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Robust and Stable Drinking Behavior Following Long-Term Oral Alcohol Intake In Rhesus Macaques

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Abstract

Face validity in animal models of alcohol abuse and dependence is often at odds with robust demonstrations of ethanol-seeking. This study determined the relative influence of ethanol and a flavorant in maintaining ethanol intake in a nonhuman primate model of “cocktail” drinking. Four year old male monkeys were maintained on a 6% ethanol/6% Tang® solution made available in daily (M-F) 1-hr sessions. Experiments determined the effect of: 1) a second daily access session, 2) concurrent presentation of the Tang® vehicle, 3) sequential presentation of the vehicle in the first daily session and the ethanol solution in the second session, 4) altering the Tang® concentration, 5) altering the ethanol concentration, and 6) removal of the flavorant. Mean daily intake (2.7 ± 0.2 g/kg/day) was stable over 7 months. Simultaneous availability of a large, but not a low-moderate, volume of the vehicle reduced ethanol intake by about 50%. Decreasing the concentration of Tang® in the first daily session reduced ethanol intake whereas intake of the standard solution was increased in the second session. Ethanol consumption was decreased by only 27% when the flavorant was removed. In summary, alterations that reduced intake in the first daily session resulted in compensatory increases in ethanol intake in the second session suggesting that animals sought a specific level of ethanol intake per day. It is concluded that models with excellent face validity (flavored beverages) can produce reliable ethanol intake in patterns that are highly consistent with ethanol-seeking behavior.

Keywords

alcohol; ethanol; nonhuman primate; rhesus macaque; self-administration

1. INTRODUCTION

The over-consumption of ethyl alcohol (ethanol; “alcohol”) remains a worldwide public health concern. For example the number of adults in the United States who abuse alcohol or are alcohol dependent rose from 13.8 million (7.4%) in 1991–1992 to 17.6 million (8.5%) in 2001–2002 as estimated by the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC; Grant et al., 2004). The abuse of alcohol among adolescent populations is also a significant and continuing problem; the Monitoring the Future survey shows that in 2005 the

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monthly prevalence of alcohol use was 17% for 8th graders, 33% for 10th graders, and 47% for 12th graders (Johnston et al., 2006). Furthermore, substantial numbers of these cohorts report that they have been drunk in the last 30 days (6% of 8th graders, 18% of 10th graders, 30% of 12th graders) as well as that they had consumed five or more drinks on one occasion in the past fortnight (11% of 8th graders, 21% of 10th graders and 28% of 12th graders). The age of initiation of alcohol drinking is important since individuals who begin drinking before age 15 are four times more likely to develop alcohol dependence than those who delay drinking until age 21 (Grant and Dawson, 1997). Evidence also suggests that adolescents who consume alcohol often exhibit enduring cognitive and brain functional impairment (Brown et al., 2000; Schweinsburg et al., 2005; Tapert et al., 2001; Tapert and Brown, 1999; Tapert and Schweinsburg, 2005). Such observations suggest a sensitive developmental window for adverse consequences of exposure and recommends additional research to fully understand the mechanisms which underlie the neurobiological and behavioral consequences of alcohol exposure.

Many controlled laboratory studies on the neurobiological and behavioral mechanisms of ethanol drinking have been conducted in rodents; nonhuman primate models are rarer but offer significant advantages for evaluating the broad spectrum of consequences of alcohol abuse (Grant and Bennett, 2003; Katner et al., 2004). Briefly, nonhuman primate models provide enhanced genetic similarity to humans, wide behavioral and social repertoires and protracted developmental stages of interest such as adolescence. Most importantly macaque monkeys exhibit relatively high preferences for consuming alcohol that appear similar to humans and unlike unselected rodent strains. Rhesus monkeys will drink low concentrations of alcohol (1 and 2% (w/v)) in tap water with minimal training history (Stewart et al., 1996) although total alcohol consumption under such conditions is typically under 0.5 grams of ethanol per kilogram of bodyweight, equivalent to about 2–3 standard drinks. It has been shown that monkeys' oral ethanol consumption can be enhanced by a variety of induction techniques, in particular the addition of a flavorant and/or sweetener to ethanol can greatly increase levels of ethanol consumption in monkeys, especially at ethanol concentrations above 2% (w/v) (Cadell and Cressman, 1972; Cressman and Cadell, 1971; Crowley et al., 1983; Crowley et al., 1990; Erwin et al., 1979; Fincham et al., 1986; Fitz-Gerald et al., 1968; Grant and Johanson, 1988; Higley et al., 1996; Shelton and Grant, 2001; Vivian et al., 1999; Williams and Woods, 1999). Such paradigms perhaps offer the best face validity since the vast majority of 8th–12th graders who report drinking alcohol also report use of “flavored drinks” (Johnston et al., 2006).

