Controlled and behaviorally relevant levels of oral ethanol intake in rhesus macaques using a flavorant-fade procedure.

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Abstract

BACKGROUND:

Flavorant-fading procedures can initiate and maintain oral ethanol intake in rodents. The present study developed a similar procedure to achieve controlled and behaviorally relevant levels of ethanol intake in monkeys.

METHODS:

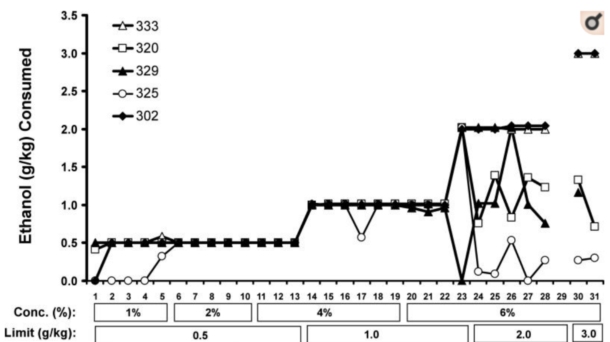
Male rhesus macaques (N = 13) were initially given the opportunity to consume 0.5 g/kg of a 1% (w/v) ethanol plus 4% (w/v) Tang solution in 1-hr limited-access sessions without the requirement of an operant response. Once consumption was stable at a particular concentration (%) and/or amount (g/kg), animals were given access to higher concentrations and/or amounts of ethanol. Animals were tested on a bimanual motor skill (BMS) task 20 and 90 min after consumption to assess behavioral impairment. Blood alcohol levels (BALs) were assessed after a session in which animals had the opportunity to consume up to 3.0 g/kg of 6% (w/v) ethanol.

RESULTS:

The gradual fading up of higher concentrations and amounts of ethanol resulted in controlled and robust levels (>2.0 g/kg) of ethanol intake in half of the subjects. Increasing the concentration of the sweetener from 4 to 6% (w/v) was effective in initiating consumption in three animals. Two monkeys required the additional step of presenting the increased-sweetener solutions after a meal (postprandial consumption) to initiate significant ethanol intake. Animals were significantly impaired on the BMS task after consumption of 2.0, 2.5, and 3.0 g/kg of ethanol. Individual consumption ranging from 0.8 to 3.0 g/kg of ethanol produced BALs of 18 to 269 mg/dl.

CONCLUSIONS:

The flavorant-fading procedure was effective in producing behaviorally relevant levels of ethanol consumption in rhesus macaques. This model facilitated a randomized-dose procedure to determine the behavioral effects of 0.5 to 3.0 g/kg of ethanol. This procedure therefore is of significant utility in determining behavioral or physiologic effects of specific doses of consumed ethanol in monkeys.



Ethanol intake (g/kg) for individual rhesus monkeys (N=5) during 1 hr limited access sessions in Experiment 1. The x-axis represents sequential drinking sessions and the different ethanol treatment stages described in Table 1.

Introduction

Forty four percent of the adult U.S. population (age 18 and over) drink alcohol and consume at least 12 drinks per year (Dawson et al., 1995). Although most individuals drink alcohol in a responsible manner, approximately 14 million Americans (17.4 percent) meet the criteria for alcohol abuse and alcoholism (Grant et al., 1994) and more than one-half of American adults have a close family member who has or has had alcoholism (Dawson and Grant, 1998). It is difficult to determine conclusively that reported perturbations in behavior, and perhaps physiology, in alcohol abusing populations are a direct result of exposure to alcohol. In many cases for example, individuals at risk for alcoholism and/or alcohol abuse may exhibit preexisting differences on the measure in question. Animal models are useful in controlling variables such as the amount of ethyl alcohol (ethanol) consumed, the duration of exposure and concurrent use of other illicit drugs. Nonhuman primates are particularly useful for evaluating the neurobiological consequences of alcoholism and alcohol abuse because many of the physiological, neuroanatomical and behavioral systems potentially affected by ethanol are more similar to humans in nonhuman primates in comparison to commonly employed rodent species. In addition, the protracted life span

of nonhuman primates makes it possible to perform more extensive and elaborate studies to determine the long-term consequences of ethanol exposure.

Previous experiments have shown that rhesus monkeys will readily consume low concentrations of ethanol (1 and 2% (w/v)) in tap water in 3 hr sessions without any training history (Stewart et al., 1996), however consumption under such conditions is typically under 0.5 grams of ethanol per kilogram of bodyweight (g/kg). This level of intake produces blood alcohol levels (BALs) of substantially less than the 0.08 BAC (i.e., 80 mg%) level which constitutes the legal limit to operate an automobile in most jurisdictions in the United States. However, oral ethanol consumption in nonhuman primates can be greatly enhanced by a variety of induction techniques. One such technique used to induce oral ethanol consumption in nonhuman primates is to gradually increase the ethanol concentration across daily 2 to 3 hr sessions until the desired concentration (e.g., 4-8%) is reached (Meisch, 1995; Stewart et al., 1996). Using such procedures, monkeys will consume an average of 1.2 to 1.5 g/kg of ethanol (Pakarinen et al., 1999; Stewart et al., 1996; Williams et al., 1998; Williams and Woods, 1999b). These studies also illustrate two findings consistent across most of the methodological techniques reviewed here. First, individual macaque consumption preferences vary widely, with some monkeys (broadly approximating 25-33%) failing to consume significant amounts (<0.5 g/kg) and some monkeys (another 25-33%) consuming large amounts (>2.0 g/kg). Second, while intake patterns within individuals are reasonably stable from day to day, it is possible to observe differences on the order of 0.5-1.5 g/kg from one session to another. Thus, the presentation of gradually increasing concentrations of ethanol to nonhuman primates is effective in inducing higher ethanol intake, however, these levels are below behaviorally relevant levels for significant fractions of the sample. Another procedure which may increase ethanol consumption in nonhuman primates is to make the ethanol available in combination with a meal. Such postprandial availability techniques are an effective way to induce ethanol consumption in both rhesus monkeys and baboons (Ator and Griffiths, 1983; Henningfield et al., 1981; Meisch and Henningfield, 1977). It has also been shown that food deprivation increases ethanol consumption in rhesus monkeys (Macenski and Meisch, 1992; Meisch and Lemaire, 1991), i.e., the majority of monkeys increased their ethanol intake when they were food restricted compared to when they were food satiated, although the average ethanol intake was only 0.25 g/kg (Meisch and Lemaire, 1991). However, in a similar study, rhesus monkeys that were slightly food restricted drank the same amount of ethanol as those that were not food restricted with an average ethanol intake of 1.5 g/kg (Pakarinen et al., 1999). These studies indicate that the presentation of gradually increasing concentrations of ethanol and post-prandial drinking can produce significant levels of ethanol intake, however, food restriction does not always enhance ethanol consumption in nonhuman primates.

