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Chronic alcohol consumption from adolescence to adulthood in mice — effect on growth and social behavior

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Abstract

Experimentation with alcohol is common during adolescence. However the long-term consequences from moderate alcohol use during adolescence development are not clear. Using a two-bottle free-choice paradigm in the home cage setting, we studied adolescent mice (4 weeks old) across a 6-week time span of the adolescence-to-adulthood development period. Adolescent mice readily reached a steady level of alcohol consumption and maintained this level throughout the 6-week period. Chronic alcohol consumption resulted in reduced growth in adolescent mice, as well as accelerated acclimation to a novel environment. During a social interaction test, similar levels of initial social investigation and subsequent habituation were observed in both the chronic alcohol and the water-only control groups. However, chronic alcohol self-administration resulted in impaired social recognition and decreased social play/fight behavior. Taken together, these results indicated that chronic alcohol consumption across adolescence development negatively impacted both physical growth and social behavior in mice, highlighting the detrimental consequences from prolonged alcohol drinking in adolescence.

Keywords

Chronic alcohol drinking; Self-administration; Adolescence; Physical growth; Social behavior; Mice

1. Introduction

Initial experience with alcohol often occurs during adolescence in humans, and experimentation with alcoholic drinks is common during this developmental stage, which can lead to prolonged drinking in some youth (Johnston et al., 2008). Alcohol consumption, especially chronic drinking, during adolescence may result in long-lasting consequences in physiological and cognitive development. Thus, it is important to better understand the relationship between adolescence alcohol consumption and potential impact on health.

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Adolescence is relatively short in duration during a normal life span. However, it is a critical period in development, in terms of brain structural and functional development (Giedd et al., 1999; Spear, 2000b), as well as the formation of social awareness (Spear, 2000b). Animal models are widely used in alcohol studies to better understand the mechanisms underlying alcohol drinking, including adolescent alcohol consumption and its long-range consequences (McBride et al., 2005). In rodents, adolescence is defined as the period around the time of sexual maturation (Odell, 1990; Spear, 2000a). Adolescent and adult rodents display marked differences in their sensitivity to acute alcohol effect on behavior (Spear and Varlinskaya, 2005). Adolescents appeared to be more sensitive than adults to the memory impairing effects of alcohol, as well as the impact of alcohol on the brain function that underlies memory formation (White and Swartzwelder, 2004; Spear and Varlinskaya, 2005). For example, compared with adult rats, adolescent rats treated with alcohol showed worse performance in spatial learning tasks known to require the functioning of the hippocampus (White and Swartzwelder, 2005).

Chronic alcohol exposure during adolescence causes alcohol-related problems in adult animals (Slawecki et al., 2001; Siciliano and Smith, 2001; McBride et al., 2005; Diaz-Granados and Graham, 2007). Chronic-intermittent binge alcohol exposure during the peri-adolescent period induced memory deficits (White et al., 2000; Schulteis et al., 2008), motor impairments (White et al., 2002), and damage in the frontal-anterior cortical regions (Crews et al., 2000). In addition, long-term alcohol exposure retarded body weight gain in mice (Lagerspetz, 1972) and rats (White et al., 2002; Silvers et al., 2003).

Alcohol exposure during development has been shown to alter social behavior in animal models just as in people. The effect of prenatal alcohol exposure on social behavior has been widely studied (Kelly et al., 2000; Lugo, Jr. et al., 2003; Lawrence et al., 2008). Spear and colleagues focused on acute (Varlinskaya et al., 2001; Varlinskaya and Spear, 2002; Varlinskaya and Spear, 2006) and chronic (7 days) (Varlinskaya and Spear, 2007) effects of alcohol on social behavior in adolescent and adult rats. However, the findings have been inconsistent. Depending on the particular paradigm and the alcohol dosing, alcohol could result in either social facilitation (increased social activity and enhanced social preference) or social inhibition (decreased social activity and avoidance of a peer). For social recognition memory, it has been shown that alcohol-exposed rats (3 g/kg alcohol via gastric intubation, from postnatal day 2-10, tested at 100 days of age) of both sexes had poorer memory of the previously encountered companion rats (Kelly and Tran, 1997). Considering the evidence of early alcohol exposure on animals' welfare later in their adult life, it is important to better understand the relationship between chronic alcohol consumption in adolescent rodents and the impact on subsequent biology and cognition in adults, including social behavior.