Although one of the significant advantages of nonhuman primate subjects is the long lifespan relatively few studies have exposed monkeys to ethanol over intervals greater than a few months. The available evidence suggests, however, that monkeys will readily consume ethanol over periods of 9–24 months (Budygin et al., 2003; Crowley and Andrews, 1987; Crowley et al., 1983; Vivian et al., 2001). The present studies were designed to determine the long term stability of ethanol consumption in periadolescent monkeys using a limited access (1 hr) ethanol-fade procedure which produced substantial ethanol consumption (>1.5 g/kg minimum; 3.0 g/kg maximum per day) in rhesus macaques (Katner et al., 2004). One of the concrete advantages to the limited time of access approach in this model is that it consistently produces the high levels of intake over a short period of time thereby resulting in high blood alcohol levels (BALs) on a consistent basis. The second major goal of this study was to determine the extent to which ethanol drinking depended on the presence of the flavorant. Despite obvious face validity inherent in this approach it is important to demonstrate in animal models that the ethanol is sought and not merely tolerated, i.e., as a side effect of a desired flavorant.

For these studies a group of rhesus macaques previously trained to consume ethanol using the fading procedure were re-induced to drink after a 14 month period of imposed abstinence. A second daily limited ethanol access session was then incorporated to determine if daily intake

increased and animals were evaluated for a further 7 months to determine if ethanol intake would increase, decrease or remain stable. Additional experiments were conducted to determine if ethanol intake was altered by the simultaneous presentation of the vehicle (Tang®) with the ethanol/Tang® solution, by presentation of the vehicle prior to the ethanol session, by alteration of the Tang® concentration in the ethanol/Tang® solution, by alteration of the ethanol concentration, or by removal of the sweetener from the ethanol solution. In total, these studies were designed to determine the factors which control ethanol intake in a flavorant-maintained oral consumption model.

2. MATERIALS AND METHODS

2.1 Animals

Four periadolescent male rhesus monkeys (*Macaca mulatta*, Chinese origin; ~4 years of age, 5–6 kg) participated in the present study. Animals (421, 426, 428, 429) were previously trained to consume ethanol (Katner et al., 2004) but had not received ethanol during a 14 month abstinence period prior to the present study. Animals were individually housed and fed twice per day in their home cages. The animals' normal diet (Lab Diet 5038, PMI Nutrition International) was supplemented with fruit or vegetables seven days per week. Chow amounts provided were as determined by the veterinarians and ranged from 250–300 grams/day for this study. Body condition scores (Clingerman and Summers, 2005) ranged from 1.75 to 2.5 out of 5.0 throughout the study. Water was available *ad libitum* in the home cage, except one hour prior to, and during, the ethanol limited access sessions. All animals were immobilized with ketamine in doses of 5–10 mg/kg (i.m.) no less than semiannually for the purposes of routine care and health monitoring. The United States National Institutes of Health guidelines for laboratory animal care (Clark et al., 1997) were followed, and all protocols were approved by the Institutional Animal Care and Use Committee of The Scripps Research Institute.

2.2 Oral Ethanol Self-Administration Procedure

The ethanol solution was made available in the home cage during daily (M-F) sessions of 1 hr duration (i.e., limited access) via normal drinking bottles beginning at 0900. To preclude water satiation at the beginning of the sessions, cage water was not available for the 1 hr period preceding ethanol availability, except as noted. The ethanol solution was the only liquid available in the home cage for the duration of the session, following which the ethanol was removed and the drinking water restored.

Oral ethanol self-administration was induced with a procedure in which the concentration (%) and/or amount (g/kg) of ethanol in a palatable solution (Tang®; Kraft Foods, Inc.; an orange flavored, sugar sweetened, powdered beverage product) was gradually increased over a series of daily limited-access sessions, as previously described (Katner et al., 2004). The stages employed for this re-induction varied slightly from the original (Table 1). After the last treatment phase for ethanol induction was completed (31 drinking sessions), animals were thereafter maintained on a 6% (w/v) ethanol / 6% (w/v) Tang® solution with a 3.0 g/kg ethanol limit as the standard condition.

2.2.1 Additional daily limited access session—An additional daily limited access session was introduced on the sixty-ninth drinking session (“Day 69”) of the present study. The usual 1 hr morning session (AM session; 6% EtOH / 6% Tang, 3.0 g/kg limit) began at 0900 and an additional 30 min afternoon session (PM session; 6% EtOH / 6% Tang, 2.0 g/kg limit) began at 1:00 pm. On Day 90 the amount of ethanol available during the PM session was increased to 3.0 g/kg. The effect of altering the timing of the PM session relative to the AM session was examined on Days 133–138 during which the start of the PM session was pseudorandomly varied between 1100, 1300 and 1500 hrs (each condition was double

determined). On Day 159 the PM session was lengthened to 1 hr of availability for the remainder of the study and on Day 168 the start of the PM session was permanently moved to 1500.