Much as with humans, the addition of a flavorant and/or sweetener to ethanol can initiate and maintain significant levels of ethanol consumption in monkeys, especially at ethanol concentrations above 2% (w/v) (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, 1999a). Rhesus monkeys have been shown to consume 0.8 g/kg of ethanol sweetened with aspartame in 60 min sessions (Higley et al., 1996), 1.1 g/kg of ethanol in orange juice in 40 min sessions (Cadell and Cressman, 1972) and 1.2 g/kg of ethanol in grape drink in 100 min sessions (Cressman and Cadell, 1971). A group of pigtail macaques consumed 1.4 g/kg of 5% ethanol in grape-flavored, saccharin sweetened Kool-Aid over 2 hrs (Crowley et al., 1983) and Japanese snow monkeys consumed 0.5 to 2.0 g/kg of ethanol, attaining BALs sometimes in excess of 100 mg% with a similar protocol (Crowley et al., 1990). The initial presentation of ascending concentrations of ethanol sweetened with aspartame later produced an average *unsweetened* ethanol intake of 1.9 g/kg in rhesus monkeys versus 0.9 g/kg ethanol in a second group of oral methadone experienced rhesus monkeys (Vivian et al., 1999) in a post-prandial procedure. These findings suggest that sweetened and/or flavored ethanol solutions facilitate oral ethanol intake in several different species of nonhuman primates, presumably by masking the aversive taste of ethanol (for review see Meisch and Stewart, 1994), however, it has also been proposed that animals may consume sweetened ethanol solutions as a result of the intrinsic reinforcing properties of sweet

solutions. See Grant and Bennett (2003) for a recent exhaustive review of alcohol self-administration in nonhuman primates.

The major hypothesis under investigation in the present study was that a flavorant-fading procedure will maintain relatively high, stable levels of ethanol intake in rhesus monkeys. Similar procedures (i.e., initially presenting the ethanol at very low concentrations in a palatable sucrose or saccharin solution and then gradually fading in greater concentrations of ethanol across a number of sessions) have been used extensively to induce oral ethanol intake in rats, including in strains which will not otherwise consume pharmacologically relevant amounts of ethanol (Katner et al., 1999; Katner and Weiss, 1999; Samson et al., 1998; Samson et al., 1989), however this procedure has less frequently been applied to nonhuman primates. The present study is innovative in adapting several techniques from previous nonhuman primate oral ethanol self-administration studies and incorporated a procedure which has not been well characterized in monkeys, a sweet solution ethanol fading procedure, to produce significant levels of ethanol intake. Since the goal was to induce and maintain consistent high levels of drinking, rather than to demonstrate evidence of ethanol-seeking, the flavorant was not faded out of the solutions. Another goal of this study was to use alternative induction techniques in animals that fail to achieve or maintain sufficiently high levels of ethanol intake using the fading procedure. Finally, this study sought to determine the behaviorally impairing effects of different amounts of orally consumed ethanol (g/kg) (i.e. dose-dependent effects) on fine motor coordination using a bimanual motor skill test.

Go to:

Methods

Animals

Five adult male rhesus monkeys (*Macaca mulatta*, Indian origin; obtained from LABS of Virginia) served as subjects (#333, 320, 329, 325, 302) in Experiment 1. The monkeys were approximately 7-8 years of age (i.e., young adult) and weighed 8-12 kg at the beginning of the study. The monkeys had previously been trained on components of a behavioral test battery (Weed et al., 1999; Taffe, 2004) and had participated in prior acute drug challenge studies with ketamine (Taffe et al., 2002b; Taffe et al., 2002c), scopolamine (Taffe et al., 2002c), nicotine and mecamylamine (Katner et al., 2004), and Δ^9-tetrahydrocannabinol; these drugs were administered at least 3 months prior to the current study. The animals' diet was restricted 5 days per week to ensure consistent behavioral responding in the test battery while maintaining adequate growth rates.

Eight peri-adolescent male rhesus monkeys (*Macaca mulatta*, Chinese origin; obtained from Covance) participated (#419, 421, 422, 425, 426, 427, 428, 429) in Experiment 2. These monkeys were approximately 3 years of age, weighed 3-5 kg at the beginning of the study and were only trained on one component of the test battery, i.e., the bimanual motor skill task. The Experiment 2 animals were fed the standard food amount established by the staff veterinarians to ensure normative growth and body maintenance, i.e., they were not restricted.

All animals were individually housed and fed in the home cage after completion of the daily ethanol access sessions. The animals' normal diet (Lab Diet 5038, PMI Nutrition International) was supplemented with fruit or vegetables seven days per week and water was available *ad libitum* in the home cage at all times. 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. 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. Although supplied originally by two different vendors, all monkeys were raised by the dam until eight months of age or greater thus no rearing effects as described in one social deprivation model (Higley et al., 1991, 1996) would apply.

Oral Ethanol Self-Administration Procedure

Ethanol solutions were made available in the home cage during daily (M-F) sessions of 1 hr duration (i.e., limited access) via normal drinking bottles.

To preclude water satiation at the beginning of the sessions, cage water was not available for the 1 hr period preceding ethanol availability. 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. Food access was determined by the experimental design but in general food was unavailable for 1 hr preceding and then during the 1 hr daily limited-access sessions.

Oral ethanol self-administration was induced with a procedure in which the concentration (%) and/or amount (g/kg) of ethanol in a palatable solution was gradually increased over a series of daily limited-access sessions. Monkeys were initially given the opportunity to consume 1% (w/v) ethanol plus 4% (w/v) Tang® in tap water with an absolute ethanol limit of 0.5 g/kg per session via a drinking bottle placed on each animal's homecage. The amount of the ethanol solution consumed during this, and all subsequent sessions, was recorded during the initial 5 min of the session, then at 10, 15, 20, 30 and 60 min after ethanol availability. Drinking bottles were tested prior to use to ensure minimal leakage and the investigators were careful to note any leakage (or bottles knocked off an animal's cage entirely) that occurred during sessions. The orange color of the solution facilitated detection of leaking or dripping during the sessions. If substantial leakage was noted during the first 20 min of the session, the volume was restored to the previously recorded level and the drinking session was continued. Note that since technical staff were in the housing room nearly continuously for this interval (multiple animals being run concurrently) detection of bottle knock-off or substantial leakage was nearly always immediate. If a reasonable determination of intake could not be made (e.g., if it occurred during the second 30 min of the hour), the data for that day were excluded.

