CONDITIONED TASTE AVERSION PRODUCED BY THE ORAL INGESTION OF ETHANOL IN THE RAT

by

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A THESIS

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INTRODUCTION

Researchers have devoted a great deal of effort attempting to develop a complete animal analogue for the human alcoholic. Such an analogue would be one in which an animal "voluntarily" drinks alcohol and over time develops physical dependence, tolerance, drug-seeking behavior, and a preference for alcohol solutions under certain conditions. Although animals can be maintained on alcohol solutions when they are the sole sources of fluid (Lester, 1961; Mello and Mendelson, 1971), historically, animals have not continued to select those solutions when alternative fluids such as water have been made available (Mardones, 1960; Myers and Carey, 1961; Lester, 1966).

Because of the tendency of most animals to drink only small amounts of alcohol solutions in concentrations greater than 5% - 7% (v/v) (Myers, 1966), a number of techniques have been developed to increase their voluntary intake. In general, the criteria of physical dependence, tolerance, and drug-seeking behavior are met by these techniques. Some of these procedures rely on preliminary repeated administrations of alcohol by artifical means. Representative of these methods is the experimenter-controlled intubation of large amounts of alcohol into the animal's stomach for a number of days. This procedure has produced physical dependence in the rat (Deutsch and Koopmans, 1973), the rhesus monkey (Ellis and Pick, 1969), and the dog (Essig and Lam, 1968). Another technique has been to inject mice with pyrazole (an inhibitor of alcohol dehydrogenase), which results in an increase in the time required to metabolize a specified

amount of alcohol, and then to administer alcohol through inhalation (Goldstein, 1972). After several days of this treatment, alcohol must continue to be made available to the mice, otherwise, they undergo a dose-related set of withdrawal symptoms.

Other investigators have utilized procedures which have occasionally resulted in animals' drinking alcohol solutions even when other fluids are available. Increased alcohol consumption has been observed when animals have been exposed to "anxiety-provoking" or "stress-inducing" situations (Masserman and Yum, 1946; Clark and Polish, 1960; Clay, 1964). Unfortunately, other investigators have been unable to replicate or extend these findings (Korman and Stephens, 1960; Persensky, Senter, and Jones, 1969). A polydipsic procedure has been used by Lester (1961) and others who reported that food-deprived animals that were required to press a lever for food pellets on an intermittent schedule of reward consumed large amounts of alcohol solutions. However, Senter and Sinclair (1967) found that the preference for alcohol solutions in a choice situation following this polydipsic regimen did not increase. It is important to note that in many of the studies which have reported increased alcohol consumption, a demonstration that the alcohol-preferring animals had developed tolerance, physical dependence, and had come to prefer alcohol to nonalcohol solutions for relatively long time-periods was never provided.

Perhaps the most obvious conclusion resulting from attempts to develop a complete animal analogue of the chronic alcoholic is that animals seldom come to prefer alcohol to nonalcohol solutions. This observation is consistent with the hypothesis that there are aversive consequences which accompany the oral ingestion of alcohol. A similar

finding has been noted in humans, to wit, young children often do not initially like alcohol solutions, but only come to prefer or tolerate them after repeated exposures.

A procedure which has often been used to demonstrate that a drug has aversive consequences accompanying its administration and/or that subsequent pharmacological effects are aversive is the taste-aversion paradigm (Berger, 1972). Operationally, a drug is administered following the ingestion of a distinctively flavored food or fluid. The drug is defined as being aversive if, after it has been paired with a specific fluid, the subsequent consumption of that fluid is decreased.

The taste-aversion procedure will be used in the experiments described in this document as a means of exploring the aversive properties of alcohol for three reasons: it has been previously used to study the effects of alcohol, it has produced relatively stable conditioned aversions, and the relevant parameters and underlying factors have been fairly well delineated (Rozin, 1969; Garcia, McGowan, and Green, 1972).

The three general questions under investigation in the research program delineated in this dissertation are: (1) Can conditioned taste aversion be produced by the oral ingestion of ethanol? (2) Are the oropharyngeal effects of alcohol by themselves sufficient to produce such an aversion or is inebriation also required? (3) Do extensive opportunities to drink alcohol serve to modify the extent of conditioned taste aversion?

Aversiveness of alcohol solutions

The findings of previous investigations of the aversiveness of alcohol are considered under three headings: (1) aversive factors accompanying the drinking of alcohol solutions, (2) aversive factors produced as a result of the route of administration of alcohol, and (3) conditioned taste-aversion experiments in which alcohol has been used as the unconditioned aversive stimulus.

A number of experimenters have concluded that there are aversive factors which accompany the drinking of alcohol solutions. Lester (1966) and Myers and Veale (1972) have reviewed a number of studies in the area of self-selection of alcohol solutions and have concluded that animals use the sense of smell and/or the sense of taste to discriminate among solutions which contain alcohol. Partial ablation or chemical interference with the normal functioning of these sensory systems was often correlated directly with either an increase in the maximum concentration of alcohol that an animal consumed, or with an increase in the amount of alcohol that was consumed. Moreover, LeMagnen and Marfaing-Jallat (1961) reported that rats that were relatively insensitive to the bitter taste of quinine, since they consumed quinine solutions whose concentration was 2.5 times higher than other animals', also drank larger amounts of 6% alcohol than the other animals. Dicker (1958) noted that after rats were treated with a drug which reduced taste discrimination (methypentynol carbamate), the concentration of alcohol they would consume preferentially was altered. Some animals drank substantial amounts of alcohol solutions at a higher concentration than usual, while others drank large amounts

of alcohol only at very reduced concentrations. Kahn and Stellar (1960) observed that the maximum concentration of alcohol which was ingested in amounts equivalent to water could be increased from 5% to 10% by removal of the olfactory bulbs. Nachman, Larue, and LeMagnen (1971) demonstrated that the removal of the olfactory bulbs reduced the aversiveness of alcohol solutions in the BALB/c mouse strain. Further, Rodgers and McClearn (1962) noted an increased consumption of alcohol solutions in the A/Crgl and BALB/cCrgl mouse strains when the anterior third of the cerebrum, including the olfactory bulbs, was removed. However, removal of only the olfactory bulbs did not increase alcohol consumption. The conclusions noted above are consistent with the hypothesis that alcohol has distinctive aversive gustatory and olfactory properties which tend to reduce its consumption, particularly when the concentration exceeds 5%-7%.

In addition, it has been suggested that the central state, or condition of inebriation, and its accompanying peripheral manifestations, e.g., loss of balance, are aversive (Lester, Nachman, and LeMagnen, 1970), and this may be a major reason why animals do not continue consistently to drink large amounts of alcohol solutions.

Barry and Wallgren (1968) have noted that there are a number of potentially aversive consequences of the methods of administration of alcohol. These authors stated that relatively high concentrations of alcohol are tolerated when ingested orally or intubated directly into the stomach; however, concentrations greater than 25% (v/v) are most likely irritating to the mouth and stomach. Intraperitoneal injections of alcohol are particularly likely to lead to painful

consequences when the solutions are not isosmotic to body fluids (12.5%, v/v). Also, when concentrations greater than 20% are injected intraperitoneally, hemorrhagic lesions and irritation to gastro-intestinal membranes may result. Intravenous injections may also have aversive consequences if the infusion rate is too fast or if the infusion time is too prolonged.

Several other investigators have reported that i.p. injections of alcohol may be aversive to rats. Freed (1967) presented data which were interpreted as supporting the notion that i.p. injections have stressful attributes, and these attributes may summate with other stressors such as electric shock. Similarly, Baum (1971) has noted the possibility that an i.p. injection of alcohol may be a traumatic experience.

There are three conditioned taste-aversion experiments in which alcohol has been used as the unconditioned aversive stimulus. In the first experiment, Lester et al (1970) utilized a procedure under which the ingestion of a 0.1% sodium saccharin solution was followed immediately by a single dose of alcohol administered, in different groups, via intracardiac catheter, intragastric tube, or intraperitoneal injection. The intraperitoneal doses ranged from 0.75 to 4.42 g/kg, but only those that produced severe intoxication or a comatose condition resulted in unequivocally significant decreases in saccharin consumption on a subsequent drinking test. Although Lester et al (1970) provided no behavioral or pharmacological criteria for severe intoxication, they were probably referring to the effects of doses of approximately 2.94 g/kg or more. Essentially the same results were obtained for the

intracardiac administration of alcohol, with the qualification that only doses of 2.94 g/kg and 4.42 g/kg were used. However, these investigators reported no significant decrease in saccharin intake when 2.94 g/kg was intubated into the stomach. Based on this latter observation, they concluded that it was unlikely that orally ingested alcohol solutions could act aversively. They further proposed that the aversive consequences of i.p. injections of alcohol were due to the central, systemic effects and not to peritoneal irritation. Support for the latter conclusion was provided by the similar results between the i.p.-injection animals and the intracardiac-infusion animals. Note that the last conclusion is based on the supposition that the intracardiac-infusion group's administration was without aversive peripheral effects.

Cappell, LeBlanc, and Endrenyi (1973) have also used a conditioned taste-aversion paradigm to investigate the aversive consequences produced by an i.p. injection of alcohol. Their procedure involved subjecting rats to 5 daily trials on which saccharin ingestion was followed by an i.p. injection of alcohol (10%, v/v). They observed a significant decrease in saccharin intake with an alcohol dose of 1.2 g/kg, which is much lower than the minimally effective dose of 2.94 g/kg in the Lester et al (1970) study. These experimenters argued against accepting a simple general toxicity explanation of their results wherein alcohol would be considered a toxic drug. Instead, they suggested consideration of the probable "interaction between behavior and the nature of the reinforcing action" of the drug in a given situation. Specifically, they suggested that the

i.p. administration of alcohol in the taste-aversion paradigm is a punishing stimulus, whereas the administration of alcohol to addicted rats is a stimulus which possesses positively reinforcing attributes.

Eckardt, Skurdal, and Brown (1974) have also demonstrated the aversiveness of i.p. injections of low doses of alcohol. When injections followed immediately the consumption of an originally preferred Kool-Aid flavor, the extent of subsequent aversion to that flavor varied directly with dosage (1.2 g/kg > 0.8 g/kg > 0.4 g/kg > 0.0 g/kg). However, i.p. injections of 1.2 g/kg administered 2-3 hr after consumption of the preferred flavor failed to produce aversion. In this study, alcohol injections were paired with Kool-Aid on four occasions, and a two-flavor preference-drinking test was used in combination with the single-flavor forced-drinking tests employed by Lester et al (1970) and Cappell et al (1973). According to Dragoin, McCleary, and McCleary (1971) and Grote and Brown (1971), the two-solution test is more sensitive than the single-solution method.

Theoretical conceptualization of the taste-aversion paradigm

An interpretation of the taste-aversion procedure in terms of classical conditioning theory has been advanced by Rozin (1969) and Garcia et al (1972). According to them, when the experimenter presents a novel flavored fluid to a fluid-deprived animal, it may be viewed as a conditioned stimulus (CS) in the Pavlovian paradigm. After consuming the fluid, the animal is usually injected or intubated with the aversive drug which therefore qualifies as an unconditioned stimulus (UCS). The pairing of the CS and the UCS is categorized as classical conditioning because the UCS is presented to the animal

regardless of its response to the CS. There may be one or more such CS-UCS pairings, but with multiple pairings, only one pairing is generally given per day. The responses of the animal to the drug, e.g., nausea, inactivity, writhing, are the UCRs, or unconditioned responses. The animal is often given water to drink for a day or so after the conditioning period to prevent any generalized sickness from interfering with its performance on the test day. Subsequent to recovery, the animal is again permitted to drink the "novel" fluid (CS). A relative reduction in the intake of that fluid is taken as supporting the hypothesis that the drug paired with the fluid had aversive consequences accompanying its administration, and/or that its pharmacological effects were aversive. Garcia et al (1972) and Rozin (1969) have concluded that the decreased intake of the CS fluid after the pairing procedure is a measure of the CR, or conditioned response.

Although Garcia et al (1972) and Rozin (1969) did not discuss the CR at length, it seems plausible that a CS that has been paired with an aversive drug probably elicits an aversive conditioned emotional response such as anxiety or revulsion. In addition, it seems reasonable to expect that the intake of the CS fluid on subsequent presentations should be reduced because not drinking or stopping drinking after a few licks would serve to reduce or eliminate the learned aversiveness for the CS.

During post-conditioning test sessions, animals have sometimes been tested with only the flavored fluid that was used during the conditioning period (Lester et al, 1970; Cappell et al, 1973). This may be described as a forced-drinking or single-solution testing procedure.

If such a procedure is used and if the solution is aversive, then the animal has been placed into an approach-avoidance conflict situation. The approach component presumably results from the internal condition of fluid deprivation combined with the external stimuli produced by the insertion of the drinking tubes into the cage by the experimenter. The avoidance component is assumed to result from a learned reaction acquired during conditioning when the drug was paired with the flavored fluid. As discussed earlier, during conditioning, the smell and taste of the distinctively flavored fluid (CS) would be followed by an aversive UCS. Subsequently, the CS would tend to elicit a conditioned aversive reaction, even when the CS is presented without the UCS. Consequently, responses that reduce or eliminate the CS should be reinforced, and the animal will turn away and not drink much of the fluid. The total amount of fluid consumed in a forced-drinking testing situation is thus viewed as the result of a competition between the tendencies to approach and drink and to cease drinking.

As noted earlier, some investigators have reported that the two-choice preference test is a more sensitive technique for detecting conditioned aversions than the forced-drinking test. In the studies of Dragoin et al (1971) and of Grote and Brown (1971), the consumption of a novel and distinctively flavored fluid was followed with an i.p. injection of a drug whose aversiveness had been previously determined. The post-conditioning tests consisted of either the simultaneous presentation of the flavored fluid in one bottle and an entirely different solution in another, or the presentation of only the flavored fluid. These experimenters concluded that a conditioned

taste-aversion outcome was more likely to be detected with a two-stimulus preference test than with a forced-drinking one-stimulus test.

In a two-stimulus preference test, the subject is able to "escape" from the conflict situation associated with the flavored fluid used during the conditioning period by drinking the alternative solution. Presumably the solution that was not used during the conditioning period would have no aversive emotional responses conditioned to it. Furthermore, the drinking of the second solution would also serve to alleviate the dehydration conditions accompanying a fluid-deprivation schedule. Thus, the observation of an increased consumption of a solution not presented during the conditioning period in the Dragoin et al (1971) and Grote and Brown (1971) studies was not surprising. However, the precise mechanism that results in the two-solution test's being more sensitive than the one-solution test remains to be clarified.