2.2.2 Choice Testing

2.2.2.1 Concurrent Choice Test: The effect of concurrent vehicle (Tang) availability on ethanol intake was examined on Days 23–27 of the induction phase. For this experiment one bottle containing Tang® only and one bottle containing 6% EtOH/Tang® were made available concurrently. Conditions tested included “High Volume Vehicle”, an amount equivalent to the volume required for an intake of 2.5 g/kg of 6% ethanol/Tang® (mean 253 mls) and “Low Volume Vehicle”, an amount equivalent to the volume required for an intake of 0.5 g/kg of 6% ethanol/Tang® (mean 50 mls). Ethanol intake under these conditions was compared with mean intake in the subsequent 4 baseline sessions (2.5 g/kg ethanol limit; 6% ethanol plus 6% Tang® only). The impact of concurrent Tang® availability was re-assessed in the AM session on Days 112–114 during which High, Middle and Low volume vehicle conditions were tested. In this latter experiment, the vehicle volumes were equivalent to those required for an ethanol intake of 3.0, 1.5 and 0.5 g/kg given the 6% ethanol/Tang® solution (mean of 343, 172, and 56 mls, respectively). Tang® Volume conditions were assessed in pseudorandomized order and intake under these choice conditions was compared with mean AM session intake in the prior 5 baseline sessions (3.0 g/kg ethanol limit; 6% ethanol plus 6% Tang® only).

2.2.2.2 Volume Test: The effect of vehicle availability during the 1 hr AM session on ethanol intake during the 30 min PM session (6% ethanol / 6% Tang; 3.0 g/kg limit) was determined on Days 90–97. Conditions presented in the AM session included Low and High volumes of 6% Tang, as above. In addition a 2% ethanol / 6% Tang® (1.0 g/kg limit, thus volume equivalent to High Tang® volume) AM condition and a 1g/kg of 6% ethanol / 6% Tang® AM condition (thus volume equivalent to Low Tang® volume) were included. Ethanol intake for the PM session under these conditions was compared with mean ethanol intake during eight baseline sessions, intermingled with the test days, in which the standard 6% ethanol / 6% Tang® (3.0 g/kg limit) solution was presented.

2.2.2.3 Ethanol Concentration Test: The effect of varying the concentration of ethanol (2, 4, 8, 16, 32% (w/v)) during AM sessions was examined on Days 186–195. The standard 6% ethanol / 6% Tang® was presented during PM sessions and the limit on ethanol intake was 3.0 g/kg for both the AM and PM sessions. Ethanol concentrations were presented in a Latin-Square design with double determination. The 32% ethanol condition had to be repeated a third time for two animals because manipulation of the spout prevented a determination of intake on one occasion each.

2.2.2.4 Tang® Concentration Test: The effect of varying the concentration of Tang® (0.5, 1, 2, 6, 10 and 14% (w/v)) in the AM ethanol solution was determined during Days 82–89. The 6% ethanol / 6% Tang® solution was available for the 30 min PM sessions. Tang® concentrations were presented in a pseudorandomized order and limits on ethanol availability were 3.0 and 2.0 g/kg for the AM and PM sessions respectively.

2.2.2.5 Unflavored Ethanol Testing: A 6% ethanol solution in tap water (i.e., no Tang) was presented to animals during both the AM and PM sessions on Days 198–199. Ethanol intake for these days was compared with baseline intake (6% ethanol / 6% Tang) in the 5 prior sessions. The limit on ethanol intake was 3.0 g/kg for both the AM and PM sessions.

2.3 Blood Alcohol Levels

Blood alcohol levels (BALs) were assessed following sessions in which animals had the opportunity to consume up to 3.0 g/kg of ethanol in the standard 6% EtOH / 6% Tang® solution. Blood samples were collected from the femoral vein under ketamine (10 mg/kg; i.m.) anesthesia immediately after the end of the limited access session on three separate occasions, i.e., after the AM session on Days 58 and 121 and after the PM session for Day 200. Serum was separated from blood cells by centrifugation and analyzed for ethanol content with an Analox AM1 ethanol analyzer (Analox Instruments USA, Lunenburg, MA). BALs were expressed as mg% (i.e., mg/dl).

2.4 Data Analysis

One-way repeated measures analysis of variance (ANOVA) was used to analyze the effects of time (month) on average total (AM + PM) daily ethanol intake as well as the effects of altering PM session time of day, concurrent choice testing, volume testing and Tang® concentration testing on ethanol intake (g/kg). Two-way repeated measures ANOVA were used to examine the effects of month on average daily AM and PM ethanol intake (g/kg) and the effects of ethanol concentration testing or unflavored ethanol testing on AM and PM ethanol intake (g/kg). Post-hoc analyses of significant main effects confirmed by the ANOVAs were conducted using the Newman-Keuls test. All analyses were performed using a commercial statistical software package (GB-STATv7.0; Dynamic Microsystems, Silver Spring MD) and in all tests the criterion for significance was $p < 0.05$.