Prior evidence suggested that monkeys may develop an aversion to ethanol (conditioned taste aversion, CTA) should they drink too much on a given occasion, particularly in the early phases of ethanol induction (Crowley et al., 1983; Higley et al., 1991). To minimize this possibility, the total amount of ethanol available (g/kg) was limited during each session. Once animals consumed a particular concentration (1 to 6%) and/or absolute amount (0.5 to 3.0 g/kg) of ethanol reliably over multiple sessions, the concentration and/or absolute amount of ethanol was gradually increased. The phases employed and their duration in days are shown in Table 1 for Experiment 1 and Table 2 for Experiment 2.

Table 1

Scheduled treatment phases for oral ethanol self-administration sessions in Experiment 1.

EtOH Concentration plus 4% (w/v) Tang®	Days	Session Limit (g/kg)
1% (w/v)	5	0.5 g/kg
2% (w/v)	5	0.5 g/kg
4% (w/v)	3	0.5 g/kg
4% (w/v)	6	1.0 g/kg
6% (w/v)	3	1.0 g/kg
6% (w/v)	6	2.0 g/kg
6% (w/v)	3	3.0 g/kg

Table 2

Scheduled treatment phases for oral ethanol self-administration sessions in Experiment 2.

EtOH Concentration plus 4% (w/v) Tang®	Days	Session Limit (g/kg)
1% (w/v)	6	0.5 g/kg
2% (w/v)	5	0.5 g/kg
4% (w/v)	5	0.5 g/kg
4% (w/v)	5	1.0 g/kg
6% (w/v)	5	1.0 g/kg
6% (w/v)	6	1.5 g/kg
6% (w/v)	5	2.0 g/kg
6% (w/v)	5	2.5 g/kg
6% (w/v)	5	3.0 g/kg

Increases in the ethanol concentrations and/or absolute amounts were not made on Mondays so that animals would not experience an increase in the concentration following the weekend layoff. Although stable levels of ethanol consumption were predicted to develop over 5 days, in some cases additional sessions were required to achieve stable intake. Therefore, increases in concentration were made T-F, depending on the individual animal. It was predicted at the outset that some animals might fail to achieve or maintain sufficiently high levels of ethanol intake, therefore alternative induction methods were employed on an individual basis in order to produce consistently high levels of intake in all animals. The first alternative technique employed was increasing the concentration of the sweetener used to adulterate the ethanol, i.e., the concentration of Tang® was increased from 4 to 6%

(w/v). If this technique was unsuccessful (Experiment 2 only), then water was withheld from the animal's homecage 2 hr before ethanol access sessions and the animal's daily food ration was made available 1 hr before the ethanol access sessions, i.e., postprandial availability.

In Experiment 1, bimanual motor skill (BMS) testing was conducted after access sessions during the course of the ethanol fading procedure (see data analysis section for details). In Experiment 2, BMS testing was performed after the completion of the fading procedure where 1.0, 1.5, 2.0, 2.5 and 3.0g/kg ethanol doses were presented to animals twice under a Latin-Square dosing schedule.

Bimanual Motor Skill Task (BMS)

This task, which involves a modification of the test board used by Brinkman (Brinkman 1981), was designed to test bimanual motor coordination, procedural learning and motivation to work for a preferred food reinforcer (raisins). Monkeys are easily trained on this task and reach a high level of proficiency. A holeboard with 15 holes is mounted perpendicular (long axis vertical) to the door of the transport or home cage. Each of the15 holes is filled with a raisin and for efficient retrieval, the monkey must push with one finger from one side and grasp the raisin from the other side, requiring bimanual dexterity. This task is therefore likely to reveal any compromised fine motor function (Fox et al., 1997; Taffe et al., 2002a; Taffe et al., 2002b; Taffe et al., 1999). The time elapsed to retrieve all 15 raisins was recorded by stopwatch. BMS testing occurred 20 and 90 min after each animal consumed their allotted amount of ethanol, rather than waiting until the end of the 1 hr access session, in order to compensate for potential differences in peak blood ethanol levels produced by differing patterns of ethanol intake between animals.

Blood Alcohol Levels

After the most preferring animals reached stable levels of consumption during the last treatment phase, a single session was scheduled with 3.0 g/kg of 6% ethanol available to all animals for the determination of BALs. Blood samples were drawn from the femoral vein under ketamine (10 mg/kg; i.m.) anesthesia to determine blood alcohol levels (BALs) reached by each animal. Samples were processed in a biocontainment P3 facility. Serum was separated from blood cells by centrifugation. 0.1µl of perchloric acid (used to deactivate any potential viruses) was added to 0.9ml of serum and analyzed for ethanol content with an Analox AM1 ethanol analyzer (Analox Instruments USA, Lunenburg, MA). BALs were corrected for dilution due to the addition of 0.1µl of perchloric acid and expressed as mg% (i.e., mg/dl). BMS results indicated that the most reliable effects of ethanol were at 20 min for Experiment 2 and 90 min for Experiment 1. Therefore, BALs were taken at 20 min for Experiment 2 and 90 min for Experiment 1 after each animal consumed the majority of their allotted amount of ethanol.

Data Analysis

For both Experiments 1 and 2, statistical analyses of the normalized BMS data (retrieval latency) were analyzed by one-way repeated measures analysis of variance (ANOVA) for the 20 and 90 min time points with the single factor of drug treatment condition. Post-hoc analyses of significant main effects confirmed by the ANOVAs were conducted with the Dunnett's procedure. All analyses were performed using a commercial statistical software package (GBSTATv7.0; Dynamic Microsystems, Silver Spring MD) and in all tests the criterion for significance was $p < 0.05$.

In Experiment 1, BMS testing was conducted during the course of the ethanol fading procedure. Since the dose conditions were not explicitly balanced during fading, BMS testing was repeated an average of 5, 4, 15, 10, 3, 2 and 2 times for the baseline, vehicle, 0.5, 1.0, 2.0, 2.5 and 3.0 g/kg conditions respectively. All 5 animals contributed to the baseline, vehicle, 0.5, 1.0, and 2.0 g/kg conditions and 3 animals consumed 2.5 and 3.0 g/kg of ethanol on at least one occasion. In Experiment 2, BMS testing was performed after the completion of the fading procedure and in this case the 1.0, 1.5, 2.0, 2.5 and 3.0g/kg ethanol doses were presented to animals using a Latin-Square design and double determination. In addition, selected doses were repeated for animals that did not consume a given dose during either the first or second presentations during a single session following completion of the Latin-Square. The 0.5 g/kg condition was presented

following each balanced determination (i.e., on the same days for all animals.)