A number of experimenters have investigated the variables of which the taste-aversion phenomenon is a function. In general, the more intense the UCS (increased duration of presentation and/or increased aversiveness), the more pronounced the subsequent aversion (Revusky, 1968; Lester et al, 1970; Eckardt et al, 1974). It has also been observed that the sooner the UCS follows the CS, the greater the conditioned aversion. However, the taste-aversion paradigm is unique among classical conditioning procedures in that some evidence of conditioning has been reported with CS-UCS intervals as long as 3 hr (Smith and Roll, 1967; Revusky, 1968; Nachman, 1970; Kalat and Rozin, 1971). Dragoin (1971) has reported a direct relation between the concentration of the CS flavor and the magnitude of the conditioned

aversion. It is probable that the latter observation can be most easily explained within the context of the CS saliency formulation of Kalat and Rozin (1970) and Kalat (1974). These investigators have proposed that the more salient, i.e., more concentrated or more novel, the CS, the more pronounced the conditioned aversion. Both Kalat and Rozin (1970) and Kalat (1974) have provided data to support this contention, and Dragoin's (1971) results are also consistent with this formulation. However, the relations between the novelty of a solution and varying concentrations of the same solution have not been specified.

EXPERIMENT I

Introduction

As noted earlier, animals drink only small amounts of alcohol solutions in concentrations above 5%-7% (v/v) (Myers, 1966). Moreover, some experimenters (Lester, 1966; Myers and Veale, 1972) have concluded that the smell and taste of alcohol solutions are aversive to the animal and therefore function to prevent ingestion of large amounts of these solutions. In addition, Lester et al (1970) have suggested that the centrally mediated concomitants of intoxication or the condition of inebriation and its accompanying peripheral manifestations, e.g., loss of balance, are aversive to the rat.

Animals do appear inebriated after drinking alcohol solutions, and it may be reasonable to assume that the same aversive central effects must be present with this method of administration as with the intracardiac infusion and the i.p. injection techniques employed

by Lester et al (1970). Thus, in addition to possible aversive tastes and smells, another explanation of why animals do not preferentially drink large amounts of alcohol solutions is that the centrally mediated systemic effects are aversive. Therefore, it seems plausible that the oral consumption of alcohol will result in conditioned taste aversion because of alcohol's aversive orosensory or aversive central effects, or both.

In opposition to the above reasoning is the hypothesis specifically advanced in Lester et al (1970) that the oral ingestion of alcohol does not have accompanying aversive central consequences because not enough alcohol is consumed "volitionally under self-selection conditions." This conclusion was based on their failure to obtain conditioned taste aversion when alcohol was intubated into the stomach at a dosage of 2.94 g/kg. Although an intubated dose of 5.15 g/kg did result in a conditioned taste aversion, these authors considered it unlikely that such high dosages would be voluntarily ingested by rats. Lester et al (1970) noted that their experimental animals appeared inebriated shortly after alcohol was introduced directly into the vascular system, whereas the intubation of alcohol into the stomach resulted in a delayed onset of inebriation. Furthermore, they suggested that the quick onset of the central effects of alcohol with i.p. injections or intracardiac infusions was correlated with the subsequently detected conditioned aversion. In contrast, the inebriation observed with the intubation of alcohol into the stomach, perhaps because of its slow onset, did not result in conditioned aversion, except at extreme doses. Kalant (1971) has documented that blood-alcohol levels increase at a reduced

rate when alcohol is intubated into the stomach because ethanol is absorbed primarily from the small intestine. One consequence of a slower increasing blood-alcohol level is an increase in the CS-UCS interval, which should serve to reduce the efficacy of conditioning.

The specific goal of the first experiment was to determine whether conditioned taste aversion to a Kool-Aid flavor could be produced by the oral ingestion of a 5% (v/v) alcohol-Kool-Aid combination. The method of administration of the alcohol was to allow the animal to drink the alcohol solution as its sole source of daily fluid during the conditioning sessions. This differs from the stomach intubation procedure used by Lester et al (1970) which, as noted above, failed to produce conditioned taste aversions, except at very high doses. In addition, it seemed likely that five pairings of ethanol with a distinctively flavored solution would be more likely to produce a conditioned aversion than the single pairing used by Lester et al (1970). Also, the sensitive two-flavor preference-drinking test (Dragoin et al, 1971; Grote and Brown, 1971) was used in addition to the single-flavor forced-drinking test employed by Lester et al (1970) since it offered increased promise of detecting low levels of conditioned aversion.

Experiment I was conducted with rats under a fluid-deprivation regimen which permitted a single, 10-min drinking session per day. After an initial session with two Kool-Aid flavors, the animals were given preference tests between the two flavors for 2 consecutive days. A preferred flavor was determined and this flavor was then mixed with alcohol to make the 5% solution which was presented to the animal

during the conditioning period. The Kool-Aid-alcohol solutions presented to the rats during the conditioning period constituted single-flavor tests. Following this period, two-flavor preference tests (identical to pre-conditioning tests) were conducted to determine whether the conditioning sessions had changed the preference for the originally preferred flavor of Kool-Aid. Neither the pre-conditioning nor the post-conditioning test solutions contained any alcohol.

The method by which the aversive drug was administered during the conditioning sessions in the above paradigm is patently different from any of those previously mentioned because the CS and UCS were combined into one solution. In the previous discussion of taste-aversion paradigms, the conditioning procedure was described as the oral ingestion of a fluid whose taste and smell served as a CS followed by the injection or intubation of an aversive drug (UCS). Most experimenters who have used the taste-aversion paradigm have chosen to separate, or at least to control precisely, the temporal presentations of the CS and the UCS. However, in Experiment I, the fluid that served as the CS was mixed with alcohol (UCS) to form a solution which was the only liquid available during a conditioning session. The technique of combining the CS and UCS into one solution is not without precedent because Barnett (1963) and Garcia and Koelling (1967) have reported that taste aversion to the CS can occur when the CS and UCS are combined in a solution or in food during conditioning. A consequence of this method is that the CS-UCS solution used during conditioning resembled the pre-conditioning and post-conditioning preference-test solutions in that it contained the preferred flavor of Kool-Aid. It differed

from the test solutions in that it also contained alcohol at a concentration of 5%.

Since rats are able to smell and taste alcohol (Lester, 1966; Myers and Veale, 1972), it follows that they may detect the alcohol and Kool-Aid combination during the conditioning sessions as the drinking tube is approached and as the fluid is consumed. Hence the odors and possibly the initial taste of the fluid, because they precede the actual ingestion of the substance, are in the position of a CS that is to be followed by a UCS. If alcohol can produce an aversive condition of intoxication, then the conditions are suitable for that condition to function as a UCS and for the smell and taste of alcohol and of Kool-Aid to become secondarily aversive. As noted earlier, some of the possible aversive properties that might accompany the rapid ingestion of alcohol solutions are central effects, and/or irritation or excessive stimulation of the taste and/or olfactory receptors and related anatomic structures.

The response of turning away or of stopping drinking the Kool-Aid+5% during the first conditioning session should be reinforced by the cessation or relative reduction of primary aversive stimulation. Similar responses during subsequent conditioning sessions should also serve to reduce primary aversive stimulation as well as to reduce the effect of any learned aversion to the alcohol solution which might have resulted from the preceding conditioning sessions. On the basis of this formulation, one might expect to observe a progressive reduction in daily intake during the conditioning period for any solution containing alcohol. However, it has been noted earlier that the forced-drinking procedure

used during conditioning sessions results in a conflict situation in that approach responses are elicited by the stimuli resulting from dehydration and the insertion of the drinking tubes into the cage, whereas avoidance responses are elicited by the aversive stimuli of the alcohol solutions. Obviously, the stimuli resulting from dehydration are reduced by drinking liquids, even an aversive fluid. Thus, the mean daily intake during conditioning of any solution containing alcohol will depend on the strength of the approach responses elicited by the stimuli accompanying dehydration and the daily presentation of fluid relative to the strength of the avoidance responses elicited by the aversive stimuli of the alcohol solution.

If the alcohol solution is sufficiently aversive so that the preferred flavor of Kool-Aid alone becomes secondarily aversive, one would predict a decrease in the intake of this flavor on post-conditioning two-flavor preference tests, as well as an increase in the intake of the nonpreferred flavor. This latter outcome seems probable because the animal will not have had the nonpreferred flavor paired with alcohol, and thus will not be in a conflict situation relative to its ingestion. The above formulations are based on the assumption that even though the acquired aversiveness may occur maximally in response to the combination of Kool-Aid and alcohol odors and tastes, it should also occur, albeit to a lesser degree, when only the Kool-Aid cues are present.

Subjects and Procedure

The subjects were 20 naive, Sprague-Dawley derived, female albino rats from Carworth Farms, Inc., Portage, Michigan. They were randomly assigned to four groups of 5 members each, and were 110 days of age at the beginning of the experiment. Throughout the study, and for 60 days prior to the start of the experiment, they were housed in group cages in a normal 12-hr, day-night cycle room. An ad lib food and water regimen was in force during this 60-day period, after which the animals were housed individually and placed on a fluid-deprivation schedule that permitted 10 min of drinking per day at the same time each day during the light cycle. For the first 3 days of fluid deprivation, the 10-min drinking sessions (water from a single drinking tube) were followed by 2-min handling and taming sessions for each animal.

Each day thereafter the animals were weighed and permitted to drink fluid for 10 min from either of two drinking tubes that projected through the mesh fronts of the cages on the right-front and middle-front areas. Food was available ad lib throughout the experiment from containers mounted against the left-front of the cages.

On Days 1 through 6 of the experimental schedule, water was presented for 10 min in one of the two drinking tubes, its position being alternated daily. On Day 7 the animals were given grape flavored Kool-Aid in one tube and orange in the other. The formula was 0.25 teaspoon Kool-Aid, 1.5 teaspoons sugar, and 1.5 cups of water at room temperature. This and other solutions were made up daily. The positions of the tubes were switched half-way through the drinking session. On Day 8, the animals were given water (the position of the full tube

being varied from where it had been on the previous water-drinking day), and on Days 9 and 10 the two Kool-Aid flavors were offered simultaneously to determine flavor preferences (positions of the flavors were alternated on the second day). Fluid consumptions were recorded, and the flavor with the highest 2-day total was designated the "preferred flavor." This flavor was used throughout the subsequent conditioning period. Water drinking was permitted on Day 11.

The procedures employed during the conditioning sessions, which were conducted on Days 12 through 21, are shown in Table 1. During this time, each animal was permitted to drink a designated fluid for 10 min per day. The only differences between the experimental and control groups were that experimental subjects had 5% ethanol added to the solution in only the Kool-Aid sessions, while the control animals had 5% ethanol in only the sugar water sessions. The experimental and control groups were each divided into two subgroups. As shown in Table 1, animals in experimental Subgroup A were presented either Kool-Aid (K) or sugar water (S) in a KSSKSKKSKS order, whereas experimental Subgroup B animals were subjected to the counterbalanced order of SKKSKSSKSK. The animals in the control subgroups were similarly counterbalanced as to order of presentation of solutions. The procedure of allowing the control animals to ingest alcohol served to control for non-associative effects, provided the control animals ingested the same, or larger doses of alcohol as the experimental animals. The sugar water solutions used during the conditioning period were made identically to the Kool-Aid formula with the exception that no Kool-Aid was added. The 5% alcohol solutions were made by adding either Kool-Aid or sugar water to 100% ethanol.

Table 1. Treatments administered during the conditioning sessions. An asterisk (*) indicates the presence of 5% ethanol, K indicates the preferred flavor of Kool-Aid, and S indicates sugar water. N = 5 in each subgroup.

	Conditioning Days									
	12	13	14	15	16	17	18	19	20	21
Experimental Groups										
Subgroup A	K*	S	S	K*	S	K*	K*	S	K*	S
Subgroup B	S	K*	K*	S	K*	S	S	K*	S	K*
Control Groups										
Subgroup A	K	S*	S*	K	S*	K	K	S*	K	S*
Subgroup B	S*	K	K	S*	K	S*	S*	K	S*	K

The conditioning period was followed by 1 day of water drinking. Sixteen days of 10-min preference testing followed to determine whether conditioned aversion had been produced by the experimental treatment. During this period, the positions of the two flavors were alternated daily.

Preference values were calculated for each 2-day period during the pre-conditioning and post-conditioning sessions. These were determined by dividing the total amount of the preferred flavor of Kool-Aid consumed over the 2-day period by the total intake for the same period. The resulting ratios reflect the animal's Kool-Aid preference over the 2-day period. The ratios were used as the measure of the dependent variable because they are independent of the absolute amount of fluid consumed, being a relative, rather than an absolute measure.

Position preference has been demonstrated to be a significant source of bias in experiments in which drinking is measured (Gillespie and Lucas, 1957). Position preference effects in this experiment were controlled by alternating daily the position(s) of the specified solution(s), and by computing preference ratios on the basis of two days' consumptions.

Results

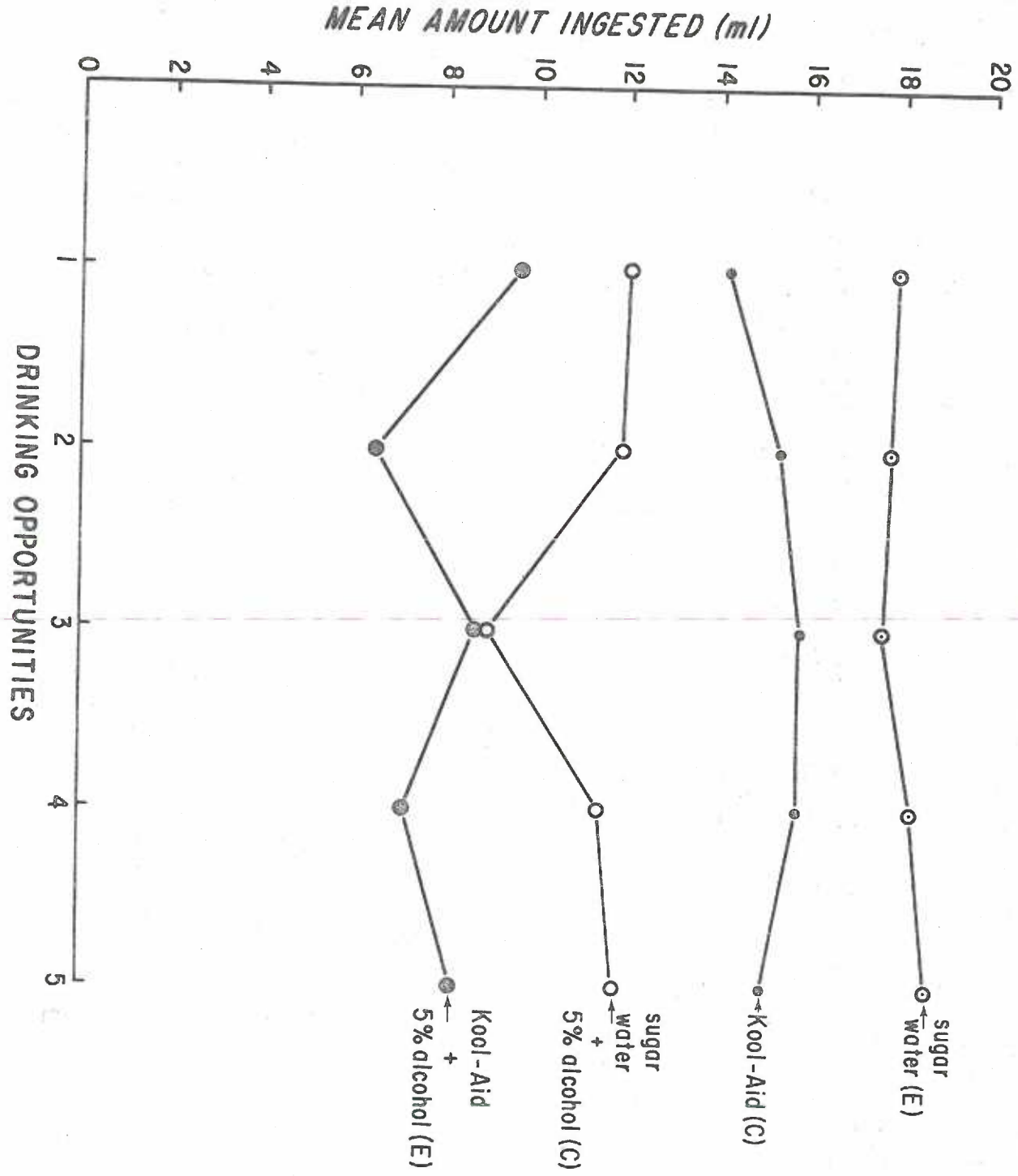
Daily intakes were recorded in ml. Preference ratios for each 2-day period were determined and constituted the data that were analyzed for the pre-conditioning and post-conditioning periods. The daily amounts consumed of Kool-Aid, Kool-Aid+5%, sugar water, sugar water+5% constituted the data analyzed for the conditioning period. These data are listed in Appendix A.