In this study we used a two-bottle free-choice paradigm in the home cage setting, and studied chronic alcohol consumption in adolescent mice (4 weeks of age) for its effect on growth and behavior in adulthood (10 weeks of age). Chronic alcohol consumption resulted in reduced growth, impaired social recognition, and decreased social play/fight behavior, demonstrating that alcohol consumption during adolescence negatively impacted both physical growth and social behavior in mice.

2. Materials and Methods

2.1. Animals

Male ICR (Institute for Cancer Research) outbred mice were obtained from Shanghai Laboratory Animal Center, Chinese Academy of Sciences, Shanghai, China. ICR outbred mice were also known as CD-1 (Caesarean Derived-1), and is a commonly used outbred mouse strain. Animals were group-housed in temperature controlled animal facilities on a 12 hr:12 hr

light-dark cycle with food and water available ad libitum. The mice used in the chronic alcohol drinking experiment (both the water-only controls and the chronic alcohol group) were singly housed and had 2 days of acclimation before starting the experiment, while mice to be used as companions in the social interaction test remained in group housing.

Principles of laboratory animal care were followed in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996), the PRC National Standards for Laboratory Animal Quality, and the Guidelines for the Use of Experimental Animals.

2.2. Chronic alcohol self-administration

For the chronic alcohol drinking experiment, the paradigm of two-bottle free-choice was used (Belknap et al., 1993; Grisell et al., 1999; Middaugh and Bandy, 2000; Roberts et al., 2001; Spanagel et al., 2002; Blizard et al., 2004; Camarini and Hodge, 2004; Khisti et al., 2006). Each cage was equipped with two drinking tubes. The drinking tubes were made from 10 ml plastic serological pipettes (Coster Stripette), with 0.1 ml graduation. The narrow tip of the pipette was cut off and a stainless steel sipper tube was connected to it through silicone tubing. The drinking tubes were placed on the slanted surface of the wire cage top, and the stainless steel tip protruded between the wires toward the interior of the mouse cage. The fitting was snug, and the tube would not move when the mouse drank from it. Standard rodent chow was available ad libitum, and was spread around both drinking tubes to avoid food-associated tube preference. Animals' body weight and food consumption were measured daily on weekdays.

Before chronic alcohol drinking began, mice (3 weeks old) were allowed to adapt to the drinking tubes with both tubes containing only water from experimental day 1-5. After the adaptation period, mice were randomly assigned to either the alcohol group or the water-only control group (n=16-18). The same housing conditions were applied to both the alcohol group and the water-only control group throughout the entire study, and all the animals were housed in the same room. For the water-only control group, both drinking tubes contained deionized water throughout the duration of the experiment. For the alcohol group, one drinking tube contained 2% alcohol solution initially (day 6 - 10) while the other contained water. On day 11, the alcohol concentration was switched from 2% to 5%, and the 5% alcohol solution was used throughout the remainder of the experiment.

The amount of water or alcohol consumption was recorded daily. The solution content (water or alcohol) was refreshed every day, and the two tubes were switched daily to avoid any side bias. To account for any liquid loss due to evaporation, separate pipettes containing alcohol or water were placed in an empty cage, and the amount of evaporation was recorded daily. Evaporated amount was subtracted from the amount of water or alcohol consumption, respectively.

2.3. Social interaction and motor activity measurements

Before behavior testing, mouse cages were transferred from the central animal housing to the behavior testing room for 5 days' adaptation. Half of the alcohol group and half of the water-only group were tested in one day, and the other halves of the two groups were tested in the next day. On the day of behavior testing, mice were first tested for social interaction, followed by motor activity measurement.