Results

Experiment 1

The sweetened ethanol fading procedure was highly effective in achieving consistent 1.0 g/kg levels of oral ethanol intake in all 5 rhesus monkeys (**Figure 1**). Four animals readily consumed 0.5 g/kg of ethanol during the 1%, 2%, and 4% ethanol treatment phases. One animal (#325) did not readily consume 1% ethanol at the beginning of the experiment, however ethanol consumption was successfully initiated in this animal by increasing the concentration of the sweetener (Tang®) from 4 to 6% (w/v) beginning on the fifth session. This animal (#325) was presented with ethanol in a 6% Tang solution for the remainder of the experiment. Thereafter, all five animals consumed 1.0 g/kg of ethanol during the 4 and 6% ethanol treatment phases.

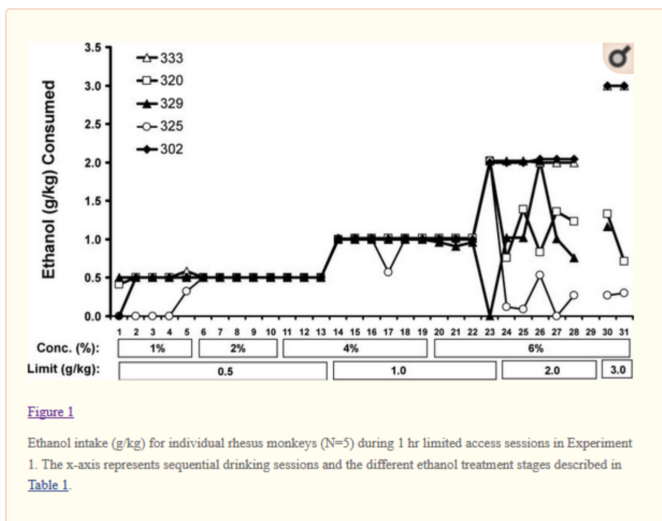


Figure 1

Ethanol intake (g/kg) for individual rhesus monkeys (N=5) during 1 hr limited access sessions in Experiment 1. The x-axis represents sequential drinking sessions and the different ethanol treatment stages described in Table 1.

To determine if the flavorant fade could induce consistent consumption above 1.0 g/kg, the amount of ethanol available in each session was further increased in steps. The results show that when animals had the opportunity to consume 2.0 and 3.0 g/kg of ethanol during the final two phases of the flavorant fading procedure, differences in amount of ethanol consumed between animals emerged (**Figure 1**). Two of five animals readily consumed 2.0 and 3.0 g/kg of ethanol. Following an initial experience with 2.0 g/kg, two additional animals consumed approximately 1.0 to 1.5 g/kg from day to day, and one animal consumed less than 0.5 g/kg. Throughout the course of the fading procedure, animals consumed the majority of their ethanol solutions during the first 5 min of the 1 hr limited access sessions (**Figure 2**), consistent with previous observations (Grant and Johanson, 1988; Henningfield et al., 1981; Henningfield and Meisch 1978; Karoly et al., 1978). A retrospective analysis of food intake based on a newly described scheme (Taffe, 2004) and inspection of the bodyweights produced no correspondence between either factor and the subsequent intake patterns. Thus, it appears that any effects of the food restriction protocols were minimal in comparison with innate individual differences.

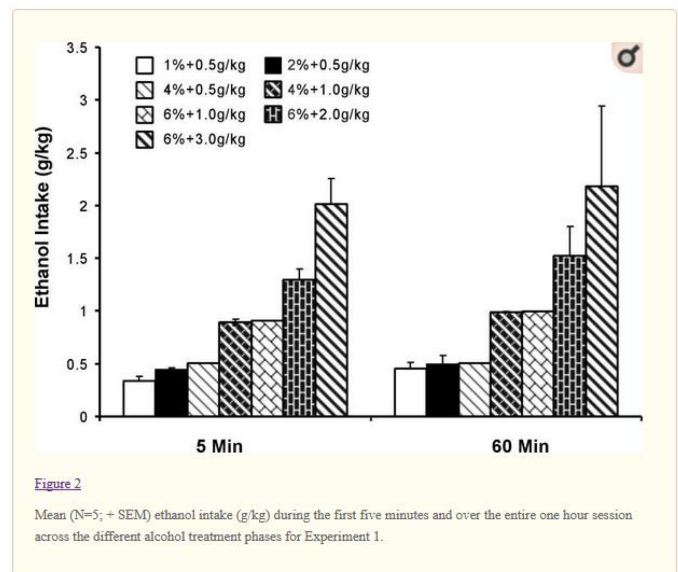


Figure 2

Mean (N=5; + SEM) ethanol intake (g/kg) during the first five minutes and over the entire one hour session across the different alcohol treatment phases for Experiment 1.

Blood samples were obtained from all animals 90 min after consumption in an ethanol session in which all animals had the opportunity to consume 3.0 g/kg of 6% (w/v) ethanol plus Tang®. As is illustrated in **Figure 6**, consumption of 3.0 g/kg in two animals produced corresponding BALs of 248 and 267 mg%, 0.8 to 0.9 g/kg in two animals produced corresponding BALs of 72 and 77 mg%, and no ethanol in one animal produced a BAL at the limit of detection (20 mg%).

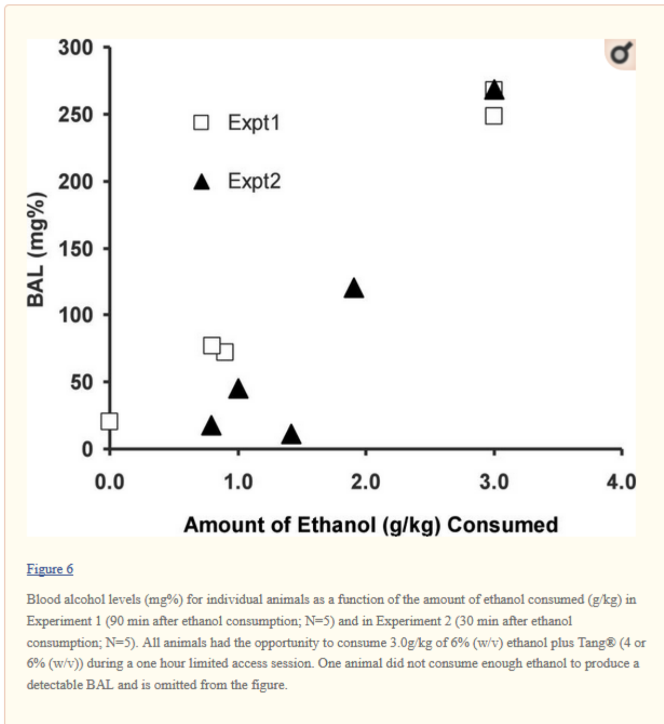


Figure 6

Blood alcohol levels (mg%) for individual animals as a function of the amount of ethanol consumed (g/kg) in Experiment 1 (90 min after ethanol consumption; N=5) and in Experiment 2 (30 min after ethanol consumption; N=5). All animals had the opportunity to consume 3.0g/kg of 6% (w/v) ethanol plus Tang® (4 or 6% (w/v)) during a one hour limited access session. One animal did not consume enough ethanol to produce a detectable BAL and is omitted from the figure.