There were no demonstrable differences between the performances of the two experimental subgroups as measured by pre-conditioning preference ratios, by first 2-day block of post-conditioning preference ratios, or by average daily g alcohol/kg of body weight self-administered during the conditioning sessions. The two control subgroups likewise performed identically. Hence, the subgroups of the two sets were combined for all further analyses.

There were no significant differences between the experimental and control groups on the pre-conditioning preference values, hence subsequent changes in preference between the groups cannot be ascribed to differences existing prior to conditioning.

Two possible measures of conditioned aversion were evaluated. The first was based on the amount of fluid ingested during the forced-drinking regimen in the conditioning sessions. The amounts of fluids consumed during this period by the experimental and control groups are shown in Fig. 1. The mean of the two experimental (E) curves (sugar water and Kool-Aid+5%) did not differ significantly from the mean of the two control (C) curves (Kool-Aid and sugar water+5%). A two-factor analysis of variance with repeated measures over drinking opportunities demonstrated that the groups did differ, however, in the quantities of specific solutions consumed during the conditioning period ($F = 94.11$, $df = 3/36$, $p < .01$). Newman-Keuls follow-up tests indicated that the experimental group drank less Kool-Aid+5% than the control group drank of Kool-Aid alone ($p < .01$), while the control group drank less sugar water+5% than the experimental group drank of sugar water alone ($p < .01$).

Figure 1. Amounts of fluids consumed during the conditioning period. The number of times an animal was permitted to drink a particular solution is indicated on the abscissa. The data for the experimental group (E) consisted of the mean intakes of sugar water and of Kool-Aid+5% alcohol, while the data for the control group (C) consisted of the intakes of Kool-Aid and of sugar water+5% alcohol. A particular point on the graph represents the mean for both subgroups even though the indicated fluid was consumed on a certain day of the conditioning period for one subgroup and on a different day for the other subgroup.


MEAN AMOUNT INGESTED (ml)
0
2
4
6
8
10
12
14
16
18
20
DRINKING OPPORTUNITIES
1
2
3
4
5
sugar water (E)
Kool-Aid (C)
sugar water + 5% alcohol (C)
Kool-Aid + 5% alcohol (E)

As Fig. 1 shows, there were no progressive decreases in intakes over the conditioning period. A progressive decrease in intake of a solution which contained alcohol would be consistent with a learned taste-aversion interpretation, because repeated pairings would enhance the conditioned aversiveness of the alcohol solution and decrease the amount of that fluid consumed during subsequent sessions. The failure to observe a progressive decrease in intake for any solution precluded, on the basis of this measure, any conclusion that a conditioned aversion had developed during the conditioning period.

A between-groups analysis of the amounts consumed on the first day of conditioning was conducted to provide information on the initial palatability of each of the four solutions used during the conditioning period. Only the first day's data were analyzed since amounts ingested on the following days would be affected by amounts consumed on the first. For example, a group that consumed a small amount on the first day would be expected to have increased thirst on the next day, and thus drink proportionately more. A difference among groups was demonstrated ($F = 12.17$, $df = 3/16$, $p < .01$). A Newman-Keuls follow-up test indicated that the Kool-Aid+5% group drank less ($p < .05$) than the Kool-Aid alone group; likewise, the sugar water+5% group drank less ($p < .01$) than the sugar water alone group. In addition, the sugar water alone group consumed more fluid than the Kool-Aid alone group ($p < .01$). The order of increasing consumption (increasing palatability) was Kool-Aid+5% < sugar water+5% < Kool-Aid < sugar water. It is interesting to note that the identical order was obtained when

the total amounts consumed over the entire conditioning period were determined (Fig. 1).

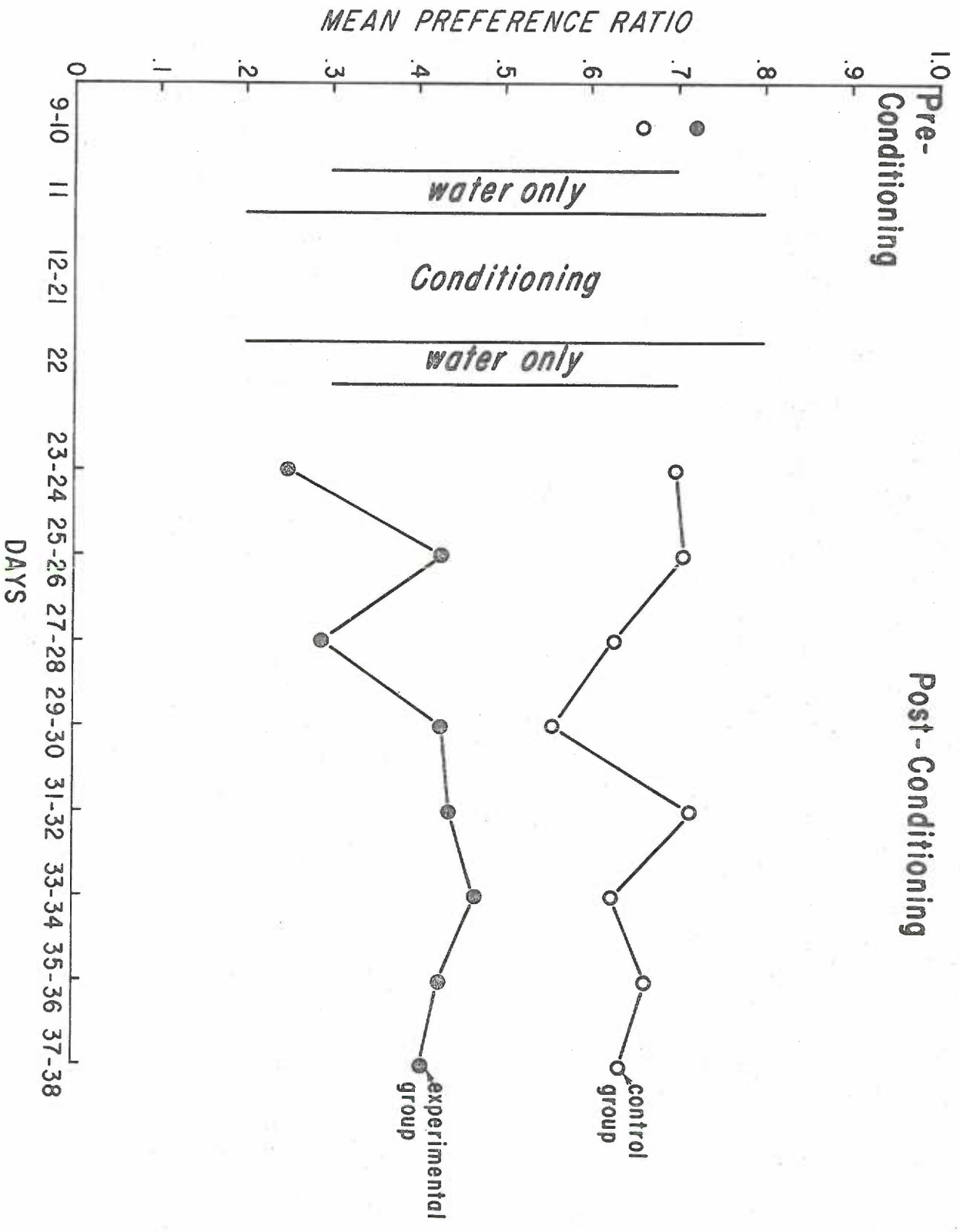
Although some of the animals that drank the alcohol solutions had difficulty in maintaining postural stability in the latter third of the 10-min drinking session, it did not appear to prevent them from continuing to drink. However, it is possible that their rate of drinking may have been reduced.

The control animals self-administered an average dosage of 2.07 g/kg/alcohol drinking day, which was larger ($F=36.57$, $df=1/18$, $p < .01$) than the dosage of 1.51 g/kg administered by the experimental animals. This difference cannot be ascribed to weight differences because there were no significant weight differences between the groups before, during, or after the conditioning period (see Appendix B).

The principal measure of conditioned aversion in this experiment involved between-group comparisons on post-conditioning preference ratios, as well as within-group analyses of pre- and post-conditioning ratios. A significant decrease in preference for the preferred flavor of Kool-Aid on post-conditioning tests would be consistent with the conclusion that the presence of the alcohol in the Kool-Aid mixture resulted in that flavor's becoming secondarily aversive.

The results of the pre-conditioning and post-conditioning preference tests are presented in Fig. 2. A two-factor analysis of variance, with repeated measures over the 2-day blocks of the post-conditioning preference-testing period indicated that the experimental animals which had the 5% alcohol mixed with the preferred flavor of Kool-Aid during the conditioning period, drank less of the preferred flavor of

Figure 2. The mean preference ratio (proportion of preferred flavor of Kool-Aid to total fluid intake) for each 2-day block during the pre-conditioning and post-conditioning sessions. The Kool-Aid solutions did not contain alcohol.


MEAN PREFERENCE RATIO
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
Pre-Conditioning
water only
Conditioning
water only
Post-Conditioning
9-10
11
12-21
22
23-24
25-26
27-28
29-30
31-32
33-34
35-36
37-38
DAYS
control group
experimental group

Kool-Aid during the post-conditioning sessions than the control animals, for whom alcohol was never mixed with Kool-Aid ($F = 22.97$, $df = 1/18$, $p < .01$). Within-group analyses of the post-conditioning preference tests indicated no significant changes over days in the drinking of either the control or the experimental group. A within-group comparison of the pre-conditioning with the first block of 2-day post-conditioning preferences for the control animals demonstrated that there was no significant change in preference as a result of the procedures these animals were subjected to during the conditioning period. On the other hand, the experimental animals displayed a large decrease in preference as a result of the conditioning procedure ($F = 47.21$, $df = 1/8$, $p < .01$). It is of interest to note that all 10 experimental subjects demonstrated a decrease in preference.

The rank-order correlation between the mean amount of alcohol drunk per day during the conditioning period and the difference between the pre-conditioning and post-conditioning preference ratios (first 2-day block) was not significantly different from zero ($rho = -.43$).

Although the experimental and control groups consumed the same total amount of Kool-Aid during the pre-conditioning preference tests, the control group's total consumption of both flavors of Kool-Aid was higher than the experimental group's on the first 2-day block of post-conditioning preference tests ($F = 26.01$, $df = 1/18$, $p < .01$). This difference resulted from a significant increase from pre- to post-conditioning tests in the control group's total consumption of both Kool-Aid flavors ($F = 30.08$, $df = 1/9$, $p < .01$), while the experimental group's total consumption of both flavors remained

unchanged. Furthermore, although there was no difference between groups in water consumption, there was an increase for both groups from the day prior to exposure to the two Kool-Aid flavors to the first day after conditioning (experimental - $F = 42.66$, $df = 1/9$, $p < .01$; control - $F = 11.25$, $df = 1/9$, $p < .01$).

Discussion

Conditioned taste aversion was demonstrated in this experiment by significant differences in preference ratios between experimental and control groups on post-conditioning preference tests, and by within-group comparisons of pre- and post-conditioning ratios. The experimental animals' significant decrease in preference for the preferred flavor of Kool-Aid on post-conditioning tests is consistent with the conclusion that the presence of the alcohol in the Kool-Aid mixture during conditioning resulted in that flavor's becoming secondarily aversive. The conditioned aversion resulted from five presentations of Kool-Aid with 5% alcohol at an average alcohol dosage of 1.51 g/kg/drinking day.

The post-conditioning preference tests, in which two flavors were presented simultaneously are not viewed as involving strong conflicts, since the nonpreferred flavor was never mixed with alcohol and thus should not have acquired significant aversive properties. Consistent with this view was the finding that the consumption of the nonpreferred flavor increased significantly from pre-conditioning to post-conditioning tests. The controls had the same number of opportunities to drink the Kool-Aid and sugar water solution, but for them the 5% alcohol was mixed with sugar water and never with Kool-Aid. Even though this

procedure during the conditioning period resulted in the control animals' administering more alcohol than the experimental rats, there was no change in preference for the controls.

The consistently reduced intake of solutions which contained alcohol relative to solutions without alcohol observed throughout the conditioning period could be due to unconditioned aversion to the alcohol solutions, and/or conditioned aversions, and/or a reduced rate of drinking in animals that ingested alcohol in sufficient quantities to become intoxicated prior to completion of the 10-min drinking period.

The learned taste-aversion interpretation developed previously would have predicted a progressive decrease in the amount of fluid consumed because it would be expected that repeated pairings would enhance the conditioned aversiveness of the alcohol solution and decrease the amount of that fluid consumed during subsequent sessions. The absence of progressive decreases in the consumption of the fluid mixed with alcohol during the forced-drinking conditioning period does not support such an interpretation, but it can perhaps be explained in terms of conflict. Recall that the forced-drinking procedure can be viewed as a conflict situation wherein the tendency to approach the drinking tube to obtain the daily fluid is opposed by a tendency to avoid the aversive alcohol solution. The lack of a progressive decrease in the consumption of either Kool-Aid+alcohol or sugar water+alcohol in the present experiment can be considered as supporting the conclusion that the relative strengths of approach and avoidance did not change during the conditioning period as a result of

CS-UCS pairings. Presumably, any increment in conditioned aversion was balanced out by comparable increases in the strength of the approach component. By contrast, Eckardt et al (1974) found a progressive decrease in the amount consumed over conditioning days at dosages of 0.8 g/kg when administered i.p., which is much less than the 1.51 g/kg administered in this experiment. The latter observation is supportive of the hypothesis of Lester et al (1970) that the administration of alcohol via i.p. or intracardiac catheter is more aversive than stomach intubation.

The observation that the control animals self-administered significantly more alcohol than the experimental animals during the conditioning period but did not change their preference, and that there were no changes in body weights during the conditioning period for either group, argue against the possibility that general malaise throughout the conditioning period served as the effective condition for the production of conditioned aversion. The results of this experiment provide no information on what is (are) the effective UCS(s), but do indicate that the rapid drinking of 5% alcohol solutions has accompanying aversive consequences.