For social interaction, the paradigm of habituation/dishabituation was used (Dluzen and Kreutzberg, 1993; Gheusi G et al., 1994; Winslow and Camacho, 1995; Ferguson et al., 2002; Choleris et al., 2004; Mineur et al., 2006). In this paradigm, the test mouse experiences four consecutive encounters with the same companion mouse, during which the test mouse

usually shows a habituation response over the four encounters, with a decrease in the extent of social interaction. During the fifth encounter, a novel companion mouse is present, which induces a dishabituation response in the test mouse with an increase in the extent of social investigation. In our study, the experimental mice had reached 10 weeks of age by the time of social interaction testing. Companion mice used in social interaction were juvenile mice of 4 weeks of age and were housed 2-3 per cage. Social interaction was conducted over 5 test encounters with 2 min per test. The inter-test interval was 10 min during which animals were returned to their home cages. Experiments were conducted in plastic cages (24×15×16cm, LxWxH) with bedding material, and the cage was covered with a piece of glass on the top. Each cage was placed inside a wooden enclosure (60×40×44cm, LxWxH) that was sound- and light-proof, illuminated from inside with a fluorescence light (5W). A video camera was mounted on the top of the wooden enclosure to record animal behavior for subsequent offline analysis. Test and companion mice never met before. Prior to the first social interaction test, the experimental mouse was placed in the testing chamber for 15 min to acclimate to the new environment.

Behavior recorded on video tapes was scored by two observers who were blind to the experimental groups. Social interaction was scored mostly as we previously described (Zou et al., 2008), with the following modifications. Three social behaviors were scored in this study:

1. Being together: cumulative time that the experimental mouse was with the companion mouse, defined as the experimental mouse approaching the companion mouse and staying within 1-cm distance of the companion mouse. During the time of being together, mice can be either stationary or engaged in any type of activity.
2. Pouncing/climbing over: cumulative time the experimental mouse pounced upon or placed its front paws onto the companion mouse.
3. Play/fight: cumulative time the experimental mouse displayed rigorous behaviors toward the companion mouse, including pushing, biting, jumping toward or onto the companion mouse.

For motor activity measurement, an automated beam break detection system (San Diego Instruments) was used as previously described (Wu et al., 2005). Animals were placed individually in plastic chambers (48×24×20cm, LxWxH) with bedding material, and the chamber was covered with a piece of glass on the top. Each chamber was placed inside a wooden enclosure (60×40×44cm, LxWxH) that was sound- and light-proof, illuminated from inside with a fluorescence light (5W). Motor activity was measured via automated detection of infrared beam breaks. Rearing behavior was detected by a second set of infrared photodetectors mounted 7.7 cm above the ground.

2.4. Statistics

Statistical analysis used unpaired t-tests to compare the chronic alcohol group and the water-only control group in locomotion, rearing and social play/fight behavior. Repeated measures two-way ANOVA with Bonferroni post tests was used to compare the chronic alcohol group and the water-only control group in body weight and food intake. When analyzing social interaction in mice from the chronic alcohol group, one-way ANOVA - Dunnett's multiple comparison tests were used to compare test 1 each with test 2, 3, 4, or 5; social interaction in mice from the water-only control group was analyzed in the same manner.

3. Results

3.1. Alcohol and liquid consumption — stable chronic self-administration of alcohol in mice

To evaluate the effect of chronic alcohol drinking in mice, we used a two-bottle free-choice paradigm to measure alcohol drinking. The time course data for the water and alcohol consumption are shown in Fig. 1. For the water-only control group, the amount of water consumption was relatively stable throughout the experiment (Fig. 1A, filled circles). For the chronic alcohol group, after mice started on the 5% alcohol solution, the amount of alcohol consumption was relatively stable with a slight increase over time (Fig. 1A, open squares), possibly compensating for an increased body weight. Indeed, weight-adjusted alcohol consumption data (Fig. 1C) indicated that, after mice started on 5% alcohol, a steady level of chronic alcohol administration was maintained throughout the remainder of the experiment. Water consumption for the alcohol group showed a slight decrease over time (Fig. 1A, open circles). When the total liquid consumption was adjusted for body weight, both the water-only control and the alcohol groups showed comparable levels of total liquid consumption throughout the entire experiment (Fig. 1B), although there was a big decrease of total liquid consumption over the first month in both groups.