Finally, monkeys were evaluated with the BMS task following ethanol consumption. The mean baseline latency to extract raisins was 14.8 (SEM= 2.1) sec. A trend for a dose-dependent slowing of performance was observed 20 minutes after consumption [$F(5,29)=1.24$; ns]. A similar effect of larger magnitude observed 90 min after consumption was statistically reliable [$F(5,29)=12.78$; $p<0.0001$]. The post hoc test confirmed that 90 min after the consumption of 3.0 g/kg of ethanol, fine motor coordination was significantly ($p<0.01$) impaired compared to the vehicle and 0.5 g/kg ethanol conditions (**Figure 3**).

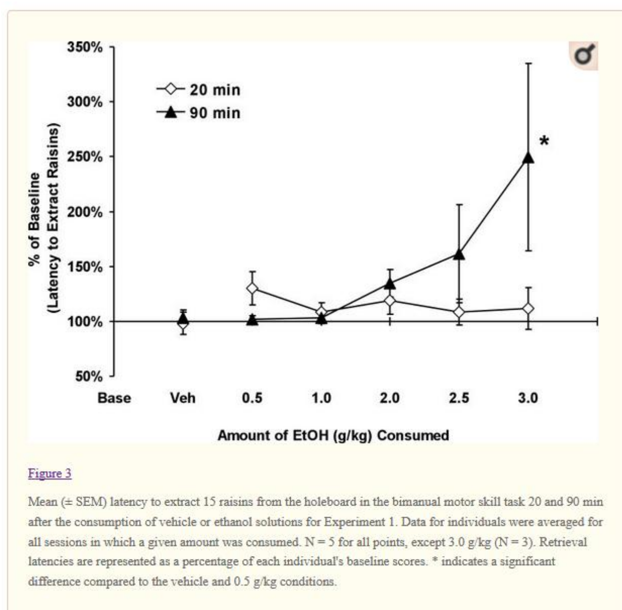


Figure 3

Mean (± SEM) latency to extract 15 raisins from the holeboard in the bimanual motor skill task 20 and 90 min after the consumption of vehicle or ethanol solutions for Experiment 1. Data for individuals were averaged for all sessions in which a given amount was consumed. N = 5 for all points, except 3.0 g/kg (N = 3). Retrieval latencies are represented as a percentage of each individual's baseline scores. * indicates a significant difference compared to the vehicle and 0.5 g/kg conditions.

Experiment 2

The second experiment was conducted in a different group of monkeys and included some procedureal modifications that were based on the results from Experiment 1. First, animals were exposed to additional treatment phases with more intermediate limits on ethanol intake over the course of the fading procedure in comparison with Experiment 1. This was based on the finding from Experiment 1 that once some animals reached the 2.0 g/kg phase, they failed to consume all of their alloted amounts of ethanol (i.e., began to titrate their dose). Second, animals that were initially reluctant to consume ethanol using the fading procedure, including increases in the concentration of the sweetener, were exposed to a post-prandial drinking procedure.

Four animals (#422, #426, #428, #429) readily consumed 0.5 g/kg of 1% ethanol during the last 3 days of the initial, 1%/0.5g/kg, phase of the ethanol-fading procedure (Figure 4A). This same group of animals also consumed their allotted amounts of ethanol during the following phases of the experiment: 2%+0.5g/kg, 4%+0.5g/kg, 4%+1.0g/kg, 6%+1.0g/kg, 6%+1.5g/kg, and 6%+2.0g/kg of ethanol. Animal #422 has slightly lower levels of intake compared to the other animals due to an error in the amount of ethanol presented, which was corrected on session 47. All animals were accidentally presented with 1.0 g/kg on two occasions during the 2%+0.5g/kg phase due to a technical error however this had no apparent effect on subsequent levels of intake. During the 6%+2.5g/kg and 6%+3.0g/kg phases, differences in the amount of ethanol consumed between animals were observed. Animals #428 and 429 consumed 2.5 and 3.0g/kg of 6% ethanol during all presentations except on one occasion for #429. Animals #422 and #426 did not consume 2.5 and 3.0 g/kg of 6% ethanol on all presentations, however, these animals did consume 3.0g/kg of 6% ethanol during the last two self-administration sessions (**Figure 4A**).

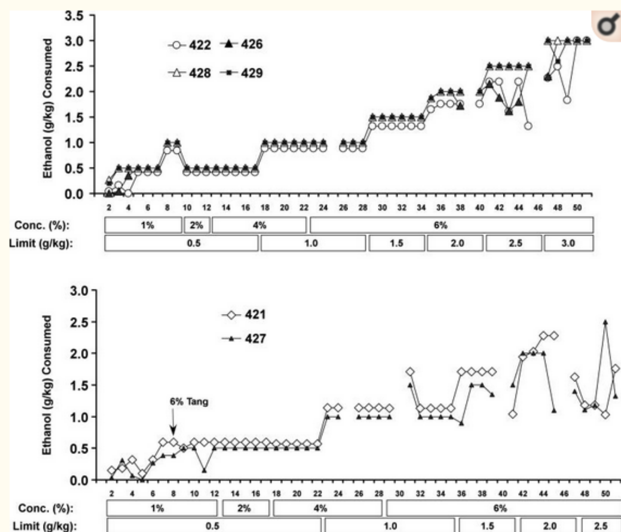


Figure 4

Ethanol intake (g/kg) for individual rhesus monkeys (N=6) during 1 hr limited access sessions in Experiment 2. The x-axis represents sequential drinking sessions and the different ethanol treatment stages described in Table 2. A) Four monkeys consumed all available alcohol after the first few days of exposure, including an inadvertent increase to 1.0 g/kg on days 7-8 ahead of schedule. Individual titration of dose was observed upon introduction of the 2.5 g/kg limit stage. B) Two monkeys consumed all available alcohol after the concentration of the flavorant was increased on day 7. Individual titration of dose was observed upon introduction of the 2.0 g/kg limit stage.