It may be noted that although the amounts of Kool-Aid consumed comprised the primary data evaluated in this experiment, in principle, the sugar water solution should also have become an aversive stimulus for the control animals since it was mixed with 5% alcohol during the conditioning period. No tests of this possibility were conducted. However, it was noted that conditioned aversions were developed to the same extent for both grape (N = 6) and orange (N = 4) flavors.

It was noted earlier that the control animal's combined consumption of preferred and nonpreferred Kool-Aid on the first 2-day block of post-conditioning tests was higher than on the pre-conditioning sessions. One explanation of this finding is that the control animals learned during the conditioning period that "safety" (Kalat and Rozin, 1973) was associated with Kool-Aid and not with sugar water. If the process of "learned safety" was operating, there should have been an increase in the consumption of the preferred flavor by the controls. This follows, because for them, the preferred flavor was never combined with alcohol, whereas sugar water was always mixed with alcohol. Analysis of the data obtained for the controls indicated a significant increase in consumption of the preferred flavor from pre- to post-conditioning. However, there was no such increase observed for the nonpreferred flavor. This finding suggested that any safety associated with the preferred flavor did not generalize to the nonpreferred flavor.

EXPERIMENT II

Introduction

The finding, in Experiment I, that learned taste aversions can be produced by combining alcohol with a preferred flavor of Kool-Aid during a conditioning period supports the conclusion that aversive consequences accompany the rapid oral ingestion of alcohol solutions. The results of that experiment, however, did not enable one to determine precisely which of the attributes of orally ingested alcohol functioned aversively. As noted earlier, alcohol may have exerted its effects because of its centrally mediated aversive consequences (Lester et al, 1970), because of its aversive orosensory qualities (Lester, 1966; Myers and Veale, 1972), because of its novelty (Amit, Ziskind, and Baum, 1973), or because of some combination of these.

Presumably the procedure followed during the conditioning period in Experiment I resulted in the subjects' experiencing both the orosensory and the centrally mediated effects of alcohol in close association with the cues provided by Kool-Aid. Orosensory stimuli were necessarily present because the animals drank the Kool-Aid+alcohol solution. Inebriation effects were assumed to be present since after drinking the 5% solution, the animals exhibited clear signs of intoxication such as difficulty in maintaining balance and lurching movements.

The specific goal of Experiment II was to ascertain whether aversive orosensory concomitants are alone sufficient to produce conditioned aversions or whether a "systemic, presumably centrally mediated, aversion-inducing effect" as Lester et al (1970) described it, must also be induced. One group was given six massed drinking trials in

10 min, while a second group was given approximately the same amount of fluid in six trials which were widely distributed throughout the day. It was assumed that the massed (10-min forced-drinking) group would experience both the orosensory stimuli and the centrally mediated intoxication stimuli whereas the distributed drinking group would experience only the orosensory stimuli. This expectation rests on the added assumption that, with distributed drinking, the concentration of alcohol in the blood failed to reach a level high enough to produce aversive central effects. In principle, animals can be given a number of small doses of a 5% alcohol solution at sufficiently long intertrial intervals to avoid intoxication, provided metabolic destruction of the alcohol from one dose occurs prior to the next dose.

Several investigators have noted that when drinking is distributed throughout the day, animals can consume alcohol solutions without any apparent signs of intoxication. Richter (1926) reported that rats whose only source of fluid was either an 8% or 16% alcohol solution ingested their daily fluid intake during 8-12 drinking bouts spaced throughout the day. On the basis of these unquantified observations, he suggested that this type of behavior would enable an animal to consume all the water required for normal maintenance, and, if the drinking bouts were sufficiently separated in time to allow complete metabolism of the previously ingested alcohol, elevated levels of blood alcohol would not occur.

After reviewing the literature on the self-selection of alcohol solutions, Lester (1966) also concluded that the amounts of alcohol ingested daily, as generally reported in the literature, do not appear

to exceed the animal's capability to metabolize the drug provided the doses are widely spaced throughout the day. Further, he noted that experimenters have not measured the temporal distribution of the drinking of alcohol solutions nor have they made periodic determinations of the blood-alcohol levels of animals for which alcohol solutions have been available continuously.

A direct comparison of the effects of alcohol ingested in a relatively short period of time (30 min) with the effects of alcohol ingested over a 24-hr period has been made by Carey (1972). He observed that rats given 30 min per day to drink their total fluid intake from a 10% alcohol solution for 10 days drank significantly less alcohol during a subsequent 24-hr, two-choice test of 10% versus water than they had originally. Even though the total volume consumed by animals which had the alcohol solution available throughout a 24-hr period was three times higher than the amount consumed by the 30-min forced-drinking group, no change in preference for alcohol was observed in the 24-hr group. Thus, the ingestion of large doses of alcohol in a short time reduced alcohol consumption, whereas the drinking of significantly larger doses over a longer period failed to diminish alcohol consumption. Carey (1972), therefore, concluded that the amount and temporal spacing of alcohol consumed during a forced-drinking situation influenced subsequent alcohol preference. Unfortunately, the lack of data on the temporal distribution of drinking of animals in the 24-hr group or on blood-alcohol levels of the 30-min and 24-hr animals precludes any clearly supported conclusion as to why the groups differed on subsequent preference tests.

Experiment II was designed to insure that all subjects consumed approximately the same amount of fluid during the conditioning period, but that all daily fluid intake occurred only during either massed or distributed presentations of the designated fluids. Similar procedures to those followed in Experiment I were used in the pre-conditioning and post-conditioning sessions and are described in detail in the procedure section of this experiment.

There are two major assumptions underlying the present experiment. The first is that a distributed drinking procedure significantly reduces or eliminates aversive centrally mediated effects. The second is that the strength of the acquired aversion to Kool-Aid should depend upon both the number of paired presentations of Kool-Aid (CS) with the aversive orosensory components of alcohol (UCS) and upon the aversiveness of the centrally mediated aversion. Since both groups had on the order of six CS-UCS pairings per day, the amount of conditioning due to that fact alone should have been the same. The longer intertrial intervals in the distributed group (2 hr) would presumably lead to superior conditioning for them. However, the more marked central effect in the massed group would presumably lead to superior conditioning for them.

The four groups required for a balanced experimental design were (1) a massed drinking experimental group which drank either a preferred flavor of Kool-Aid+5% alcohol or a sugar water solution during six massed drinking trials in a single daily 10-min period, (2) a massed control group which had sugar water+5% alcohol on six occasions during a 10-min session but never had the alcohol mixed with the preferred flavor of Kool-Aid, (3) a distributed drinking experimental group that

received six widely spaced opportunities to drink its preferred flavor of Kool-Aid+5%, but was never allowed to ingest more than 2 ml on any one trial, (4) a distributed control group which was allowed to drink the same amount, at the same times, as the distributed experimental group, but the alcohol was mixed with sugar water, not with Kool-Aid. The counterbalancing of the groups and the control procedures for position effects were identical to those used in Experiment I.

As indicated above, the distributed groups had six daily opportunities to drink their designated fluids, successive trials being separated by a 2-hr period. The maximum fluid consumed on any one of these trials was 2 ml. This value was chosen because the average daily intakes of the Kool-Aid+5% animals in Experiment I were never greater than 12 ml. Another reason for limiting the amount of alcohol that could be consumed on any one trial was that the metabolism of alcohol proceeds at a fixed rate. The lowest reported rate of metabolism in an adult rat is .27g/kg/hr (Aull, Roberts, and Kinard, 1956), and the highest rate is .30 g/kg/hr (Kalant, 1971). Regardless of which value is closer to being correct, if the amounts of alcohol consumed on any one trial by the distributed groups were small, there would be a significantly lower level of blood alcohol in those groups than in the massed groups. For example, a 200 g rat which consumed 2 ml of a 5% alcohol (v/v) solution would administer a dosage of .395 g/kg. If the alcohol were metabolized at .270 g/kg/hr, this dosage should be completely metabolized by the end of the 2-hr intertrial interval used for the distributed drinking animals in the second experiment. The latter calculations are based on the assumption that the alcohol is present in the vascular

system as quickly when it is absorbed from the digestive tract as when it is injected i.p. or i.v.; clearly this is not the case. Nevertheless, a reduced peak blood-alcohol level should be observed in the animals subjected to a distributed drinking procedure relative to the rapid ingestion by the massed animals.

In that the massed experimental group in this experiment was treated in nearly an identical manner as was the experimental group of the first experiment, it was predicted that aversion would be exhibited. The two groups differed only in that the experimental group in Experiment I was permitted to drink continuously for 10 min whereas the massed group's drinking was broke-up into six variable time periods which together totaled 10 min. The experimental group in the first experiment never drank more than 12 ml of the Kool-Aid+5% solution on any conditioning day, and this solution was consistently consumed in lesser amounts than the other solutions (see Fig. 1). Based on the latter observation, it was predicted that animals in groups other than the massed experimental group would consume quantities equal to or larger than the maximum amount consumed by any animal in the massed experimental group. Therefore, in the present experiment the largest amount of Kool-Aid+5% drunk by any animal in the massed experimental group (up to and including 12 ml) determined the maximum amount every animal in the other groups was allowed to consume for that day. This procedure resulted in the massed experimental animals' drinking from 0-12 ml per day, whereas all other animals could drink a quantity equal to the maximum amount consumed by any animal in the massed experimental group. In that

larger amounts of the alcohol solution would be consumed by every group other than the massed experimental group, this technique should increase the possibility of obtaining conditioned aversion in the distributed experimental group as well as non-associative effects in both control groups.

If centrally mediated aversive effects are necessary for the development of conditioned taste aversions, then the massed experimental group should be the only to exhibit a significant post-conditioning decrement in its consumption of the preferred flavor of Kool-Aid. The preferences of all other groups should not differ significantly and should change but little from their pre-conditioning levels. But if orosensory stimuli alone are sufficiently aversive to effect conditioned aversions, then both the massed and the distributed experimental groups should show significant decrements in their choice of the preferred flavor of Kool-Aid during the post-conditioning tests. If both central and orosensory components are aversive, then decrements in preference should be exhibited by both the massed and the distributed experimental groups, but the massed group should exhibit a greater decrement in preference because of a larger additional central effect.

Subjects and Procedure

The subjects were 40, naive, Sprague-Dawley derived, female albino rats from Carworth Farms, Inc., Portage, Michigan. They were randomly assigned to four groups of 10 members each, and were 120 days old at the beginning of the experiment. Throughout the study, and for 7 days prior to the start of the experiment, they were housed in

individual cages in a normal 12-hr, day-night cycle room. An ad lib food and water regimen was in effect during this 7-day period, after which the animals were placed on a fluid deprivation schedule that permitted 10 min of drinking per day at the same time each day during the light part of the cycle. For the first 3 days of fluid deprivation, the 10-min sessions (water from a single drinking tube) were followed by individual 2-min handling and taming sessions.

Each day thereafter the animals were weighed and permitted to drink fluid for 10 min from either of two drinking tubes (one of which was empty) that projected through mesh fronts of the cages on the right-front and middle-front areas. Food was available ad lib throughout the experiment from containers mounted against the left-front of the cages.

On Days 1 through 3 of the experimental schedule, water was presented for 10 min in one of the two drinking tubes, its position being alternated daily. To accustom the animals to the Kool-Aid solutions, they were given grape-flavored Kool-Aid in one tube and orange-flavored Kool-Aid in the other on Day 4. The positions of the tubes were switched half-way through the drinking session. The formula for the Kool-Aid solution was identical to the one used in Experiment I (0.25 teaspoon Kool-Aid, 1.5 teaspoons sugar, and 1.5 cups of water at room temperature). On Day 5, the animals were given water (the position of the full tube being varied from where it had been on the previous water-drinking day), and on Days 6 and 7 the two Kool-Aid flavors were offered simultaneously to determine flavor preferences. The positions of the flavors were changed on Day 7.

Fluid consumptions were recorded in ml, and the flavor with the highest 2-day total was designated the "preferred flavor" for each animal. This flavor was used throughout the subsequent 10 conditioning days.

Conditioning sessions were given on Days 8 through 17. The treatments of the four major groups and eight subgroups are summarized in Table 2. As the table indicates, animals in all groups had the same number of opportunities to drink the preferred flavor of Kool-Aid and the sugar water solution so as to preclude any subsequent differences among them arising from differential exposures during the conditioning period. Animals in both experimental groups had 5% ethanol mixed with the preferred flavor of Kool-Aid and never with the sugar water, whereas subjects in both control groups had 5% ethanol mixed with the sugar water and never with the preferred flavor of Kool-Aid. Rats in the distributed groups drank their daily intake in six trials at a 2-hr intertrial interval. However, they were never allowed to consume more than 2 ml on any one trial. The sugar water solutions used during the conditioning period were made identically to the Kool-Aid formula with the exception that no Kool-Aid was added. The 5% alcohol solutions were made by adding either Kool-Aid or sugar water to 95% ethanol.

The groups were equated for daily fluid in the following manner: First, animals in the subgroup of Group I which were scheduled to drink the preferred flavor of Kool-Aid+5% (K*) were allowed to drink that solution in six variable time periods totaling 10 min (massed drinking), the only restriction being that no subject was allowed to

Table 2. Treatments administered during the taste-aversion conditioning sessions. An asterisk (*) indicates the presence of 5%, K indicates the preferred flavor of Kool-Aid, and S indicates sugar water. N = 5 in each subgroup

	Conditioning Days									
	8	9	10	11	12	13	14	15	16	17
Group I - Massed Experimental										
Subgroup A	K*	S	S	K*	S	K*	K*	S	K*	S
Subgroup B	S	K*	K*	S	K*	S	S	K*	S	K*
Group II - Massed Control										
Subgroup A	K	S*	S*	K	S*	K	K	S*	K	S*
Subgroup B	S*	K	K	S*	K	S*	S*	K	S*	K
Group III - Distributed Experimental										
Subgroup A	K*	S	S	K*	S	K*	K*	S	K*	S
Subgroup B	S	K*	K*	S	K*	S	S	K*	S	K*
Group IV - Distributed Control										
Subgroup A	K	S*	S*	K	S*	K	K	S*	K	S*
Subgroup B	S*	K	K	S*	K	S*	S*	K	S*	K

drink more than 12 ml. These animals were subjected to six distinct drinking periods because all massed animals had their tubes withdrawn and inserted six times during their 10-min drinking sessions. This procedure served to equate massed and distributed groups for the number of insertions and withdrawals of drinking tubes during any single conditioning day, and therefore equated them also for the number of CS-UCS pairings, provided the subject drank on each occasion when the tube was inserted. The maximum amount consumed by any animal in this group was determined and designated as the criterial quantity. Second, animals in Group II, as well as those in the other subgroup of Group I which were scheduled to drink sugar water, were all allowed to ingest up to the criterial amount of their specified fluids in their 10-min drinking period. Third, subjects in Groups III and IV were also allowed to drink the criterial amount in six separate drinking trials, an equal amount being consumed on each trial. At no time were rats in Groups III and IV allowed to drink more than 2 ml per trial. The latter restriction limited the maximum total intake per conditioning day for all groups to the criterial amount which could vary to a maximum of 12 ml.

