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-h 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 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, and 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 g of ethanol per kilogram of bodyweight, equivalent to about two to three 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, 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 typically have high preferences for consuming alcohol that appear similar to 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 g of ethanol per kilogram of bodyweight, equivalent to about two to three 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, 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 primates 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 h) 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 depends 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 that 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, and 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 7 days per week. Chow amounts provided were as determined by the veterinarians and ranged from 250 to 300 g/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 1 h 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 h duration (i.e., limited access) via normal drinking bottles beginning at 09:00 h. To preclude water satiation at the beginning of the sessions, cage water was not available for the 1-h 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 69th drinking session (“Day 69”) of the present study. The usual 1 h morning session (AM session; 6% EtOH/6% Tang®, Table 1 Ethanol treatment phases for the re-induction of oral ethanol self-administration

<table>
<thead>
<tr>
<th>Ethanol concentration (% w/v)</th>
<th>Days</th>
<th>Session limit (g/kg)</th>
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<tbody>
<tr>
<td>1</td>
<td>3</td>
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<td>4</td>
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The indicated concentration of ethanol was incorporated in the 6% (w/v) Tang® vehicle.
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 ml) 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 ml). Ethanol intake under these conditions was compared with mean intake in the subsequent four 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 ml, respectively). Tang® volume conditions were assessed in pseudorandomized order and intake under these condition choices was compared with mean AM session intake in the prior five 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 h 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 1 g/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, and 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 five 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 on 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 (Fig. 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 (±S.E.M.) of 1.9 ± 0.2 g/kg of ethanol over the last 5 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
Fig. 2. (A) The mean (N=4±S.E.M.) daily ethanol intake over 7 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 in 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±S.E.M.) ethanol intake in the second session is presented; the first session was 09:00–10:00 h for all conditions. A significant difference from the other two conditions is indicated by *.

month is illustrated in Fig. 2. The data include only “standard” days in which none of the experimental manipulations described below were performed. Mean (±S.E.M.) 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 (±S.E.M.) ethanol intake (Fig. 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 3 p.m. compared to 11 a.m. or 1 p.m.

3.2. Blood alcohol levels

The consumption of the 6% ethanol/6% Tang® solution resulted in BALs (Fig. 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.

Fig. 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 h 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.

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; Fig. 4A] and second [F(3,15)=9.55; p<0.004; Fig. 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 (Fig. 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 (Fig. 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
Fig. 4. The effect of concurrent vehicle availability on mean (N=4; ±S.E.M.) ethanol intake during the AM 1 h 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 four 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 five baseline sessions (3.0 g/kg ethanol limit; ethanol only). The * indicates a significant difference relative to all other conditions.

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 Fig. 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 Fig. 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 h sessions) over 180 days of maintenance after a 90-day induction period.
while ethanol intake during the AM session gradually decreased, phase, ethanol intake during the PM session gradually increased, in this approach did not apparently lead to reduced overall improved experimental control over the timing of drinking inher-
in these prior studies. Thus, an important outcome was that the result in total daily intake.

represents a 2-day average of ethanol intake during testing. The * indicates a intake for 5 days prior to testing, while the 6% ethanol (No Tang®) condition represents a 2-day average of ethanol intake during testing. The * indicates a significant difference in total daily intake.

Fig. 6. The effects of altering AM and/or PM access conditions on mean (N = 4; ±S.E.M.) 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 2-day average of ethanol intake during testing. The * indicates a significant difference in total daily intake.

(Vivian et al., 2001) and pigtail macaques consumed 1.76 g/kg in 2 h sessions over 450 consecutive sessions across approximately 2 years (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-

Simultaneous choice tests were performed in order to deter-

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-h 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 even immediately after drinking Tang®.

Alteration of the concentration of ethanol in the solution (the higher 16 and 32% conditions are equivalent to the concentra-
tion of ethanol in sherry and distilled spirits, respectively) during the AM session had no significant effect on ethanol consump-
tion. 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®. 

Fluid/Tang® manipulations.

Stewart et al., 1996; Vivian et al., 1999; Williams et al., 1998). Together these data suggest that the concentration of ethanol is not a

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intake were 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-h 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 concentra-
tion of ethanol in sherry and distilled spirits, respectively) during the AM session had no significant effect on ethanol consump-
tion, 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 substan-
tial 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. Also notice 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 on 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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