Two additional animals, #421 and #427, did not consume 0.5 g/kg of 1% ethanol during the initial phase of the fading procedure, however ethanol consumption was successfully initiated in these animals by increasing the concentration of the sweetener (Tang®) from 4 to 6% (w/v), as is illustrated in Figure 4B. These animals (#421 and #427) were presented with ethanol in a 6% Tang solution for the remainder of the experiment. Thereafter, these two animals consumed their allotted amounts of ethanol during the following phases of the fading procedure: 1%+0.5g/kg, 2%+0.5g/kg, 4%+0.5g/kg, 4%+1.0g/kg, 6%+1.0g/kg, 6%+1.5 g/kg of ethanol. Animal #421 had slightly higher levels of ethanol intake due to an error in the calculated amount of ethanol presented this animal which was corrected on session 48. Variable ethanol intake was observed in these two animals during both the 6%+2.0g/kg and 6%+2.5g/kg phases of the experiment, however three to four days of stable intake were observed in these subjects during the presentation of 6%+2.0g/kg of ethanol (**Figure 4B**). During the randomized presentation of doses for BMS testing, however, animal #421 consumed 3.0 g/kg of ethanol on one occasion and #427 consumed 3.0 g/kg of ethanol on two occasions.

The two remaining animals, #419 and #425, did not initially consume the available ethanol, even when the concentration of Tang® used to sweeten the ethanol was increased (Figure 5). Ethanol consumption in these two animals was successfully initiated, however, by postprandial presentation of the solutions (Figure 5). Animal #419 only consumed between 0.15 to 0.22 g/kg of ethanol during the first seven days of the ethanol fading procedure when presented with 1%+0.5g/kg of ethanol, however once postprandial drinking was initiated (session 16), ethanol intake gradually increased to approximately 0.4g/kg in this animal during the 1%+0.5g/kg and 2%+0.5g/kg phases. After 13 days of postprandial drinking, this animal's intake stabilized at 0.5 g/kg during the 2%+0.5g/kg and 4%+0.5g/kg phases. For the remaining phases, 4%+1.0g/kg, 6%+1.0g/kg and 6%+1.5g/kg, this animal's intake ranged from 0.7 to 1.0g/kg. Animal #425 only consumed 0 to 0.13 g/kg of ethanol during the first 14 days of the fading procedure, however following the introduction of postprandial availability (session 15), ethanol intake gradually increased to 0.5g/kg when 1% ethanol was presented. Thereafter, animal #425 consistently drank all ethanol presented to him during the 2%+0.5g/kg, 4%+0.5g/kg, 4%+1.0g/kg, 6%+1.0g/kg, and 6%+1.5g/kg phases. When 6%+2.0g/kg was presented to animal #425, he drank 2.0g/kg on two occasions and between 1.0 and 1.25g/kg on 4 occasions (**Figure 5**).

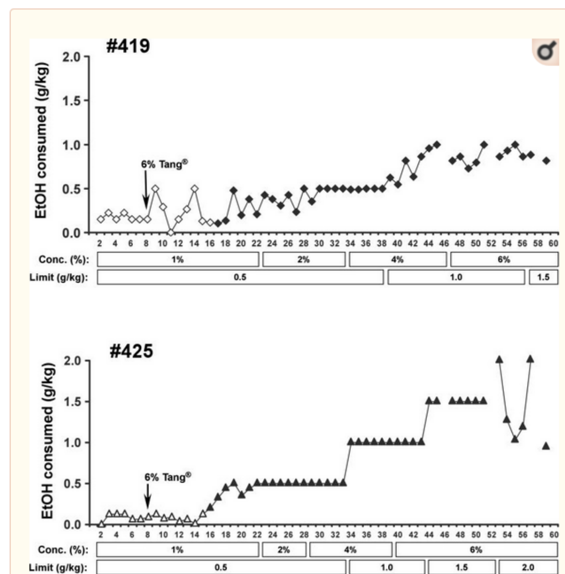


Figure 5

Ethanol intake (g/kg) for individual less-preferring rhesus monkeys (N=2) during 1 hr limited access sessions in Experiment 2. The x-axis represents sequential drinking sessions and the different ethanol treatment stages described in Table 2. These individuals were reluctant to consume until post-prandial access conditions were instituted (indicated by filled symbols).

Blood samples were taken from the six well-consuming animals in Experiment 2 (i.e., excluding #419 and #425) 30 min after consumption in a session in which all animals had the opportunity to consume 3.0 g/kg of 6% (w/v) ethanol plus Tang®. As is illustrated in **Figure 6**, the consumption of 3.0, 1.9, 1.0 and 0.8 g/kg of ethanol produced BALs of 269, 120, 45, and 18 mg%, respectively in four of the subjects. One animal appeared to consume 1.4 g/kg of ethanol, however, the corresponding BAL was only 12 mg% suggesting undetected leakage of the solution or a recording error. Another animal had a BAL of 50 mg%, however the g/kg of ethanol consumed could not be determined due to leakage from the ethanol bottle and therefore this animal was not included in **Figure 6**.

After the ethanol induction and BAL determination session, the six well-consuming animals were evaluated with the BMS task following the presentation of different doses of ethanol using a Latin-Square design. Each dose was made available on two occasions and the behavioral data were grouped by the amount of ethanol actually consumed. **Figure 7** shows that the amount of ethanol presented to animals prior to BMS testing could be controlled to a large degree by limiting the amount of ethanol available during individual sessions. The mean baseline latency to extract raisins was 30.8 (SEM= 2.2) sec. Ethanol consumption slowed BMS performance 20 min later [$F(6,41)=2.78$; $p<0.03$], but the slowing observed 90 min after consumption was not statistically reliable [$F(6,41)=1.16$; ns]. The post hoc test confirmed that fine motor coordination was significantly impaired relative to the 0.5 g/kg ethanol condition 20 min after the consumption of 2.0, 2.5 and 3.0 g/kg of ethanol (**Figure 8**).

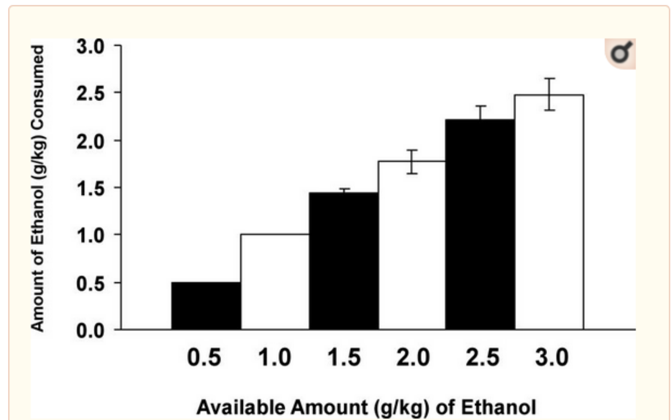


Figure 7

Average ethanol intakes (N=6; + SEM) for animals in Experiment 2 (n=6), when presented with different amounts of 6% (w/v) ethanol plus Tang® (4 and 6% (w/v)) in a randomized dose procedure prior to bimanual motor skill testing. The 1.0-3.0g/kg ethanol doses were presented twice to each animal using a Latin-Square design. The 0.5 g/kg condition was presented following each balanced determination (i.e., on the same days for all animals.)