3. RESULTS

3.1 Ethanol Induction and Maintenance

The ethanol fading procedure was successful in re-inducing animals to consume ethanol (Figure 1). Once the amount of ethanol available was 2.0 g/kg or greater, animals appeared to self-regulate their ethanol intake as in the prior study (Katner et al., 2004). The group consumed an average ($\pm$ SEM) of 1.9 ± 0.2 g/kg of ethanol over the last five days of the initial, single daily session phase (Days 54–58). The mean daily ethanol intake during the AM and PM limited access sessions, as well as the mean total (AM + PM) daily ethanol intake for each month is illustrated in Figure 2. The data include only “standard” days in which none of the experimental manipulations described below were performed. Mean ($\pm$ SEM) total daily ethanol intake across this 7 month period was 2.7 ± 0.2 g/kg and monthly intake was stable; no main effect of month was confirmed in the analysis of daily intake. Intake during the AM session was significantly greater than intake during the PM session (main effect of session time [$F_{(1,55)} = 29.32$; $p < 0.02$]) and this relationship was altered over time (significant interaction between session time and month [$F_{(6,55)} = 29.19$; $p < 0.0001$]). Post hoc analysis confirmed that AM session intake was significantly greater than PM intake for months 1–4 and 6. Delaying the start of the second session increased mean ($\pm$ SEM) ethanol intake (Figure 2B). The ANOVA confirmed a main effect of second session time [$F_{(2,11)} = 10.37$; $p < 0.02$] and the post hoc test confirmed that ethanol intake was significantly greater when ethanol was presented at 3pm compared to 11am or 1pm.

3.2 Blood Alcohol Levels

The consumption of the 6% ethanol / 6% Tang® solution resulted in BALs (Figure 3) that are consistent with other macaque studies (Green et al., 1999; Vivian et al., 2001) as well as our prior report (Katner et al., 2004). A linear fit to the data estimates that the consumption of 1.0, 1.5, 2.0 and 2.5 g/kg of ethanol corresponds with BALs of 74, 98, 122 and 146 mg%, respectively.

3.3 Choice Testing

3.3.1 Concurrent choice test—Animals consistently consumed the Tang® vehicle first then drank some of the 6% ethanol / 6% Tang® solution when the ethanol solution was presented concurrently with larger and smaller volumes of Tang® vehicle (main effect of choice condition on ethanol intake in the first [$F_{(2,11)} = 5.61$; $p < 0.05$; Figure 4A] and second [$F_{(3,15)} = 9.55$; $p < 0.004$; Figure 4B] concurrent choice tests). Post hoc tests further confirmed that ethanol intake was only significantly reduced under the High Volume condition in comparison with the other conditions for each of the studies. Ethanol intake in the first and second High Volume conditions averaged 0.9 and 0.7 g/kg respectively.

3.3.2 Volume testing—Presentation of different volumes of Tang® or the dose-limited Tang® / ethanol solution during the AM session increased intake during the PM session for all conditions (Figure 5). The ANOVA confirmed a main effect of the AM session manipulations on PM ethanol intake [$F_{(4,19)} = 6.93$; $p < 0.004$] and the post hoc analyses confirmed that the smaller and larger volumes of Tang® vehicle, and 1.0 g/kg of 2% ethanol / Tang® during the AM sessions significantly increased PM session ethanol intake compared to ethanol intake during the PM session under baseline conditions.

3.3.3 Ethanol concentration testing—Altering the concentration of ethanol in the solution during the AM session did not significantly alter ethanol intake during the AM session nor during the PM session in which the standard 6% ethanol / 6% Tang® solution was presented (Figure 6A). In addition there was no significant effect on total daily ethanol intake.

3.3.4 Tang® concentration testing—Altering the concentration of Tang® in the solution during the AM session decreased ethanol intake for the AM session [$F_{(5,23)} = 12.12$; $p < 0.0001$] and increased intake for the PM sessions [$F_{(5,23)} = 7.62$; $p < 0.002$] as is illustrated in Figure 6B. Post hoc comparisons confirmed that presentation of the 6% ethanol / 0.5% Tang® or 6% ethanol / 1% Tang® solution during the AM session significantly decreased ethanol intake compared to standard 6% Tang® / 6% AM ethanol condition. In addition, post hoc comparisons confirmed that the presentation of 6% ethanol / 1% Tang® during the AM session significantly increased PM ethanol intake under standard 6% ethanol / 6% Tang® availability. Total daily intake was not significantly changed by any Tang® concentration in the AM session.

3.3.5 Unflavored ethanol testing—Removing the Tang® from the ethanol solution in both daily sessions reduced intake by about 27% as is illustrated in Figure 6C. A main effect of Tang® on ethanol intake was confirmed by the ANOVA [$F_{(1,15)} = 40.39$; $p < 0.008$] but there was no effect of session nor an interaction.