3.2. Reduced growth from chronic alcohol consumption

Body weight for the chronic alcohol and the water-only control groups was recorded (Fig. 2A). Compared with that for the water-only control group, body weight for the chronic alcohol group showed significantly reduced growth [$F(1,203)=11.74$, $P=0.0018$, $p<0.05$]. Specifically, body weight measures of the alcohol group were significantly lower than those of the controls for Day 27 ($t=3.546$, $p<0.01$), Day 34 ($t=3.981$, $p<0.001$), Day 41 ($t=3.775$, $p<0.01$), Day 48 ($t=4.095$, $p<0.001$), and Day 55 ($t=5.034$, $p<0.001$). Analysis also showed that there was interaction between alcohol consumption and time [$F(7,203)=12.98$, $p<0.001$]. This indicated that as the time went on, alcohol effect made the body weight difference between the two groups to become more pronounced.

Daily food intake is shown in Fig. 2B. Overall, the chronic alcohol group consumed comparable amount of food compared with that of the water-only group [$F(1,72)=0.35$, P value=0.5608].

3.3 Accelerated acclimation of locomotor activity from chronic alcohol consumption

To determine whether chronic alcohol intake had any effect on mobility, mice were tested for their motor activity. Fig. 3A showed the time course of locomotor activity during the 120-min observation period. During the initial exploratory phase (0 - 20 min) when the mouse was first placed in the novel environment of testing chamber, both groups showed similar levels of total locomotor activity (Fig. 3B). For the subsequent acclimation phase (20 - 60 min), mice from the alcohol group displayed significantly less activity (Fig. 3C, $t=2.416$, $df=29$, $p<0.05$), suggesting accelerated acclimation for the alcohol group. After mice were acclimated to the test chamber, there was little activity in either group (60 - 120 min, Fig. 3A), and there was no difference in the total activity during this phase (data not shown).

Rearing activity was also recorded (Fig. 3D). Cumulative rearing was comparable during the initial exploratory phase (Fig. 3E). For the subsequent acclimation phase, rearing activity showed a moderate reduction (20 - 60 min, Fig. 3D); however, the difference did not reach statistical significance (Fig. 3F, $t=1.971$, $df=29$, $p=0.058$).

3.4 Impaired social recognition from chronic alcohol consumption

To evaluate whether chronic alcohol intake affected social behavior, we used a habituation/dishabituation paradigm (Dluzen and Kreutzberg, 1993; Gheusi G et al., 1994; Winslow and

Camacho, 1995). This paradigm allowed the quantification of initial social investigation, habituation during subsequent encounters, and social recognition.

Social interaction data are shown in Fig. 4. During the initial test of social investigation, both the chronic alcohol and the water-only control groups showed a virtually identical extent of social interaction with the companion mouse, as measured by the cumulative time of the two mice staying together during the 2-min testing period (Fig. 4, test 1). During the subsequent tests of social encounter with the same companion mouse, both the chronic alcohol and the water-only control groups displayed habituation, with less and less time spent together with the companion mouse (Fig. 4, tests 2 - 4). By the fourth encounter, the amount of social investigation by the test mouse was significantly lower than that for the first investigation for the alcohol group [$F(4, 80)=3.069$, $p<0.05$, compared with test 1], as well as for the water-only control groups [$F(4, 65)=2.149$, $p<0.05$, compared with test 1].

To access social recognition, a novel companion mouse was used in the fifth encounter test. Mice in the water-only control group recognized the novel companion mouse being new, as the extent of social interaction increased to a level that was similar to that of the first social investigation test [$F(4, 65)=2.149$, $p>0.05$, compared with test 1]. For mice in the chronic alcohol group, however, the extent of social interaction with the novel companion mouse remained low [$F(4, 80)=3.069$, $p<0.05$, compared with test 1].

3.5 Reduced social play/fight behavior from chronic alcohol consumption

We observed that the experimental mice sometimes displayed aggressive play/fight behavior toward the juvenile companion mice. To determine whether chronic alcohol had any effect, we recorded the behavior of pouncing/climbing over the companion mouse (Fig. 5A) and the play/fight behavior with the companion mouse (Fig. 5B). There was no statistical difference for the pouncing/climbing over behavior between the two groups. For the play/fight behavior, however, mice in the chronic alcohol group showed a significant decrease compared with the water-only control group (Fig. 5B, *, $p<0.05$). These data suggest that chronic alcohol self-administration resulted in a significant decrease in play/fight behavior.