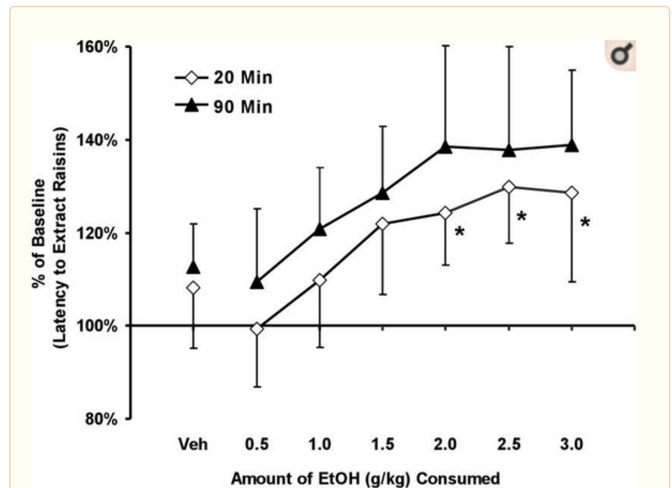


Figure 8

Mean (N=6; ± SEM) latency to retrieve 15 raisins from the holeboard in the bimanual motor skill task 20 and 90 min after the consumption of vehicle or ethanol solutions for Experiment 2. Data for individuals were averaged for all sessions in which a given amount was consumed. * indicates a significant difference compared to the 0.5 g/kg condition.

Discussion

The results of this study show that the presentation of gradually increasing concentrations and amounts of sweetened ethanol is effective in producing behaviorally relevant levels of ethanol intake in rhesus macaques. The ethanol drinking-induction process was assisted in less-preferring animals by the employment of alternative techniques such as increasing the concentration of the sweetener used to adulterate the ethanol or postprandial presentation of the solutions. The results of Experiment 2 show that once significant and stable levels of ethanol consumption were attained using the fading procedure, the amount of ethanol

consumed by each animal during a given session could be closely controlled by limiting the amount of ethanol presented. The ability to control animal's intake in this fashion enabled the determination, in a randomized dose procedure, that the consumption of 2.0, 2.5 and 3.0 g/kg of ethanol impairs fine motor coordination as measured in the bimanual motor skill task. Finally, it is a significant strength that the findings generalized across two groups of animals who differed significantly in terms of age, behavioral training , experimental history with drug challenge and food restriction status. That is, the proportion of high, medium and low-consuming individuals was similar in each group and the one alternate technique attempted in each group was similarly effective. This model is therefore of significant use for conducting controlled behavioral pharmacological studies of the effects of ethanol in macaque monkeys.

Previous work has shown that nonhuman primates display large individual differences in their propensity to self-administer ethanol (Crowley and Andrews, 1987; Crowley et al., 1983; Higley and Bennett, 1999; Meisch and Lemaire, 1991). Such findings are well illustrated by a report from Vivian and colleagues in which cynomologus monkeys allowed to consume unsweetend ethanol over 180 long-access sessions displayed individual stability across several treatment conditions. The animals in that study could be divided into three separate groups consuming minimal (<1.0 g/kg; N=4), moderate (1.0 to 3.0 g/kg; N=7) and high (>3.0 g/kg; N=5) amounts of alcohol on a consistent basis (Vivian et al., 2001). Critically apparent in that study is the observation that large differences in intake *between sessions* may be observed on both a group and individual basis. In other studies in which sweetened alcohol solutions were made available for shorter intervals (1-2 hrs), similar intake patterns were observed however mean intakes were frequently in the 0.8-1.5 g/kg range (Fahlke et al., 2000; Grant and Johanson 1988; Higley et al., 1998; Higley et al., 1996; Vivian et al., 1999). Provision of ethanol solutions without a sweetener or flavorant may result in intakes substantially below 1.0 g/kg (Macenski and Meisch, 1992; Stewart et al., 1996). The present study showed that a flavorant fade procedure was effective in getting 12 of 13 monkeys to consume 1.0 g/kg of ethanol reliably within a one hour session. The remaining monkey would consume between 0.8 and 1.0 g/kg by the time the study was discontinued for this animal. Seven of 8 animals evaluated (Experiment 2) would consume 1.5 g/kg of ethanol reliably. Once the amount of ethanol available was increased to 2.0 g/kg, 7 of 12 monkeys reliably consumed all of the ethanol during the induction phase. These data show the effectiveness of the flavorant fading procedure in getting high proportions of male rhesus macaques to consume amounts of ethanol that produce BALs in the relevant 80-100 mg% range within a one-hour access session. In part this success depended on employing alternative techniques for less-preferring individuals. For example, increasing the concentration of the sweetener from 4 to 6% (w/v) was effective in inducing significant levels of ethanol intake in one animal in Experiment 1 and two animals in Experiment 2. Post-prandial availability of the solutions initiated ethanol consumption in two additional animals (Experiment 2) which did not consume the ethanol solutions when the concentration of the sweetener was increased. These observations are important since one of the goals of the study was to produce significant levels of oral ethanol intake in all subjects regardless of their predisposition to consume ethanol. In other words, one of the unique benefits of this approach lies in increasing consumption in the less-preferring individuals. Another critical outcome of the present study was that consumption was highly stable from session to session when the amount of ethanol available was under that animal's apparent titration threshold. This ability to control the amount a given animal consumed is critically important for determining the behavioral, physiological or other acute effects of specific ethanol doses.

One limitation with the present procedure was that approximately half of animals appear to titrate their intake when 2.0 g/kg or greater amounts are available. Similarly, mean ethanol intake under the randomized presentation schedule in Experiment 2 was equivalent to the total amount available up to 1.5 g/kg but not higher. This level of intake, as a minimum, is considerably higher than in previously reported studies and the consistency of intake across sessions is also superior. That is, prior studies report both significant numbers of individuals who do not consume more than 1.0 g/kg and more-preferring individuals who consume less than 1.5 g/kg on a significant proportion of their sessions. Although the present procedure did not appear to dramatically

increase group mean consumption this may be because the upper limit of consumption in the more-preferring individuals was not determined (3.0 g/kg was the maximum available in a session). It is thus likely that if greater amounts of ethanol were presented our group mean intake might have been higher albeit at the expense of the greater variability reported in other studies.

