased vocalizations with age and experience as reported previously (Harding & Velotta, 2011), we found that Young EtOH animals vocalized less overall during forced consumption. These effects did not persist in the absence of ethanol.

In adult rats, the effects of chronic ethanol consumption on 50-kHz vocalizations have been examined during reproductive behaviors (Cagiano et al., 1998). In this study, male rats received low levels (3%) of ethanol or no ethanol (0%) continuously in water bottles for 8 weeks, and no differences in 50-kHz vocalizations were observed between groups. Similarly, no differences were seen in the frequency of vocalizations between Adult EtOH and Adult Control groups when ethanol was administered repeatedly via gavage. Taken together, these findings suggest that ultrasonic vocalizations may be more sensitive to ethanol during adolescence vs. adulthood. Reductions in this behavior during adolescence could produce consequences in terms of reproductive success, including communicating with and attracting potential mates.

One possible mechanism that may account for the changes in behavior associated with forced ethanol consumption is a reduction in testosterone (T). In long-term alcoholics, the suppression of T, testicular atrophy, and reductions in sexual performance coincide (Emanuele & Emanuele, 1998; Lloyd & Williams, 1948; Van Thiel & Lester, 1979; Van Thiel, Lester, & Sherins, 1974), and similar reductions in hormone levels have been observed with acute ethanol exposure (Gordon, Altman, Southren, Rubin, & Lieber, 1976). Likewise, in rodents, ethanol suppression of T is quite rapid, with significant reductions observed after a single injection of ethanol in adults (Steiner, Holloran, Jabamoni, Emanuele, & Emanuele, 1996) and adolescents (Przybycien-Szymanska et al., 2010). Sexual communication, motivation, and performance are all dependent on circulating levels of T (Harding & Velotta, 2011; Hull, Meisel, & Sachs, 2002; Meisel & Sachs, 1994). While we did not measure T levels directly during forced consumption of ethanol, we did remove peripheral structures, which are known to reflect

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**Fig. 4.** Mean number of 50k-Hz vocalizations in response to soiled bedding from sexually receptive females. Mean number of vocalizations (±SEM) for each group during treatment (3, 5, 7 weeks) and after abstinence (Post Test).
circulating levels of T (Armagan et al., 2006). Seminal vesicles were smaller in Young EtOH rats compared to Young Controls, although the size difference did not reach statistical significance. Perhaps T levels were reduced during consumption and returned to normal by the end of the study, since seminal vesicles were not removed until about 10 weeks after the last ethanol administration. Other research has reported that T levels and seminal vesicle weights are suppressed with alcohol consumption when measurements are made soon after treatment (Oliva et al., 2006).

It is also possible that long-term effects on behavior are mediated by ethanol-mediated changes in the wiring of the adolescent brain. In humans, alcohol consumption during adolescence is associated with reductions in gray matter and increases in white matter (Blakemore et al., 2010), particularly in areas known to support cognition and behavior, including the prefrontal cortex and sensory regions of the brain (Spear, 2015; Windle, 2016). Drinking, may produce long-term effects on brain and behavior research. Furthermore, the research lends support to the growing interest in the basis for future studies.

In summary, our results suggest that binge-like patterns of forced alcohol consumption beginning in the critical period of adolescence can be detrimental to emerging reproductive behaviors in male rats. Specifically, males administered ethanol repeatedly via gavage during adolescence and young adulthood showed longer latencies than controls in sexual performance tests, consistent with what was previously reported in adult animals and humans. Adult animals had effects that were more modest during forced consumption, and the long-term effects did not reach statistical significance. There is also evidence for impaired communication in response to sexual cues, reflected by a suppression in the number of vocalizations (overall) in the Young EtOH group. Finally, perhaps of most interest, males in the Young EtOH group seemed unable to differentiate and/or prefer sexually receptive vs. non-receptive females well after the cessation of alcohol exposure.

Whether these effects are due to changes in the structure of reward pathways or sensory regions of the brain could be a topic for future research. Furthermore, the research lends support to the growing body of evidence that alcohol consumption during adolescence, a critical period of development when many people begin binge drinking, may produce long-term effects on brain and behavior (Spear, 2015; Windle, 2016).

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References