The 10 conditioning days were followed by 4 days of 10-min preference testing, the positions of the two flavors being alternated daily.

An additional 15, naive, female, albino rats from Carworth Farms, Inc. served as subjects to determine whether different blood-alcohol levels resulted from the massed and from the distributed drinking procedures. The subjects were of the same age and weight as the

animals used in Experiment II. They were subjected to the identical paradigm utilized in Experiment II, up to and including the first day of conditioning.

On the first day of conditioning, five animals were given six massed drinking trials with Kool-Aid+5% solution for 10 min, the only restriction being that no animal was allowed to consume more than 12 ml. Upon completion of the six-trial 10-min session, the blood-alcohol levels of these animals were determined from samples taken at 5, 25, 65, 95, 125, and 185 min. The enzymatic assay for determining the blood-alcohol level as well as the surgical technique used for obtaining the venous blood samples are described in Appendix C. Another five animals were allowed to drink 2 ml of the Kool-Aid+5% solution prior to having their blood-alcohol levels determined from samples taken at the same time periods as the massed experimental animals. The final five animals were permitted to drink 2 ml of the Kool-Aid+5% solution in each of six trials, with successive trials being separated by 2 hr. Following consumption of the last 2 ml, blood-alcohol levels were determined for this group with samples taken at the same time periods as the previously mentioned two groups.

Results

The preference ratios for each 2-day period during the pre- and post-conditioning sessions were calculated in the same manner as in Experiment I, i.e., the amount of preferred flavor of Kool-Aid consumed over the 2-day period was divided by the total intake for that period. The resulting value reflects the strength of an animal's preference for one of the two flavors of Kool-Aid. These ratios were derived from data listed in Appendix D.

The subgroups were combined for all analyses of the four principal groups, massed experimental (I), massed control (II), distributed experimental (III), and distributed control (IV).

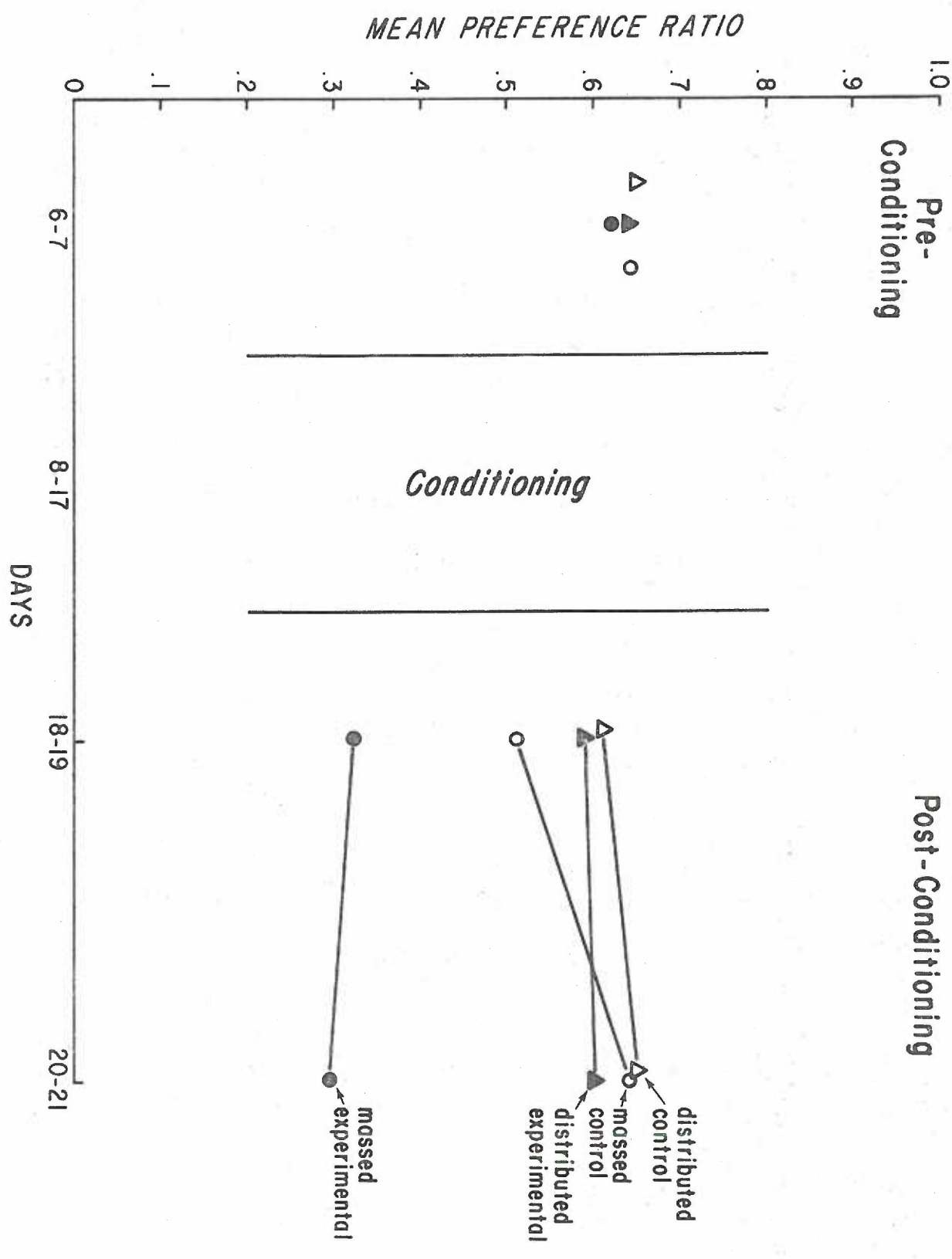
There were no significant differences among the pre-conditioning preference values for the four groups, hence any subsequent differences between them cannot be ascribed to differences that existed prior to conditioning.

Measures of conditioned taste aversion

The major measure of conditioned aversion was based on between-group comparisons of post-conditioning preference ratios, as well as on within-group contrasts of pre- and post-conditioning ratios. A significant decrease in preference for the preferred flavor of Kool-Aid on post-conditioning tests would be consistent with the conclusion that the presence of the alcohol in the Kool-Aid mixture resulted in that flavor's becoming secondarily aversive.

The results of the pre-conditioning and post-conditioning preference tests are presented in Fig. 3. A two-factor analysis of variance, with repeated measures over the 2-day blocks of the post-conditioning tests yielded differences among the groups ($F = 13.05$, $df = 3/36$, $p < .01$). A Newman-Keuls follow-up test showed that the massed experimental group differed from each of the distributed groups ($p < .01$) and from the massed control group ($p < .02$), while none of the other groups differed statistically from one another. A within-group comparison of the pre-conditioning and the post-conditioning preferences for the massed experimental animals indicated a decrease in preference following the conditioning procedure

Figure 3. The mean preference ratio (proportion of preferred flavor of Kool-Aid to total fluid intake) for each 2-day block during the pre-conditioning and post-conditioning sessions.


MEAN PREFERENCE RATIO
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
Pre-
Conditioning
Conditioning
Post-Conditioning
6-7
8-17
18-19
20-21
DAYS
distributed
control
massed
control
distributed
experimental
massed
experimental

($F=23.27$, $df=1/9$, $p < .01$). However, none of the other groups of animals displayed any change in preference from the pre- to the post-conditioning tests.

A second measure of conditioned aversion was provided by the amounts of fluids ingested during the forced-drinking regimen utilized for the conditioning sessions. The daily mean intakes for the four groups during the conditioning period are summarized in Table 3. A visual inspection of these values indicates that the massed experimental group consumed less of its assigned Kool-Aid+5% solution than the other groups consumed of theirs. In no instance was a progressive decrease in intake observed in any solution over the conditioning period.

A progressive decrease in consumption of a solution which contained alcohol would be consistent with a learned taste-aversion interpretation, because repeated pairings should enhance the conditioned aversiveness of the alcohol solution and decrease the intake of that fluid during subsequent sessions. The failure to observe a progressive decrease in intake for any solution precluded, on the basis of this measure, any conclusion that a conditioned aversion had developed during the conditioning period.

A Kruskal-Wallis one-way analysis of variance revealed significant group differences in the amounts of alcohol consumed during the conditioning period ($H=20.73$, $p < .01$). The massed experimental animals self-administered 1.69 g/kg/drinking opportunity, whereas all other groups of animals administered larger doses, distributed experimental group-2.04 g/kg, distributed control group - 2.04 g/kg, massed control group - 2.06 g/kg. These differences in dosage cannot be ascribed

Table 3. Mean amounts of fluids in ml consumed during the conditioning period. The number of times an animal was allowed to drink a particular solution is indicated at the top of the table. The tabled values represent means of consumptions on different days for the two subgroups.

Preferred Flavor of Kool-Aid

Group	Concentration of alcohol	Drinking Opportunities				
		1	2	3	4	5
Massed Exptl.	5%	8.9	8.5	9.9	8.8	9.8
Massed Control	0%	11.6	10.0	11.5	11.0	11.0
Distr. Exptl.	5%	12.0	10.0	11.5	11.0	11.0
Distr. Control	0%	12.0	10.0	11.5	11.0	11.0

Sugar Water

Group	Concentration of alcohol	Drinking Opportunities				
		1	2	3	4	5
Massed Exptl.	0%	12.0	10.0	11.5	11.0	11.0
Massed Control	5%	10.7	10.0	11.3	11.0	11.0
Distr. Exptl.	0%	12.0	10.0	11.5	11.0	11.0
Distr. Control	5%	12.0	10.0	11.5	11.0	11.0

to weight differences among the groups since there were no demonstrable weight differences before, during, or after the conditioning period (see Appendix E).

The rank-order correlation between the mean dosage of alcohol administered per drinking opportunity for each of the massed experimental subjects during the conditioning period and the difference between each animal's preference ratio on the first 2-day block of post-conditioning tests relative to its pre-conditioning preference ratio was not significantly different from zero (rho = +.20).

There were no significant differences in the total consumption of the two flavors of Kool-Aid on the first 2-day block of post-conditioning preference tests among the four groups. However, all groups, except the massed experimental group, demonstrated a significant increase from the amount consumed during the pre-conditioning preference tests (distributed experimental group - $F=103.3$, $df=1/9$, $p < .01$; distributed control group - $F=25.71$, $df=1/9$, $p < .01$; massed control group - $F=23.37$, $df=1/9$, $p < .01$).

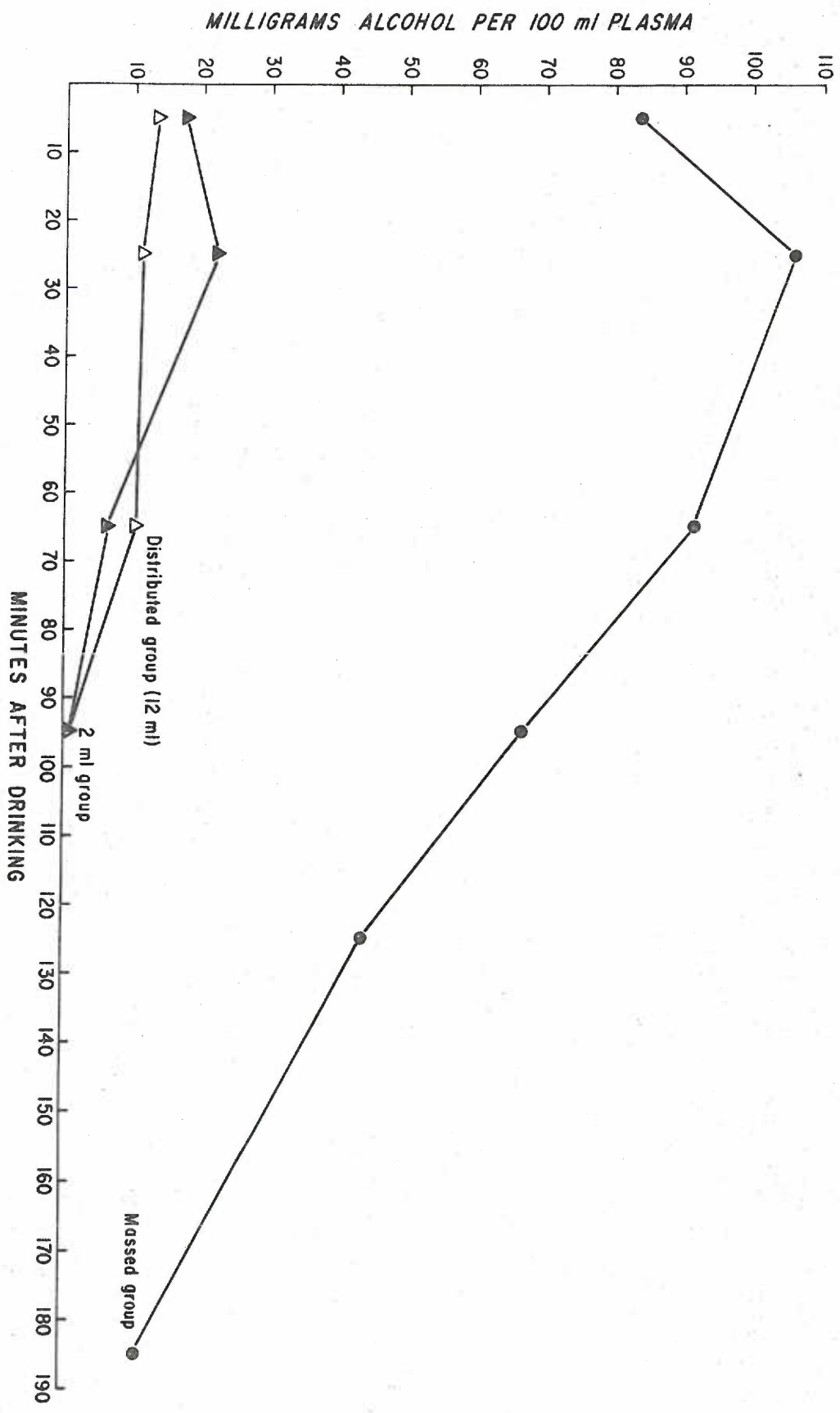
Blood alcohol

Presumably, the dynamics of the central concomitants of alcohol consumption can be approximated by monitoring blood-alcohol levels. If the effective determinant in the taste-aversion paradigm of this experiment was an aversive centrally mediated state, then it follows that the massed drinking should have resulted in a higher peak blood-alcohol level than distributed drinking. More specifically, it would be expected that the massed drinking procedure resulted in a higher peak blood-alcohol level than that attained at the completion of the last drinking trial in the distributed procedure. Even though the

animals given six distributed trials were expected to consume more alcohol than the experimental animals given six massed trials (see results of massed experimental animals versus distributed experimental animals presented in Table 3), it was predicted that these distributed animals would have significantly lower peak blood-alcohol levels because of the metabolic destruction of ethanol between trials at the rate of approximately .27 g/kg/hr. The difference between the blood-alcohol levels determined after the first 2-ml trial and after the sixth trial should provide information on the rate of accumulation of alcohol in the blood which occurred as a result of the distributed drinking procedure.