4. DISCUSSION

The present results show that an ethanol-fading procedure with limited access sessions produces consistently high levels of voluntary oral ethanol intake, across many months, in adolescent male rhesus monkeys. The drinking behavior was very stable as was demonstrated in a series of concurrent and sequential choice manipulations. Overall the findings show that monkeys seek to consume pharmacologically relevant levels of ethanol and closely regulate their daily intake. This model is therefore highly useful for additional study of the etiology and consequences of adolescent drinking behavior.

The fading procedure was successful in re-inducing animals to consume approximately 2.0 g/kg of ethanol and once the amount of ethanol available was 2.0 g/kg or greater animals appeared to self-regulate their ethanol intake as observed in our previous study (Katner et al., 2004). The blood alcohol levels (~80–180 mg%) determined on three occasions suggest that the amount

of ethanol consumed was pharmacologically relevant. Total daily ethanol consumption averaged 2.7 g/kg and remained relatively stable over a period of 7 months when two access sessions were provided. Few studies have examined ethanol consumption in nonhuman primates over protracted periods, however, cynomolgus macaques have been shown to reach stable levels of ethanol intake (2.2 g/kg/day in 16–22 hr sessions) over 180 days of maintenance after a 90 day induction period (Vivian et al., 2001) and pigtail macaques consumed 1.76 g/kg in 2 hr sessions over 450 consecutive sessions across approximately 2 yrs (Crowley and Andrews, 1987; Crowley et al., 1983). The present study found that the use of two discrete access sessions resulted in intakes of similar magnitude to what was reported in these prior studies. Thus an important outcome was that the improved experimental control over the timing of drinking inherent in this approach did not apparently lead to reduced overall ethanol exposure. Over the course of the 7 month maintenance phase ethanol intake during the PM session gradually increased, while ethanol intake during the AM session gradually decreased, resulting in similar levels of intake for the two sessions. It was also found in a specific experiment with mixed order of inter-session interval that moving the second access session to the latest time of the day enhanced ethanol intake in the second session. This suggests that animals are more likely consume greater amounts of ethanol if the second limited access session is available at a more distant time relative to the first limited access session.

Simultaneous choice tests were performed in order to determine if animals would continue to consume ethanol when given the choice between ethanol and Tang® solutions. These tests found that the concurrent presentation of small to moderate volumes of Tang® and ethanol had no effect on ethanol consumption. Although the simultaneous presentation of large volumes of Tang® and ethanol resulted in statistically reliable reductions, ethanol intake was still 0.7–0.9 g/kg, equivalent to ~3–4 standard drinks. In all cases the Tang® vehicle was consumed first and the ethanol/Tang® solution second. Together these findings support the hypothesis that while Tang® is the preferred reinforcer animals still seek to consume significant amounts of ethanol even immediately after drinking Tang.

Sequential choice tests were performed in order to further investigate the potential role of acute fluid satiety which was identified in the concurrent choice test. Increases in ethanol intake were observed during the PM session following AM presentation of small and large volumes of the Tang® vehicle or of 1.0 g/kg of ethanol, although the latter effect was only statistically reliable when 1 g/kg was provided in 2% solution (with the volume being equivalent to the larger volume Tang condition). Nevertheless these findings further suggest that the reductions in ethanol intake observed during concurrent choice testing with large volumes of vehicle were possibly due to animals' unwillingness or inability to consume this amount of fluid within a 1 hr period. Furthermore the compensatory increases in ethanol intake for the PM session, in response to experimentally-induced reductions in AM ethanol intake, suggest that animals sought a consistent amount of ethanol per day. This effect was further explored with additional sequential choice manipulations.

Alteration of the concentration of ethanol in the solution (the higher 16 and 32% conditions are equivalent to the concentration of ethanol in sherry and distilled spirits, respectively) during the AM session had no significant effect on ethanol consumption, similar to findings of previous monkey studies (Stewart et al., 1996; Vivian et al., 1999; Williams et al., 1998). Together these data suggest that the concentration of ethanol is not a significant factor in determining intake in monkeys. It should be recognized however that the current animals had a substantial history of ethanol drinking prior to these tests (185 days), which may have aided their ability to overcome the putatively aversive taste of high concentrations of ethanol. It is also of note that when the animals were presented with the higher concentrations of ethanol (16 and 32%), and therefore fluid/Tang® satiety might predict increased consumption, animals

continued to titrate their ethanol dose. This further supports the hypothesis that monkeys seek a consistent amount of ethanol per day in this model.

In contrast to the effect of *ethanol* concentration the concentration of the *flavorant* did influence ethanol intake. When the concentration of Tang® was limited to 0.5–1% during the AM session, ethanol intake was reduced from approximately 2.0 to 1.0 g/kg. Ethanol intake during the following PM session was enhanced which is, again, consistent with compensatory drinking. Only lowered Tang® concentration had any effect in this experiment since the 10 and 14% Tang® conditions did not reliably change ethanol intake. This latter observation is consistent with the general conclusion that monkeys closely regulate their ethanol intake in this model.