4. Discussion

In this study, we employed a paradigm of chronic alcohol self-administration, and investigated its effect on mice during their adolescence development. Mice initiated alcohol drinking at 4 weeks of age, and chronic drinking lasted for about 6 weeks. This time frame in mice corresponded to the development stage of adolescence-to-adulthood in humans, therefore allowing evaluation of the impact of adolescence alcohol consumption on subsequent physiology and behavior in adulthood.

Our study is unique in that we took into account a number of factors when studying alcohol effect on mouse physiology and social behavior: 1) most studies of long-term alcohol effect on social behavior and/or adolescence development used rats (Slawecki et al., 2001; Siciliano and Smith, 2001; Varlinskaya and Spear, 2007; Schulteis et al., 2008; Maldonado et al., 2008), which was the major impetus for us to undertake the present study, since mice are increasingly more common in alcohol studies due to the availability of numerous transgenic mouse models; 2) the method of alcohol intake in our study was by chronic 2-bottle choice self-administration, whereas other studies tend to use alcohol vapor (Slawecki et al., 2001; Diaz-Granados and Graham, 2007; Schulteis et al., 2008), alcohol as the sole source of fluid (Siciliano and Smith, 2001), or intragastric administration (Maldonado et al., 2008), which are forms of forced alcohol exposure rather than free-choice alcohol self-administration; 3) we used moderate alcohol concentration to better model the youth drinking behavior in human (beer and wine, rarely long-term consumption of high proof alcoholic drinks), whereas other

studies tend to use higher alcohol concentrations; 4) we used a home-cage setting where mice had free access to alcohol all the time, again in an attempt to better model the situation of opportunistic alcohol consumption in human, whereas other studies tend to use binge, limited time access, or intermittent alcohol protocols; 5) our study allowed the mice to access alcohol throughout their adolescence development, whereas other studies rarely covered such an extended period of adolescence development; 6) we followed the long-term alcohol consumption with physiological and behavioral characterizations, whereas other studies in mice rarely carry out these analyses; 7) in particular, no previous study had examined social memory as a consequence of long-term alcohol self-administration.

When using mice as animal models, C57BL6 mice are the most widely used ones. We chose ICR mice for two reasons: 1) ICR is an outbred mouse strain, and there exists certain inter-individual variability — similar to the outbred nature of the human species. Therefore, using an outbred mouse strain to study alcohol effect would more closely model the effect of alcohol on growth and behavior in humans. 2) As an inbred strain, C57BL6 mice drink considerably more alcohol than other mouse strains. Thus, C57BL6 may represent only a special case regarding alcohol drinking; using ICR would add breadth to the knowledge on alcohol effect in mice.

Our results indicated that with the free access paradigm in a home cage setting, adolescent mice readily achieved a steady level of alcohol consumption and maintained alcohol consumption at this level into the young adulthood (Fig. 1). These data suggested that in the home cage environment, alcohol self-administration did not escalate over time. This is in contrast to the stepwise procedure where the alcohol concentration is increased stepwise by the investigator, and higher concentrations of alcohol resulted in substantially increased alcohol consumption in rodents (Lankford et al., 1991). Our data indicated that with the home cage environment and the same moderate concentration of alcohol (5%), alcohol consumption by adolescent mice did not increase over time.

It is note-worthy that even though the level of alcohol consumption remained steady, chronic alcohol consumption appeared to retard adolescence growth, in that the body weight gain for the chronic alcohol mice was significantly reduced compared with that of the water-only control group (Fig. 2A). Furthermore, statistical analysis showed that there was significant interaction between alcohol consumption and time, indicating that as the time went on, alcohol effect made the body weight difference between the two groups to become more pronounced. We also monitored food intake, and the two groups showed comparable levels of food intake (Fig. 2B). Since alcohol consumption added to the caloric content of the diet, mice drinking alcohol would have more caloric intake compared with the water-only control group. In this regard, it is even more remarkable that mice in the alcohol group displayed reduced growth. Taken together, these results suggested that chronic alcohol consumption was detrimental to adolescence development. This notion is consistent with other reports. Using a similar paradigm for alcohol drinking, Siciliano et al. (Siciliano and Smith, 2001) reported a reduction of body weight gain in male rats that were given 10% alcohol during periadolescent development (postnatal day 21-70). In an earlier study (Lagerspetz, 1972), shorter periods of chronic alcohol exposure (1-2 g/kg daily for 8 days) during adolescence also suppressed body weight gain. Alcohol has previously been shown to exert profound effects on various endocrine systems (Van Thiel and Gavalier, 1990; Emanuele and Emanuele, 1997). It is well-documented that growth hormone is reduced by alcohol in humans and animals (Redmond, 1981; Valimaki et al., 1990; Tentler et al., 1997; Steiner et al., 1997). It has been reported that alcohol has inhibitory effects on the GHRH-GH-IGF-I axis in rats (Soszynski and Frohman, 1992). Since the GHRH-GH-IGF-I axis plays an essential role in nutrient metabolism and growth, the effects of alcohol are likely to result in physiological consequences. Thus, growth reduction under