In addition, the results of Experiment 2 suggest that additional animals in Experiment 1 may have consumed 2.0 g/kg consistently had the intermediate 1.5 g/kg stage been employed. In the overall design, the amount of ethanol animals were exposed to during each session was limited and gradually increased over the course of the fading procedure. This strategy was based on the hypothesis that over consumption in relatively naïve animals might lead to an aversion to the taste of ethanol. Evidence of such a conditioned taste aversion (CTA) has been reported in a study where accidental water deprivation of pigtail macaques prior to an ethanol self-administration session resulted in increased intake (4.5 g/kg of ethanol) and severe intoxication (Crowley et al., 1983). Prior to this session, animals consumed 2.4 g/kg, but following this episode consumption was suppressed for many sessions. Evidence for a similar effect can be seen for three of the subjects in Experiment 1 who consumed 2.0 g/kg on one occasion and thereafter failed to consume all of the available ethanol. This may also be supported by the finding that proportionally more animals consumed higher amounts of ethanol in Experiment 2 in comparison with Experiment 1, likely due to the inclusion of the additional 1.5 g/kg limit on ethanol intake. Given the sample sizes, this promising outcome is clearly a matter for additional study since consistent intake of 2.0 g/kg and greater in more than half of individuals would appear to represent a significant advance.

Finally, and perhaps most importantly, this study was successful in designing a protocol that facilitates examination of the behavioral effects of controlled ethanol doses. Few available studies have determined the behavioral effects of oral ethanol intake in nonhuman primates, although many report signs of intoxication anecdotally or reference BALs in comparison with humans for an indication of behavioral relevance. Here, significant impairment in the ability to extract raisins from a holeboard was observed following the consumption of ethanol doses of 2.0 g/kg or greater, and a reasonably linear dose-response function was observed in rhesus macaques. The consumption of 2.0 g/kg corresponds to a BAL of about 125 mg% (Figure 6; also see Vivian et al., 2001) which is slightly higher than the statutory limit for automobile operation in the United States. Since the 80 mg% BAL has been associated with behavioral impairment in humans it might be proposed that the BMS task is insensitive to the effects of alcohol. However, a more likely explanation is that rhesus macaques are behaviorally less sensitive to the performance impairing effects of alcohol in comparison with humans. For example in one study, 2.2 g/kg of ethanol (i.v.) was required to produce observable intoxication leading to BALs of 243 to 282 mg% (Barr et al., 2003). Furthermore, a 2.0 g/kg intragastric dose of ethanol (producing BALs > 100 mg%) was required to disrupt rhesus monkeys performing a visual tracking task where human subjects were impaired on the same task following consumption of 1.0 g/kg of ethanol (Ando et al., 1987). In total these findings indicate that rhesus macaques may be less sensitive than humans to the motor impairing effects of ethanol. Rhesus monkeys, or the macaque genus, may be uniquely insensitive in this respect. For example adult male squirrel monkeys administered 1.0 g/kg of ethanol via gavage stagger noticeably (Weerts et al., 1993), although unfortunately, BALs were not reported in this study making it difficult to compare these findings with others. Another study reported fine motor impairment associated with intake of 2.0 g/kg of ethanol in squirrel monkeys who achieved BALs of 70-75 mg% after this dose (Kaplan et al., 1882). Thus the present BMS results are most likely a result of a species difference in behavioral sensitivity rather than a result of an insensitive assay.

In terms of ethanol dose, the effects on bimanual motor coordination were similar regardless of whether they were observed during the course of the induction procedure (Experiment 1) or in a Latin-square randomized-dose design after induction (Experiment 2). Some inconsistencies were noted however. In Experiment 1, animals were significantly impaired on the BMS task 90 min after ethanol consumption, while in Experiment 2 animals were most reliably impaired 20 min after ethanol consumption. This outcome is unlikely to be

due to measurement during the induction phase versus the randomized design following induction (Experiment 2), since the BMS data collected during the fading procedure in Experiment 2 indicated that animals were also most reliably impaired 20 min after ethanol consumption (data not shown). The Experiment 1 animals were modestly food restricted, whereas the Experiment 2 animals were fed under normative conditions, however this might have predicted an opposite effect, i.e., the restricted animals absorbing the ethanol solutions more quickly. This effect may reflect developmental differences, since the Experiment 1 animals were well into adulthood, whereas the Experiment 2 animals were post-pubescent young adults. Furthermore, animals in Experiment 1 were of Indian origin and animals in Experiment 2 were of Chinese origin, therefore, it is possible that genetic differences, e.g., in ethanol metabolism, between these two groups (amounting to a strain difference) might account for their disparate timing of sensitivity to ethanol's motoric effects. Although the present study was not designed to test such variables, future studies to examine factors which have a significant impact on the time course of ethanol's effects on behavioral performance are clearly warranted.

The present findings demonstrate the utility of our ethanol fading procedure to examine the effects of controlled ethanol doses on fine motor skill as measured by the BMS task. Furthermore, once animals have been maintained at significant levels of ethanol intake after the induction phase of the ethanol fading procedure, the BMS task could also be used to examine the effects of behavioral tolerance over the course of animals' self-administration history (i.e. after chronic ethanol exposure) or the effects of ethanol withdrawal/abstinence. Similarly, it would be of interest to determine the effects of acute ethanol doses, or protracted exposure, on other behavioral tasks (Weed et al., 1999; Taffe, 2004). In the future it would be useful to include control groups in order to determine which factors in the present study contributed to ethanol drinking behavior, what innate characteristics predispose animals to consume different amounts of ethanol, and what factors contribute to individual differences in the behavioral sensitivity to ethanol. For example, the timing and the amount of food presented to animals could influence ethanol consumption levels. Is ethanol itself reinforcing in these animals and to what degree? Does the age or genetic background of the animals influence their drinking behavior and their sensitivity to the behavioral effects of ethanol? Although some of these questions have been addressed in previous studies, these are important questions that could be addressed with the incorporation of additional control groups and manipulations with the flavorant-fading procedure employed here.

In conclusion, the present study demonstrates that a flavorant-fading procedure, in combination with some alternative techniques, can induce groups of rhesus monkeys to consume significant amounts of ethanol. These data also show that group mean alcohol intake may be elevated, primarily by inducing low-preferring animals to consume more. In addition, this induction procedure produced sufficiently reliable consumption patterns to facilitate examination of the behavioral effects of controlled doses of consumed ethanol. Thus, this model offers considerable face validity, and practical utility, for investigating the behavioral effects of ethanol consumption.

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