Finally, although the presentation of unflavored ethanol in tap water reduced ethanol intake, levels of ethanol intake still remained high with total daily intake of 1.9 g/kg or ~8–10 standard drinks. Continued consumption of this amount of ethanol despite the removal of the sweetener strongly supports the interpretation from the other experimental manipulations that animals were seeking the pharmacological effects of the ethanol itself. Although in the present study the sweetener was only removed for two consecutive days a subsequent study in different animals found a similar effect when ethanol was presented in tap water for 15 consecutive sessions (unpublished data). Such findings are also consistent with a prior study in which rhesus macaques that drank large amounts of aspartame-sweetened ethanol continued to do so when the flavorant was removed (Vivian et al., 1999).

In conclusion the present study examined the role of several factors regulating ethanol intake within a “cocktail” model of consumption in periadolescent rhesus macaques. The findings suggest strongly that a flavorant may facilitate, but does not exclusively motivate, high levels of ethanol drinking in monkeys; the parallels with human adolescent drinking are unmistakable. Although the animals consumed less ethanol when the concentration of the flavorant was decreased to low or nonexistent levels, intake levels were still likely to be pharmacologically significant. On a methodological level, it was shown that this experimental procedure is effective in producing significant and stable levels of ethanol intake in rhesus macaques over a protracted period. This demonstrates the significant utility of this approach for the examination of ethanol’s long term effects in nonhuman primates. The simplicity of this procedure and the temporal control over drinking are also seen as unique advantages for researchers seeking to determine the effects of chronic ethanol exposure. The paradigm is, for example, readily adapted to studies as diverse as investigating the long-term cognitive effects of adolescent alcohol drinking, determining influences of immunodeficiency virus disease progression or characterizing potential pharmacotherapy for alcohol abuse.

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References

- Brown SA, Tapert SF, Granholm E, Delis DC. Neurocognitive functioning of adolescents: effects of protracted alcohol use. *Alcohol Clin Exp Res* 2000;24:164–171. [PubMed: 10698367]
- Budygin EA, John CE, Mateo Y, Daunais JB, Friedman DP, Grant KA, Jones SR. Chronic ethanol exposure alters presynaptic dopamine function in the striatum of monkeys: a preliminary study. *Synapse* 2003;50:266–268. [PubMed: 14515345]
- Cadell, TE.; Cressman, R. Group social tension as a determinant of alcohol consumption in *Maccaca mulatta*. In: Goldsmith, EI.; Moor-Jankowski, J., editors. *Medical primatology, Part III, Infectious*