As expected, the massed experimental animals drank less of the Kool-Aid+5% (Md=8 ml) than the distributed experimental subjects (Md=12 ml). Also, the massed group self-administered a median dosage of 1.40 g/kg which was significantly less than the median dosage of 2.20 g/kg administered by the distributed animals ($U=0.0$, $p < .01$). The blood-alcohol curves for the three groups are presented in Fig. 4. A Kruskal-Wallis one-way analysis of variance of the peak blood-alcohol levels demonstrated significant differences among the three groups ($H=10.82$, $p < .01$). A follow-up analysis of this finding was conducted using Mann-Whitney U tests. It was shown that the massed group differed from both of the other groups ($p < .01$), whereas the other two groups were not demonstrably different. The finding that the blood-alcohol level of the distributed group did not increase after six trials spaced throughout the day, relative to the level observed after the first trial, supported the conclusion that all or almost all of the .37 g/kg consumed

Figure 4. Median blood-alcohol curves for fluid-deprived animals permitted to drink a Kool-Aid+5% solution. The massed group of animals was presented the solution for 10 min, the distributed group was allowed to drink only 2 ml at each of six trials, with each trial being separated by 2 hr, and the "2-ml group" was permitted to consume only 2 ml of the alcohol solution. N = 5 subjects in each group.


MILLIGRAMS ALCOHOL PER 100 ml PLASMA
10
20
30
40
50
60
70
80
90
100
110
MINUTES AFTER DRINKING
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
Distributed group (12 ml)
2 ml group
Massed group

during any one trial was metabolized prior to the next trial. The data for these animals are listed in Appendix F.

Discussion

A conditioned taste aversion was demonstrated in this experiment by the significant differences in preference ratios between the massed experimental group and all the other groups on post-conditioning preference tests, as well as by within-group comparisons of pre- and post-conditioning ratios. The massed experimental animals' significant decrease in consumption of their preferred Kool-Aid flavor on post-conditioning tests is consistent with the conclusion that the presence of the alcohol in the Kool-Aid mixture during conditioning resulted in that flavor's becoming secondarily aversive. The conditioned taste aversion exhibited by the massed experimental but not by the other groups resulted from five presentations of the preferred flavor of Kool-Aid+5% with a mean alcohol dosage of 1.69 g/kg/drinking day. This value approximates the 1.51 g/kg dosage which produced conditioned taste aversion in Experiment I.

As noted earlier, the two-stimulus post-conditioning preference tests are not viewed as a conflict situation because the nonpreferred flavor was never mixed with alcohol, and thus should not have acquired aversive properties. Consistent with this formulation is the finding that consumption of the nonpreferred flavor significantly increased during the post-conditioning preference tests for the massed experimental animals. The massed control animals had the same number of opportunities as the experimental animals to drink the Kool-Aid and the sugar water solution, but for them the 5% alcohol was mixed with sugar water and never with Kool-Aid. Even though this procedure resulted in the massed control

animals' ingesting more alcohol than the experimental animals during the conditioning period, there was no change in preference for the massed control animals.

The observations that the massed control animals drank significantly more alcohol than the experimental animals during the conditioning period but did not alter their preference, and that there were no changes in body weights during the conditioning period for either group, argue against the possibility that a general malaise throughout the conditioning period served as the effective determinant of the conditioned aversion.

The failure to demonstrate conditioned taste aversion in the distributed experimental animals as well as the finding of conditioned aversion in the massed experimental animals support and provide a quantitative basis for Carey's (1972) assertion that the amount and temporal spacing of alcohol consumption during a forced-drinking situation determines whether aversive consequences will accompany alcohol ingestion. Carey (1972) based his conclusion on the observation that when alcohol was available continuously, relatively large amounts were ingested without altering subsequent alcohol consumption, whereas the consumption of smaller amounts in short time periods did reduce alcohol preference on subsequent tests.

The absence of conditioned taste aversion in the distributed animals is interpreted as supporting the hypothesis that the orosensory stimuli that accompany the drinking of 5% alcohol solutions are not sufficiently aversive by themselves to produce conditioned aversions. Were oropharyngeal stimulation alone sufficient to produce a conditioned aversion, the distributed animals that drank more alcohol than the massed subjects (see Table 3) and hence had more aversive (UCS) stimulation, should have

exhibited more conditioned taste aversion than the massed group. Furthermore, the assumption that the distributed animals experienced more orosensory stimulation than did the massed animals can also be supported by consideration of the phenomenon of adaptation. The reduction in sensitivity to taste stimulation with continuous exposure to a solution occurs in 1-5 min, while the reduction in sensitivity to olfactory stimulation occurs in 20-90 sec (Geldard, 1972). Thus, it would be expected that the massed drinking animals would experience a greater degree of adaptation in their 10-min drinking period than the distributed drinking animals in each of their short drinking periods, which were never longer than 1 min. Hence, it seems reasonable to assume that the massed experimental animals experienced a lower average level of orosensory stimulation (UCS intensity) than did the distributed experimental subjects. In spite of this, the massed subjects demonstrated a conditioned aversion, while the distributed subjects did not alter their preference.

In addition, the massing of the conditioning trials in the massed group should have been less effective in producing conditioned aversion than temporally separating the conditioning trials as in the distributed procedure.

The demonstration that the rapid ingestion of alcohol in a 10-min drinking period resulted in significantly greater peak blood-alcohol levels than distributed drinking of even larger total doses of alcohol is consistent with the hypothesis that a centrally mediated aversive state, as measured by peak blood-alcohol levels, was the determinant of the conditioned taste aversion observed in the massed experimental animals. Thus, an explanation of the absence of a conditioned taste aversion in

the distributed experimental animals is that their blood-alcohol levels were much lower than those of the massed experimental animals. This result was expected because the distributed animals were allowed to drink only a relatively small amount of the Kool-Aid+5% at each of the six trials per day, and the trials were separated by 2 hr which was sufficient to insure metabolic destruction of most of the ingested alcohol prior to the next trial. The lack of a conditioned taste aversion with distributed drinking of alcohol occurred even though these animals self-administered significantly more total alcohol than did the massed experimental animals.

Thus, the results of Experiment II are consistent with the suggestion of Lester et al (1970) that the central concomitants accompanying the administration of alcohol have aversive consequences. However, it is also plausible that the novelty of the drug state (Amit et al, 1973), or the combined effects of orosensory and centrally mediated effects of alcohol may be the determinants of conditioned aversions. Consequently, the results of Experiment II cannot be interpreted as indicating unambiguously that conditioned taste aversions are due solely to centrally mediated aversive states.

The absence of a progressive decrease over conditioning days in the consumption of any fluid which contained alcohol was inconsistent with a learned taste-aversion interpretation. Such an interpretation would have predicted a progressive decrease in the amount of fluid consumed because it would be expected that repeated pairings would enhance the conditioned aversiveness of the alcohol solution and decrease the amount of that fluid consumed during subsequent sessions. The consistently reduced intake of the Kool-Aid+5% solution by the massed experimental animals could be due to

unconditioned aversion to the alcohol solution and/or to conditioned aversion. Reduced intakes were not observed in animals that drank sugar water+5% in 10-min periods, nor were they observed in any solution ingested by those animals subjected to the distributed drinking procedure.

The absence of progressive decreases in the consumption of the fluid mixed with alcohol during the forced-drinking conditioning period can perhaps be explained in terms of competing tendencies. Recall that the forced-drinking procedure can be viewed as a conflict situation, wherein the tendency to approach the drinking tube to obtain the daily fluid is opposed by a tendency to avoid the aversive alcohol solution. The lack of a progressive decrease in the consumption of either Kool-Aid+alcohol or sugar water+alcohol in the present experiment could be viewed as the result of increasing aversion opposed by an increasing tendency to drink, motivated by progressively increasing thirst.

EXPERIMENT III

Introduction

The results of Experiments I and II showed that five presentations of a 5% alcohol solution were sufficiently aversive to produce a conditioned taste aversion for the Kool-Aid flavor mixed with alcohol when approximately 1.5 g/kg was self-administered within a 10-min/day drinking period. In contrast, when drinking trials were distributed throughout the day (Experiment II), even significantly larger daily doses of alcohol failed to produce a conditioned aversion. These findings support the conclusions that the orosensory stimuli accompanying the drinking of 5% alcohol solutions are not sufficiently aversive by themselves to produce a conditioned taste aversion and that a condition of intoxication is necessary. Were the orosensory components sufficient, the animals given distributed drinking trials should also have exhibited an aversion to their preferred flavor of Kool-Aid. This follows because they drank more Kool-Aid+5% during each of the 5 conditioning days than did the massed drinking subjects, and therefore, presumably had even stronger unconditioned stimulation than did the massed animals. The finding that the massed drinking of alcohol solutions resulted in higher peak blood-alcohol levels than the distributed drinking of even larger dosages is consistent with the conclusion that the primary determinant of conditioned aversion in Experiments I and II was an aversive central state.

In discussing the results of Experiments I and II, it was suggested that the centrally mediated state produced by alcohol may be aversive only when an animal has not experienced it on a number of previous

occasions. This interpretation was based on Amit and Baum's (1970) suggestion that the experience of being in a novel drug state for a pharmacologically naive rat is aversive. Thus, it is plausible that the alcohol consumed by the experimental group of Experiment I and by the massed experimental group of Experiment II may have resulted in a conditioned taste aversion to the accompanying flavor of Kool-Aid because the novelty of the concomitant central state made it aversive. Although the novelty of the drug state on the second day of conditioning was doubtless less than on the first day, the total novelty effect of 5 days of exposure to the mixture of alcohol and the preferred flavor of Kool-Aid might have been sufficiently aversive to produce a conditioned taste aversion to that flavor on the post-conditioning two-flavor preference tests. In general, however, it would be reasonable to assume that the greater the number of exposures to alcohol the less should be the novelty of the central concomitants of intoxication. Therefore, a relatively large number of prior exposures to alcohol might be expected to reduce or eliminate the conditioned taste-aversion phenomenon demonstrated in the massed drinking experimental groups of Experiments I and II.

In addition, there are other important reasons for being concerned with the effects of numerous alcohol-drinking sessions. Repeated exposures to alcohol are often cited as a factor contributing to human alcoholism (Lester, 1966), and an experimental procedure which results in animals' being given a number of opportunities to drink only alcohol solutions might provide important data on subsequent positive or negative reinforcing properties which come to be associated with alcohol.

Also, the few studies directed towards elucidating the specific effects and mechanisms-of-action of previous exposures to alcohol have appeared to be paradoxical and have not involved the administration of comparable dosages of alcohol. Further, the repeated presentations of an alcohol solution prior to its use as an aversive compound in the taste-aversion procedure is analogous to the presentations of UCSs prior to their inclusion in a classical conditioning paradigm. Although the effects of pre-conditioning UCSs on subsequent tasks have been evaluated adequately for some classical conditioning paradigms, there are few data available on the effects of this procedure on the development of conditioned taste aversions.

Experiment III was thus designed to evaluate the effects of repeated alcohol-drinking opportunities on the subsequent development of conditioned taste aversion. A preferred flavor of Kool-Aid was first determined, after which the animals drank either alcohol or sugar water for 20 days. The animals were then subjected to a conditioning procedure during which the preferred flavor of Kool-Aid was mixed with alcohol. This period was followed by preference tests between the originally presented flavors of Kool-Aid. A sufficient number of groups were included so as to study dose-response phenomena in both pharmacologically naive subjects and in "experienced" subjects.

Experimental data on the effects of repeated presentations of alcohol

Richter and Campbell (1940) determined the amounts of water and of an alcohol solution, whose concentration was increased daily, that were consumed by nonfluid-deprived rats. Their experimental procedure consisted of repeated 24-hr simultaneous presentations of both solutions

with the concentration of alcohol starting at .01% (w/v) and increasing in daily steps of .01%. These investigators found that equal amounts of the alcohol solution and the plain water were consumed when the alcohol concentrations ranged from .01% to 1.4%. When the concentration ranged from 1.8% to 4.8%, the rats preferred the alcohol solutions to water, and a slight preference for alcohol persisted at 6%. More water than alcohol was drunk at 6.5%, and only minimal amounts of alcohol were consumed at concentrations of 7% or above. Thus, a gradual increase in the concentration of alcohol did not result in more water's being consumed than alcohol at concentrations below 6.5%, with a decided preference for alcohol existing from 1.8% to 4.8%. Previously, Richter (1926) had noted that rats drank the same total amount of an 8% alcohol solution in a 24-hr-forced-drinking situation as they did of water. However, Richter (1953) reported that subsequent to being restricted to an 8% alcohol solution for 265 days rats preferred water to alcohol solutions regardless of the alcohol concentration. On the basis of Richter's results, it would seem that prolonged exposure to an 8% alcohol solution in a forced-drinking situation resulted in low concentrations of alcohol becoming aversive, while contrarily, as discussed previously, a gradually increasing concentration of alcohol (over days) resulted in no aversion below 6.5%.

Myers (1961) reported that when food and fluid-deprived rats were allowed to bar press for a 5% (v/v) alcohol solution, water, or a food pellet, they selected water over the alcohol solution. This preference, however, was reversed if the animals were restricted to a continuously available 5% alcohol solution for at least 10 days prior to the

preference tests. Contrary to the results obtained with 5% alcohol solutions, Myers noted that animals which had been previously exposed to continuously available 20% alcohol solutions, uniformly rejected 20% alcohol solutions in favor of water during testing. Therefore, repeated opportunities to drink a 5% alcohol solution resulted in a subsequent preference for 5%; in contrast, the repeated drinking of 20% alcohol solutions did not result in a subsequent preference for 20% alcohol. In addition, Myers observed that when exposures and testing conditions were reversed, i.e., 5% exposure--20% test, and 20% exposure--5% test, animals given repeated opportunities to drink 5% refused the 20% solution, whereas the animals that had repeated opportunities to drink 20% pressed the lever for 5% in preference to water. Thus, Myers found that 180 days of exposure to a continuously available 20% alcohol solution resulted in more bar pressing for a 5% alcohol solution than for water under fluid deprivation conditions even though the animals would not press for 20%.

Myers and Carey (1961) permitted rats to drink either 5% or 20% alcohol solutions for either 30 or 120 days. The animals were then tested in a similar manner to the procedure used by Myers (1961) except for two variations. For half the subjects, the alcohol solutions increased from 5% in 1% steps on successive daily test sessions, and for the other subjects, the alcohol solutions decreased from 15% in 1% steps on successive daily test sessions. The animals in the 5% and 20% groups did not differ significantly in their responses, and the data from the two groups were combined for further analysis. The subjects that were tested with decreasing alcohol

concentrations (15% with 1% decreases per day) did not manifest a preference for alcohol until the 4% level was reached, whereas animals given increasing alcohol concentrations demonstrated a preference for 6% alcohol solutions. Also, the shorter the exposure period (30 versus 120 days), the more quickly the animals changed their preference from alcohol to water, and this change occurred at a lower concentration.

Myers' conclusions are consistent with the notion that rats do not initially prefer alcohol solutions in concentrations greater than 5%. However, the repeated drinking of alcohol solutions can apparently alter this initial aversion as a function of the concentration of the exposure solution, the duration of the exposure period, and the concentration of alcohol used during the exposure period versus the concentration of alcohol used during the testing period.