chronic alcohol consumption in adolescent mice may be related to alcohol-induced endocrine perturbation.

We examined the alcohol effect on behavior at the end of the chronic drinking period. We conducted behavioral measurements while mice still had access to alcohol, rather than imposing an abstinence period. We chose this paradigm to avoid the confound of alcohol withdrawal, since imposing an abstinence period could illicit physiological withdrawal from chronic alcohol, thus impacting animals' behavior (Kampov-Polevoy et al., 2000).

In humans, moderate alcohol drinking at social gatherings tends to ease anxious feelings and facilitate social interaction. In this regard, we observed chronic alcohol mice displayed accelerated acclimation to the novel environment in motor activity testing chambers (Fig. 3). One possible explanation for this phenomenon is that it may parallel the human social situation mentioned above. Alternatively, the accelerated acclimation in alcohol mice can be interpreted as a reduced interest in the novel environment. This latter interpretation is consistent with our findings in mouse social interaction (see below).

Chronic alcohol consumption showed virtually no effect on animals' initial social investigation behavior toward a companion mouse, or on their subsequent habituation when the same companion mouse was present (Fig. 4, tests 1-4). Given this lack of difference between the alcohol and water-only control groups, it was striking that the chronic alcohol mice displayed no sign at all of social recognition when a novel companion mouse was present (Fig. 4, test 5, open square). This was in contrast to the robust social recognition of the novel companion mouse by the water-only control group (Fig. 4, test 5, filled circle). Such a profound difference in distinguishing a novel partner suggested an altered ability of social recognition in the chronic alcohol mice, possibly an impairment in social memory due to chronic alcohol consumption. An alternative interpretation, one that is in agreement with the "reduced interest" hypothesis (see motor activity discussion above), is that chronic alcohol consumption rendered mice less interested in a companion mouse upon repeated encounters (faster acclimation to social interaction), regardless of whether it was the same or a novel companion mouse.

Aggressive play/fight behavior toward a companion mouse also reflected the effect of chronic alcohol consumption. Mice in the chronic alcohol group showed a significant decrease in play/fight behavior (Fig. 5B). The reduced aggressive play/fight behavior from the chronic alcohol mice may underscore a decreased interest toward the companion mouse. Another consideration is that mice in this study have been single-housed for seven weeks, and the aggressive play/fight behavior in the water-only control group was more prominent than that observed in group-housed mice in our previous studies (Zou et al., 2008). This suggested that isolation may have increased the baseline level of aggression in single-housed mice, which is consistent with other reports (Lister and Hilakivi, 1988; Varlinskaya and Spear, 2008). Our study also found that chronic alcohol reduced aggressive behavior, which confirmed similar observations by other groups. Studies in rodents showed that, for mice with low to intermediate baseline levels of aggressive behavior (such as group-housed mice), moderate doses of alcohol increased aggressive behavior, while high concentration of alcohol reduced it; on the other hand, for mice with high baseline level of aggressive behavior (such as single-housed mice), alcohol reduced aggressive behavior (Blanchard et al., 1987; Hilakivi and Lister, 1989). Thus, social context (such as housing condition), alcohol administration paradigm (acute or chronic), and doses of alcohol all exerted influence on aggressive behavior.