- diseases, oncology, pharmacology, and toxicology, cardiovascular studies, Third Conference on Experimental Medicine and Surgery in Primates. Karger: New York, Lyons, France; 1972. p. 250-259.
- Clark JD, Gebhart GF, Gonder JC, Keeling ME, Kohn DF. Special Report: The 1996 Guide for the Care and Use of Laboratory Animals. *Ilar J* 1997;38:41–48. [PubMed: 11528046]
- Clingerman KJ, Summers L. Development of a body condition scoring system for nonhuman primates using *Macaca mulatta* as a model. *Lab Anim (NY)* 2005;34:31–36. [PubMed: 15861156]
- Cressman RJ, Cadell TE. Drinking and the social behavior of rhesus monkeys. *Q J Stud Alcohol* 1971;32:764–774. [PubMed: 5000180]
- Crowley TJ, Andrews AE. Alcoholic-like drinking in simian social groups. *Psychopharmacology (Berl)* 1987;92:196–205. [PubMed: 3110841]
- Crowley TJ, Weisbard C, Hyding-MacDonald MJ. Progress toward initiating and maintaining high-dose alcohol drinking in monkey social groups. *J Stud Alcohol* 1983;44:569–590. [PubMed: 6685212]
- Crowley TJ, Williams EA, Jones RH. Initiating ethanol drinking in a simian social group in a naturalistic setting. *Alcohol Clin Exp Res* 1990;14:444–455. [PubMed: 2378430]
- Erwin J, Drake D, Kassorla E, Deni R. Drinking of ethanol by adult and infant rhesus monkeys (*Macaca mulatta*). *J Stud Alcohol* 1979;40:301–306. [PubMed: 109702]
- Fincham JE, Jooste PL, Seier JV, Taljaard JJ, Weight MJ, Tichelaar HY. Ethanol drinking by vervet monkeys (*Cercopithecus pygerythrus*): individual responses of juvenile and adult males. *J Med Primatol* 1986;15:183–197. [PubMed: 2874229]
- Fitz-Gerald FL, Barfield MA, Warrington RJ. Voluntary alcohol consumption in chimpanzees and orangutans. *Q J Stud Alcohol* 1968;29:330–336. [PubMed: 5662826]
- Grant BF, Dawson DA. Age at onset of alcohol use and its association with DSM-IV alcohol abuse and dependence: results from the National Longitudinal Alcohol Epidemiologic Survey. *J Subst Abuse* 1997;9:103–110. [PubMed: 9494942]
- Grant BF, Dawson DA, Stinson FS, Chou SP, Dufour MC, Pickering RP. The 12-month prevalence and trends in DSM-IV alcohol abuse and dependence: United States, 1991–1992 and 2001–2002. *Drug Alcohol Depend* 2004;74:223–234. [PubMed: 15194200]
- Grant KA, Bennett AJ. Advances in nonhuman primate alcohol abuse and alcoholism research. *Pharmacol Ther* 2003;100:235–255. [PubMed: 14652112]
- Grant KA, Johanson CE. Oral ethanol self-administration in free-feeding rhesus monkeys. *Alcohol Clin Exp Res* 1988;12:780–784. [PubMed: 3064639]
- Green KL, Szeliga KT, Bowen CA, Kautz MA, Azarov AV, Grant KA. Comparison of ethanol metabolism in male and female cynomolgus macaques (*Macaca fascicularis*). *Alcohol Clin Exp Res* 1999;23:611–616. [PubMed: 10235296]
- Higley JD, Suomi SJ, Linnoila M. A nonhuman primate model of type II excessive alcohol consumption? Part 1. Low cerebrospinal fluid 5-hydroxy indoleacetic acid concentrations and diminished social competence correlate with excessive alcohol consumption. *Alcohol Clin Exp Res* 1996;20:629–642. [PubMed: 8800378]
- Johnston, LD.; O'Malley, PM.; Bachman, JG.; Scheulenberg, JE. Monitoring the Future national survey results on drug use, 1975–2005. Volume I, Secondary school students (NIH Publication No 06-5883). Bethesda, MD: National Institute on Drug Abuse; 2006. p. 715
- Katner SN, Flynn CT, Von Huben SN, Kirsten AJ, Davis SA, Lay CC, Cole M, Roberts AJ, Fox HS, Taffe MA. Controlled and behaviorally relevant levels of oral ethanol intake in rhesus macaques using a flavorant-fade procedure. *Alcohol Clin Exp Res* 2004;28:873–883. [PubMed: 15201630]
- Schweinsburg AD, Schweinsburg BC, Cheung EH, Brown GG, Brown SA, Tapert SF. fMRI response to spatial working memory in adolescents with comorbid marijuana and alcohol use disorders. *Drug Alcohol Depend* 2005;79:201–210. [PubMed: 16002029]
- Shelton KL, Grant KA. Effects of naltrexone and Ro 15-4513 on a multiple schedule of ethanol and Tang® self-administration. *Alcohol Clin Exp Res* 2001;25:1576–1585. [PubMed: 11707632]
- Stewart RB, Bass AA, Wang NS, Meisch RA. Ethanol as an oral reinforcer in normal weight rhesus monkeys: dose-response functions. *Alcohol* 1996;13:341–346. [PubMed: 8836321]
- Tapert SF, Brown GG, Kindermann SS, Cheung EH, Frank LR, Brown SA. fMRI measurement of brain dysfunction in alcohol-dependent young women. *Alcohol Clin Exp Res* 2001;25:236–245. [PubMed: 11236838]

- Tapert SF, Brown SA. Neuropsychological correlates of adolescent substance abuse: four-year outcomes. *J Int Neuropsychol Soc* 1999;5:481–493. [PubMed: 10561928]
- Tapert SF, Schweinsburg AD. The human adolescent brain and alcohol use disorders. *Recent Dev Alcohol* 2005;17:177–197. [PubMed: 15789866]
- Vivian JA, Green HL, Young JE, Majerksy LS, Thomas BW, Shively CA, Tobin JR, Nader MA, Grant KA. Induction and maintenance of ethanol self-administration in cynomolgus monkeys (*Macaca fascicularis*): long-term characterization of sex and individual differences. *Alcohol Clin Exp Res* 2001;25:1087–1097. [PubMed: 11505038]
- Vivian JA, Higley JD, Linnoila M, Woods JH. Oral ethanol self-administration in rhesus monkeys: behavioral and neurochemical correlates. *Alcohol Clin Exp Res* 1999;23:1352–1361. [PubMed: 10470978]
- Williams KL, Winger G, Pakarinen ED, Woods JH. Naltrexone reduces ethanol- and sucrose-reinforced responding in rhesus monkeys. *Psychopharmacology (Berl)* 1998;139:53–61. [PubMed: 9768542]
- Williams KL, Woods JH. Naltrexone reduces ethanol- and/or water-reinforced responding in rhesus monkeys: effect depends upon ethanol concentration. *Alcohol Clin Exp Res* 1999;23:1462–1467. [PubMed: 10512311]

Figure 1.

Re-induction of ethanol drinking in periadolescent monkeys. Mean ($N=4$; $\pm$ SEM) ethanol intake (g/kg) is presented for 1 hr limited access sessions. The x-axis represents sequential drinking sessions; the different ethanol treatment stages (see Table 1) are indicated below the axis.

Figure 2.