One obvious difference between Richter's and Myers' results is that the animals maintained by Richter for 265 days on 8% alcohol did not prefer alcohol at any concentration, whereas rats maintained for 180 days on 20% alcohol by Myers preferred 5% to water. Myers and Carey (1961) supported the Myers' (1961) conclusion by the observation that the combined groups of animals given either 5% or 20% alcohol solutions for either 30 or 120 days preferred alcohol at 4% or 6% depending on whether the test solutions decreased from 15% or increased from 5% respectively.

Veale and Myers (1969) have investigated the effects of repeated presentations of a continuously available alcohol solution on subsequent preference tests between water and alcohol. These authors reported that animals restricted to either a 12% or 15% (v/v) alcohol

solution for 10 days drank little alcohol on subsequent preference tests regardless of the concentration. On the other hand, if water was also available to the rats during exposures to increasing concentrations of alcohol (from 3% to 30%), they consumed two to three times more alcohol during the seventh sequence than during the first.

As previously mentioned in Experiment II, Carey (1972) has reported that the amount and temporal distribution of alcohol consumption during a pre-test period affects the amount of alcohol consumed during subsequent 24-hr, water-versus-alcohol preference tests. This conclusion was based on the following observations. Ten daily 30-min drinking sessions of 5% alcohol (v/v) resulted in no change in preference in a 24-hr water-versus-5% alcohol test. Contrarily, 10 opportunities to drink a 10% alcohol solution for 30 min/day resulted in a significant decrement in preference for a 10% alcohol solution in a 24-hr water-versus-10% test. However, animals that were given ten 24-hr drinking periods, rather than 30-min drinking periods, to the 10% alcohol solution did not alter their preference.

One apparent and important conclusion from the above studies is that the aversive consequences which accompany the oral consumption of alcohol are demonstrable at a lesser concentration if the alcohol is consumed in a brief period than if it is continuously available.

Baum (1969, 1970, 1971) has investigated the effects of alcohol on performance in an avoidance paradigm. He reported (1969) the seemingly "paradoxical" finding of increased resistance to extinction of an avoidance response in rats that were given an i.p. injection of

alcohol (1.2 g/kg) prior to extinction testing. Baum stated that this finding was unexpected because the alcohol was suppose to reduce conditioned fear and thereby decrease extinction performance. Subsequently, Amit and Baum (1970) attempted to explain the paradoxical finding by positing that the experience of being in a novel drug state for a pharmacologically naive rat is aversive. Further, this aversive stimulation was assumed to function to increase resistance to extinction in an avoidance paradigm by increasing conditioned fear. Amit, Ziskind, and Baum (1973) have extended this latter formulation to include specifically the administration of alcohol. In their study, one group of animals received i.p. alcohol injections (1.2 g/kg) for 14 days prior to avoidance training, while another group of animals received saline injections. Just prior to extinction testing, all animals were given i.p. injections of alcohol at 1.2 g/kg. The animals which had previously received saline injections showed increased resistance to extinction, whereas those animals which had previously received i.p. injections of alcohol did not demonstrate increased resistance to extinction. These investigators proposed that the novelty of the drug state was aversive to the saline-injected animals and hence acted to increase conditioned fear which resulted in perseveration of the avoidance response. Further, they concluded that repeated administrations of alcohol prior to being subjected to the avoidance paradigm reduced this inherent aversiveness which accompanies a novel drug state, and thus no increase in conditioned fear was postulated to have occurred in the alcohol-injected animals.

Based on the above studies, it appears that alcohol solutions above a certain concentration have aversive consequences for pharmacologically naive rats when consumed orally or injected i.p. However, after a number of previous experiences with alcohol, the aversive consequences of alcohol tend to be reduced. The latter statements must be qualified in that there are some levels of alcohol (8% in Richter, 1936; 20% in Myers, 1961; 12% in Veale and Myers, 1969; 10% in Carey, 1972) which appear to maintain their aversiveness over long periods of time. The mechanism underlying the empirical process of stimulus-neutralization by repeated exposures to alcohol remains unknown.

Permitting rats to drink alcohol solutions prior to subjecting them to the taste aversion procedure of Experiment I is analogous to giving animals pre-conditioning presentations of a UCS prior to the use of that UCS in a classical conditioning paradigm. In general, it has been found that after a number of pre-conditioning administrations of the UCS, the incorporation of the same UCS into a classical conditioning paradigm results in a reduced rate of conditioning. MacDonald (1946) presented a UCS to human subjects 50 times prior to its inclusion in a classical conditioning procedure. She used either electric shocks to the finger or puffs of air directed at the cornea as UCSs. The effect of this pre-conditioning exposure technique was to reduce the probability of obtaining CRs to the CS. Likewise, Taylor (1956) reported that 50 UCS-only trials prior to its inclusion into a classical conditioning procedure with human subjects resulted in a decrease in the number of CRs observed during the conditioning

period. More recently, Siegel and Domjan (1971) and Mis and Moore (1973) have reported that pre-conditioning presentations of the UCS retarded acquisition of CRs induced by the same UCS during a subsequent conditioning period. Both groups of investigators employed rabbits as subjects and classically conditioned a nictitating membrane response. In addition, Siegel and Domjan (1971) obtained consistent results when rats were used in a CER paradigm. Also, Mis and Moore (1973) demonstrated that a CR developed faster when there was a 24-hr delay between pre-conditioning administrations of the UCS and conditioning, than when there was no delay.

There are a number of investigators who have reported that repeated presentations of the UCS by itself resulted in a decrease in the response strength of the UCR. For example, Kellogg (1941) observed in dogs a decrease in the magnitude of the UCR with repeated exposures to electric shock. Similarly, Seward and Seward (1934) found that during repeated electric shocks to human subjects, the PGR, inspiration rate, and general body movements tended to return to pre-shock levels.

Elkins (1974) has reported a reduced conditioned taste aversion when animals were given pre-conditioning exposures to the UCS. In addition, he noted that the extent of conditioned aversion was an inverse function of the number of pre-conditioning UCSs.

Experiment III was designed to evaluate the effects of extensive opportunities to drink alcohol solutions on the subsequent production of conditioned taste aversion by the oral ingestion of alcohol. Three different concentrations of alcohol were used in an attempt to discern dose-related levels of conditioned aversion in both pharmacologically

naive and experienced subjects. Animals that drank only alcohol solutions for 20 days prior to conditioning were designated as experienced, whereas the animals that drank sugar water during the same period were designated as pharmacologically naive. During the conditioning period, experienced animals were given Kool-Aid+ alcohol at the same concentrations they had received during the preceding 20-day period. Pharmacologically naive animals were given the same Kool-Aid+alcohol concentrations as the experienced animals.

The concentrations of alcohol were chosen so as to include a presumably preferred concentration of alcohol, as well as two purported aversive concentrations. More precisely, some animals were allowed to consume 3% alcohol, which was selected to maximize the likelihood that its consumption would be positively reinforcing. The assumption that 3% would be positively reinforcing was based on Richter and Campbell's (1940) study wherein rats preferred solutions ranging from 1.8% - 4.8% to water. The remaining subjects drank either 5% or 7% alcohol. These concentrations were assumed to be aversive and hence capable of serving as negative reinforcers. The assumption that 5% alcohol would be aversive was based on the finding in Experiments I and II that the rapid consumption of 5% alcohol resulted in conditioned taste aversion.

Subjects and Procedure

The subjects were 80 naive, Sprague-Dawley derived, female albino rats from Carworth Farms, Inc., Portage, Michigan. The animals were randomly assigned to eight groups of 10 members each, and were 110 days of age at the beginning of the experiment. Throughout the study, and for 7 days prior to the start of the experiment, the animals

were housed individually in a normal 12-hr, day-night cycle room. An ad lib food and water regimen was utilized during this 7-day period, after which the animals were placed on a fluid deprivation schedule that permitted 10 min of drinking per day at the same time each day during the light cycle. For the first 3 days of fluid deprivation, the 10-min drinking sessions (water from a single drinking tube) were followed by 2-min handling and taming sessions for each animal.

Each day thereafter the animals were weighed and permitted to drink for 10 min from either of two drinking tubes that projected through the mesh fronts of the cages on the right-front and middle-front areas. Food was available ad lib throughout the experiment from containers mounted against the left-front of the cages.

On Days 1 through 5 of the experimental schedule, water was presented for 10 min in one of the two drinking tubes, its position being alternated daily. On Day 6, the animals were given grape flavored Kool-Aid in one tube and orange in the other. The formula was 0.25 teaspoon Kool-Aid, 1.5 teaspoons sugar, and 1.5 cups of water at room temperature. The positions of the tubes were switched half-way through the drinking session. On Day 7, the animals were given water to drink, and on Days 8 and 9 the two Kool-Aid flavors were presented simultaneously to determine preferences. The positions of the flavors were switched on the second day. Daily fluid consumptions were recorded in ml. The flavor with the highest amount consumed over the 2-day preference period was designated the "preferred flavor." This flavor was used during the subsequent 10 conditioning days.

On Days 10 through 29, the pre-conditioning period, the animals were allowed access to only their prescribed solutions. The four solutions were 3% alcohol, 5% alcohol, 7% alcohol, and sugar water (see left side of Table 4). During this pre-conditioning period, animals in four of the groups drank sugar water, animals in two of the groups drank 7% alcohol, and the remaining animals consumed either 3% or 5% alcohol. All alcohol solutions were made by adding sugar water to 95% ethanol until the desired concentrations were attained. The formula used for the sugar water solution was identical to the Kool-Aid formula with the exception that no Kool-Aid was added. Throughout the 20-day pre-conditioning period, the position of the fluid-containing tube was alternated daily.

Conditioning sessions were given on Days 30 through 39. The treatments of the counterbalanced groups are summarized in the right side of Table 4. Hereafter, all groups are designated in the following manner: two numbers are separated by a dash with the first number denoting the concentration of alcohol (ranging from 0% to 7%) that the animals drank during the 20-day pre-conditioning period, and the second number specifying the concentration of alcohol they drank throughout the subsequent conditioning period. The four groups of animals that drank sugar water (0% alcohol) during the 20-day period are collectively designated as pharmacologically naive at the start of the conditioning period, whereas animals that drank alcohol solutions are designated as experienced.

During the conditioning period, animals in all groups had the same number of opportunities to drink the preferred flavor of Kool-Aid

Table 4. Treatments administered during the pre-conditioning and conditioning sessions. An asterisk (*) indicates the presence of ethanol, K indicates the preferred flavor of Kool-Aid, and S indicates sugar water. N = 5 in each subgroup.

Pre-Conditioning days 10 - 29	Concentration of alcohol		Conditioning days 30	31	32	33	34	35	36	37	38	39
Group - 3%-3% (S*)												
Subgroup A	3%	3%	K*	S	S	K*	S	K*	K*	S	K*	S
Subgroup B	3%	3%	S	K*	K*	S	K*	S	S	K*	S	K*
Group - 5%-5% (S*)												
Subgroup A	5%	5%	K*	S	S	K*	S	K*	K*	S	K*	S
Subgroup B	5%	5%	S	K*	K*	S	K*	S	S	K*	S	K*
Group - 7%-7% (S*)												
Subgroup A	7%	7%	K*	S	S	K*	S	K*	K*	S	K*	S
Subgroup B	7%	7%	S	K*	K*	S	K*	S	S	K*	S	K*
Group - 7%-7%/C (S*)												
Subgroup A	7%	7%	K	S*	S*	K	S*	K	K	S*	K	S*
Subgroup B	7%	7%	S*	K	K	S*	K	S*	S*	K	S*	K
Group - 0%-3% (S)												
Subgroup A	0%	3%	K*	S	S	K*	S	K*	K*	S	K*	S
Subgroup B	0%	3%	S	K*	K*	S	K*	S	S	K*	S	K*
Group - 0%-5% (S)												
Subgroup A	0%	5%	K*	S	S	K*	S	K*	K*	S	K*	S
Subgroup B	0%	5%	S	K*	K*	S	K*	S	S	K*	S	K*
Group - 0%-7% (S)												
Subgroup A	0%	7%	K*	S	S	K*	S	K*	K*	S	K*	S
Subgroup B	0%	7%	S	K*	K*	S	K*	S	S	K*	S	K*
Group - 0%-7%/C (S)												
Subgroup A	0%	7%	K	S*	S*	K	S*	K	K	S*	K	S*
Subgroup B	0%	7%	S*	K	K	S*	K	S*	S*	K	S*	K

and the sugar water solution. For the subjects in the control groups (7%-7%/C and 0%-7%/C), alcohol was mixed with sugar water only, never with Kool-Aid; for rats in the experimental groups, alcohol was mixed with Kool-Aid and never with sugar water.

Prior to conditioning, animals in Groups 3%-3%, 5%-5%, and 7%-7% were given 20 days of drinking sugar water+alcohol at the same concentration as that which was mixed with the preferred flavor of Kool-Aid during the subsequent conditioning period. Group 7%-7%/C was the control group for the above subjects, and animals in this group were given 7% alcohol mixed with sugar water. Seven per cent was chosen for the control animals so as to insure that they would be exposed to the maximum intensity of the UCS presented to any subject drinking Kool-Aid+alcohol. Thus, any possible non-associative effects should be equally likely in both experimental and control subjects.

As Table 4 indicates, animals in Groups 0%-3%, 0%-5%, and 0%-7% had 20 days of drinking sugar water solutions prior to conditioning. During the conditioning period, these animals were given their preferred flavor of Kool-Aid mixed with alcohol (3%, 5%, or 7%). Group 0%-7%/C was the control group for these animals, and subjects in this group were given 7% alcohol mixed with sugar water. As in the experienced control group, 7% was chosen because it was the maximum intensity of UCS presented to any subject in the above three groups.

Thus, it was possible to detect any effects of 20 days of drinking alcohol solutions on the development of conditioned aversions by comparing groups 3%-3%, 5%-5%, and 7%-7% with groups 0%-3%, 0%-5%, and 0%-7%.

The 10 conditioning days were followed by 10 days of preference tests during which the two Kool-Aid flavors were simultaneously presented; the positions of the two Kool-Aid flavors were alternated daily.

An additional 30, naive, female albino rats purchased from Carworth Farms, Inc. were used to determine whether different peak blood-alcohol levels resulted from drinking different concentrations of Kool-Aid+alcohol during the conditioning period, and whether 20 previous days of drinking only alcohol solutions had any effect on the peak blood-alcohol levels observed in pharmacologically naive rats. The subjects were of the same age and weight as the animals used in Experiment III. Six groups of five animals each were subjected to the same paradigm, up to and including the first day of conditioning as the following groups: 0%-3%, 0%-5%, 0%-7%, 3%-3%, 5%-5%, and 7%-7% (see Table 4).

On the first day of conditioning, animals were permitted to drink a Kool-Aid+alcohol solution for 10-min. Upon completion of the 10-min period, the blood-alcohol levels of these animals were determined from samples taken at 5, 25, 65, 95, 125, 185 min. The enzymatic assay for determining the blood-alcohol level as well as the surgical technique used for obtaining the venous blood samples are described in Appendix C.