Adolescence, for humans as well as for various mammalian species, represents a developmental phase with emerging patterns of distinctive behaviors, including age-related increase in social behavior and risk-taking/novelty-seeking. These behaviors are mainly due to developmental processes in the adolescent brain (Spear, 2000b). Adolescent risk-taking/novelty-seeking

increases the possibility of experimenting with alcohol and other drugs, and at the same time also subjects adolescents to their harmful effects. In this study, with a rather moderate alcohol concentration of 5%, long-term chronic consumption throughout mouse adolescence development resulted in accelerated motor acclimation to a novel environment and impaired social recognition. As a possible explanation, a common theme in these observations was that chronic alcohol consumption during adolescence led to a reduced interest in the surrounding environment, both physical (testing chamber) and social (companion mouse).

In summary, using a home-cage 2-bottle free-choice paradigm for alcohol self-administration, we studied chronic alcohol effects in mice across the developmental stage from adolescence to adulthood. Mice showed stable levels of alcohol self-administration over the 6 weeks of alcohol drinking, but with reduced growth. When placed in a novel environment, chronic alcohol mice displayed accelerated acclimation in motor activity. During social interaction, the chronic alcohol mice showed the same extent of activity as the water-only control mice in both initial social investigation and subsequent habituation. However, social recognition was impaired in the chronic alcohol mice; they also showed a reduced play/fight behavior. Taken together, these results indicated that chronic alcohol consumption across adolescence development negatively impacted both physical growth and social behavior in mice, highlighting the detrimental consequences from prolonged alcohol drinking in adolescence.

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Fig. 1.

Stable chronic self-administration of alcohol in mice. (A) Water or alcohol consumption in the alcohol group vs. the water-only control group. Data are shown for daily alcohol or water consumption (ml/day, average over 5 days). (B) Total liquid consumption for the alcohol group (alcohol + water, open squares) and the water-only group (water + water, filled circles) are shown (averaged over 5 days, ml/kg/day). (C) Alcohol consumption (g/kg/day) for the chronic alcohol group, shown as grams of alcohol per kilogram mouse body weight. Data are expressed as mean $\pm$ S.E.M. ($n=17$ for the alcohol group, $n=14$ for the water-only control group).

Fig. 2. Reduced growth from chronic alcohol consumption. (A) Average body weight. (B) Food intake (g/kg/day). Data for Day 1 represent the average food consumption for that day. Subsequent time points represent average value over a week. Open squares, chronic alcohol group; filled circles, water-only control group. Data are expressed as mean $\pm$ S.E.M. (n=17 for the alcohol group, n=14 for the water-only control group). *, significant difference compared with the water-only control group ($p < 0.05$, repeated measures two-way ANOVA with Bonferroni post tests).

Fig. 3.

Accelerated acclimation of motor activity from chronic alcohol consumption. Upper panels (A, B and C): locomotion; lower panels (D, E and F): rearing. Time course of locomotion (A) and rearing (D) are shown as beam breaks per 5min. Cumulative locomotion (B) and rearing (E) for the initial period of exploration (0-20 min) are shown. For the subsequent period of acclimation (20-60 min), cumulative locomotion (C) and rearing (F) are shown. Data are expressed as mean $\pm$ S.E.M. (n=17 for the alcohol group, n=14 for the water-only control group). *, significant difference compared with the water-only control group ($p < 0.05$, unpaired t-test).

Fig. 4.

Social recognition is impaired from chronic alcohol consumption. The paradigm of habituation/dishabituation was used to measure social recognition. For tests 1-4, the same companion mouse was paired with an experimental mouse. For encounter test 5, a novel companion mouse was paired with the experimental mouse in order to access social recognition of the experimental mouse. Open squares, the alcohol group; Filled circles, the water-only control group. Data are expressed as mean $\pm$ S.E.M. ($n=17$ for the alcohol group, $n=14$ for the water-only control group). #, significant difference compared with the first test of the same group ($p<0.05$, one-way ANOVA - Dunnett's multiple comparison tests, for the alcohol group and the water-only control group, respectively).

Fig. 5. Reduced play/fight behavior from chronic alcohol consumption. Social investigative behaviors of an experimental mouse toward a juvenile companion mouse were observed for the first 4 tests where the same companion mouse was used in the pairing. (A) Pouncing and climbing over the companion mouse. (B) Play/fight behavior. Data are expressed as mean $\pm$ S.E.M. (n=17 for the alcohol group, n=14 for the water-only control group). *, significant difference compared with the water-only control group (p<0.05, unpaired t-test).