A) The mean ($N=4$; $\pm$ SEM) daily ethanol intake over seven months is presented for AM and PM limited access sessions. A significant change in AM intake relative to the first month is indicated by * and a significant change in PM intake relative to the first month is indicated by #. A significant difference between AM and PM intake for a given month is indicated by @. B) The effect of altering the start time of the second session on mean ($N=4$; $\pm$ SEM) ethanol intake in the second session is presented; the first session was 0900–1000 for all conditions. A significant difference from the other two conditions is indicated by *.

Figure 3.

Blood alcohol levels (BALs; mg%) are presented for individual animals ($N = 4$) as a function of the amount of ethanol consumed. All animals had the opportunity to consume 3.0 g/kg of 6% (w/v) ethanol plus 6% (w/v) Tang® during a 1 hr limited access session. Blood samples were collected from the same animals immediately after the end of the limited access session on three separate occasions. For sessions 58 and 121 the samples were taken after the AM session and for session 200, BALs were taken after the PM session. Linear regression equation: $y = 48.06x + 25.84$; $r = 0.67$.

Figure 4.

The effect of concurrent vehicle availability on mean ($N=4$; $\pm$ SEM) ethanol intake during the AM 1 hr limited access session is illustrated for the first and second concurrent choice tests. A) The first test was conducted during Days 23–27. The High Tang® Volume was an amount equivalent to the Tang® volume required for 2.5 g/kg ethanol and the Low Tang® Volume was an amount equivalent to the Tang® volume required for 0.5 g/kg ethanol. Intake under choice conditions is compared with mean intake in the subsequent 4 normal sessions in which ethanol/Tang® solution was provided (2.5 g/kg ethanol limit). The * indicates a significant difference from baseline intake. B) The second test was conducted during Days 112–144. The High Tang® Volume condition in this case was equivalent to the Tang® volume required for

3.0 g/kg ethanol (the maintenance condition at that time) and the Low and Mid Tang® Volumes were equivalent to that required for 0.5 and 1.5 g/kg ethanol, respectively. The baseline was derived from mean intake in the prior 5 baseline sessions (3.0 g/kg ethanol limit; ethanol only). The * indicates a significant difference relative to all other conditions.

Figure 5.

The effects of altering AM access conditions on mean ($N=4$; $\pm$ SEM) ethanol intake in the AM and PM sessions are illustrated. In all conditions, animals had the opportunity to consume 6% ethanol plus Tang® with a 3.0 g/kg limit in the PM session. In the Lo and Hi Veh conditions, volumes of Tang® without ethanol, equivalent to that of the ethanol/Tang® solution required for intake of 0.5 and 3.0 g/kg ethanol, respectively, were presented in the AM session. Grey bars for the Lo and Hi Veh conditions during the AM session represent the vehicle volume translated to putative g/kg ethanol units for comparison. In the 2% ethanol condition, a 2% ethanol plus Tang® (1.0 g/kg ethanol limit) solution was presented in the AM session, while in the 1 g/kg condition, 1 g/kg of a 6% ethanol plus Tang® was presented in the AM session. PM session intake under these conditions is compared with mean intake for baseline sessions in which the normal 6% ethanol / 6% Tang® (3 g/kg limit) solution was presented in the AM. A significant difference from the PM baseline condition is indicated by the *.

Figure 6.

The effects of altering AM and/or PM access conditions on mean ($N=4$; $\pm$ SEM) ethanol intake are illustrated. **A) Ethanol concentration testing.** The effect of varying the concentration of ethanol in the 6% Tang® solution during AM sessions is presented. The standard 6% ethanol / 6% Tang® solution was presented in the PM sessions. Each condition was repeated twice. No significant differences in intake were observed. **B) Tang® concentration testing.** The effects of varying the concentration of the Tang® used to flavor the 6% ethanol solution was altered for the AM session and the standard 6% ethanol / 6% Tang® solution was available in the PM sessions. Significant differences from the AM session under baseline (6% ethanol plus 6% Tang) conditions are indicated by *, while a significant difference from the PM session

following the AM baseline is indicated by #. C) Unflavored ethanol testing. The effect of removing the Tang® from the ethanol solution in both the AM and PM sessions is illustrated. Intake for the baseline (6% ethanol / 6% Tang) condition represents average ethanol intake for 5 days prior to testing, while the 6% ethanol (No Tang) condition represents a two day average of ethanol intake during testing. The * indicates a significant difference in total daily intake.

Table 1

Ethanol treatment phases for the re-induction of oral ethanol self-administration. The indicated concentration of ethanol was incorporated in the 6% (w/v) Tang® vehicle.

Ethanol Concentration	Days	Session Limit (g/kg)
1% (w/v)	3	0.5 g/kg
2% (w/v)	3	0.5 g/kg
4% (w/v)	3	0.5 g/kg
4% (w/v)	3	1.0 g/kg
6% (w/v)	3	1.0 g/kg
6% (w/v)	3	1.5 g/kg
6% (w/v)	4	2.0 g/kg
6% (w/v)	4	2.5 g/kg