Results

From daily intakes, preference ratios for each 2-day period were determined for each animal and constituted the data that were analyzed for the pre-conditioning and post-conditioning preference tests(Appendix I). The amounts of sugar water or sugar water+alcohol consumed each day constituted the data for the 20-day pre-conditioning period(Appendix G), and the daily intakes of Kool-Aid, Kool-Aid+alcohol, sugar water, sugar

water+alcohol(Appendix H) constituted the data analyzed for the conditioning period.

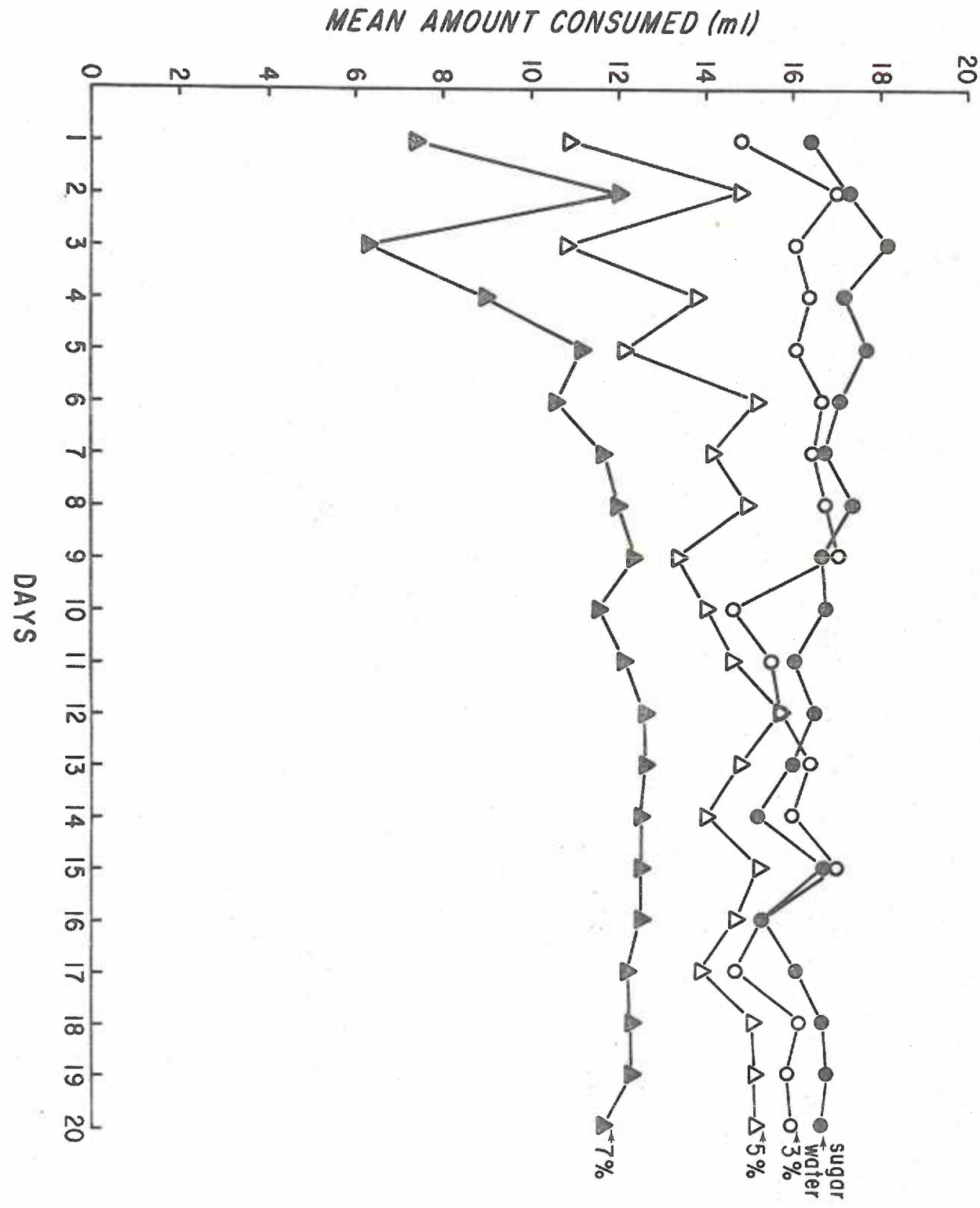
There were no significant differences among groups with respect to the pre-conditioning preference ratios, hence subsequent differences in preferences among the groups cannot be ascribed to differences existing prior to the 20-day pre-conditioning period.

Twenty-day pre-conditioning period

The daily mean amounts of fluids consumed during this period are represented graphically in Fig. 5. For purposes of this figure, the four groups that drank only sugar water (0%-3%, 0%-5%, 0%-7%, 0%-7%/C) were combined into one group, as were the two groups that drank sugar water+7% (7%-7% and 7%-7%/C). The justification for combining these groups was that there were no differences in consumption among the four groups that drank sugar water over the total period, nor were there any differences in consumption between the two groups that drank sugar water+7%.

The drinking behavior of the eight groups during the 20-day period was evaluated by means of a two-factor analysis of variance, with repeated measures over days. A main effect of days ($F=7.53$, $df=19/1368$, $p < .01$), and a main effect of alcohol concentration, 0%, 3%, 5%, and 7% were demonstrated ($F=40.48$, $df=7/72$, $p < .01$). Newman-Keuls follow-up tests showed that when daily intakes were collapsed across days, each of the 7% groups drank less than the 5%-5% group (both $ps < .05$) and less than the 3%-3% group and the sugar water group (all $ps < .01$). There were no demonstrable differences among the 0% (sugar water), 3%, and 5% groups. However, analysis of intakes

Figure 5. Daily mean amounts of fluids consumed during the 20-day period prior to conditioning. The composite sugar-water group was composed of 40 subjects in Groups 0%-3%, 0%-5%, 0%-7%, 0%-7%/C. The composite sugar water+7% alcohol group contained 20 subjects from groups 7%-7% and 7%-7%/C, and each of the two remaining groups, 3%-3% and 5%-5%, was composed of 10 animals.


MEAN AMOUNT CONSUMED (ml)
0
2
4
6
8
10
12
14
16
18
20
DAYS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
sugar water
3%
5%
7%

on Day 1 alone indicated the following order of increasing consumptions, 7% < 5% < 3% (all ps < .01). There were no differences in intakes among the 3%-3% group and the four sugar water groups. On Day 20, the 5%-5% group was no longer drinking significantly less than the 3% and sugar water groups, whereas each of the 7% groups drank less than the 5%-5% animals (both ps < .03) and less than the 3%-3% and sugar water groups (all ps < .01). The significant days by alcohol concentration interaction ($F = 3.79$, $df = 133/1368$, $p < .01$) can be explained in part by the significant increase in consumption from Day 1 to Day 20 by the 7%-7% ($F = 33.35$, $df = 1/1368$, $p < .01$), 7%-7%/C ($F = 20.06$, $df = 1/1368$, $p < .01$), and 5%-5% ($F = 26.89$, $df = 1/1368$, $p < .01$) groups, while no changes occurred in the intakes of either the 3%-3% group or the four sugar water groups.

As is clear from Fig. 5, none of the above changes in intakes over the 20-day period occurred as progressive decreases; rather, the only changes were increases. A progressive decrease in a solution which contained alcohol would be consistent with a learned taste-aversion interpretation, because repeated pairings would enhance the conditioned aversiveness of the alcohol solution and decrease the amount of that fluid consumed during subsequent sessions. The failure to observe a progressive decrease in any solution precluded, on the basis of this measure, any conclusion that a conditioned aversion had developed during the 20-day period.

It appears that throughout the pre-conditioning period the ingestion of sugar water+alcohol varied inversely with concentration. The animals drank less 7% than 5%, and at least initially, consumed less 5% than 3%.

However, animals drank as much 3% as they did of sugar water. Thus, the magnitude of aversive consequences accompanying the rapid ingestion of alcohol solutions can be described as 7% > 5% > 3%, there being no direct evidence that 3% was aversive. The observation that animals that drank 5% and 7% solutions increased their intakes over the 20-day period is consistent with the hypothesis that over days the stimuli produced by these initially aversive concentrations of alcohol attained some tendency to elicit approach and drinking responses because they were the only solutions available.

The amounts of alcohol consumed during the 20-day period were converted into g/kg dosage levels which were subjected to separate analyses. The main effect of alcohol concentration was highly significant ($F = 123.12$, $df = 3/36$, $p < .01$), with follow-up tests indicating that the daily dosage of 1.62 g/kg ingested by the 3%-3% animals was less than the 2.44 g/kg dosage for the 5%-5% animals ($p < .05$). Each of the 7%-7% groups consumed more alcohol (both ps $< .01$) than the 5% animals (7%-7%/C - 2.77 g/kg; 7%-7% - 2.89 g/kg). There were no differences between the two 7%-7% groups. Follow-up tests also showed that although there were no differences in doses among any of the groups on Day 1, on Day 20, the order of significant differences was 3% < 5% < 7% (all ps $< .01$). The significant interaction between days and alcohol concentration ($F = 2.80$, $df = 57/684$, $p < .01$) can be explained by the observation that the 5%-5% group and both of the 7%-7% groups increased their dosage between Day 1 and Day 20 (all ps $< .01$), whereas the 3%-3% animals demonstrated no difference. Thus it is apparent that the alcohol dosage was a direct function of concentration.

The observation that the groups differed with respect to dosages cannot be ascribed simply to weight differences among the groups. Although the weights of all groups increased significantly during the 20-day period ($F = 263.65$, $df = 1/72$, $p < .01$), there were no reliable between-group weight differences either at the start or at the end of the period (see Appendix J).

Conditioning period

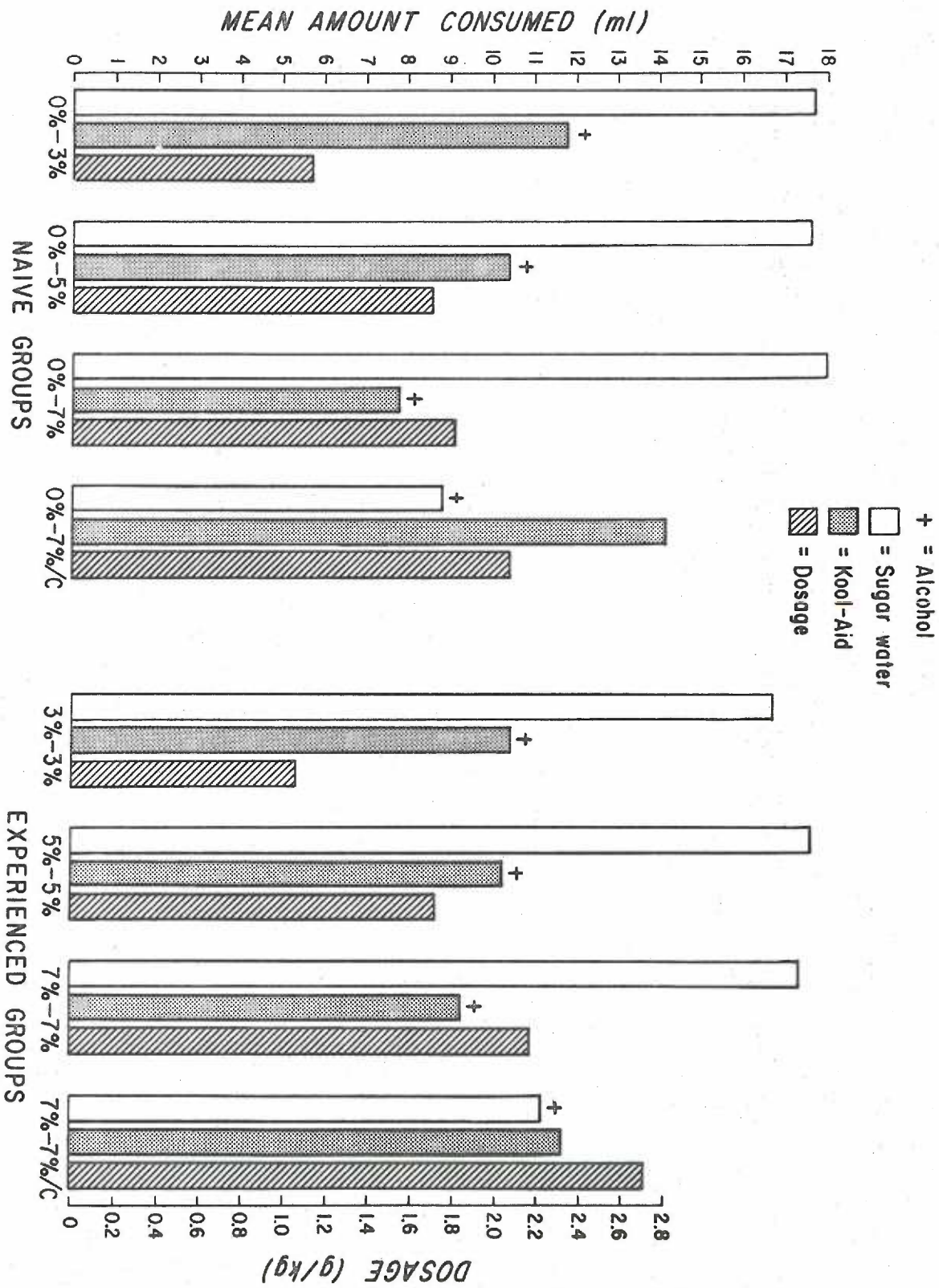
The effects of 20 previous opportunities to drink alcohol solutions was assessed, in part, by comparing the daily alcohol intakes of pharmacologically naive and experienced subjects at the same concentrations during the conditioning period.

It was noted previously that the presentation of alcohol and sugar water solutions during the 20 days prior to conditioning can be viewed, in principle, as a conditioning procedure wherein the sugar water solution and the smell and taste of alcohol qualify as the CSs and the aversive central state accompanying the rapid drinking of alcohol is the UCS. Thus, the sugar water solution as well as the smell and taste of alcohol should have become secondary aversive stimuli for the experienced animals. However, it is also possible that some positive reinforcing properties should have become associated with alcohol solutions. The positive component would have resulted from the daily reduction of thirst by the drinking of alcohol solutions. In that for 20 days alcohol solutions were the only fluid available, alcohol should have acquired some positive reinforcing properties. Therefore, it is possible to view the consumption of alcohol solutions in a forced-drinking situation (20-day pre-conditioning and conditioning

periods) as a result of competing positive and negative reinforcing events. Specifically, to the extent that the taste and smell of alcohol have become more negatively than positively reinforcing as a result of the 20-day period, a reduced intake of alcohol solutions in such subjects would be predicted relative to pharmacologically naive subjects drinking the same concentration of alcohol. To the extent that the taste and smell of alcohol have become more positively than negatively reinforcing, an increased alcohol intake would be expected. Data bearing on this are shown in Fig. 6.

The observation that the 0%-3% group drank more of the Kool-Aid+3% solution than the 3%-3% group ($F = 6.93$, $df = 1/18$, $p < .05$) indicated that 20 days of drinking sugar water+3% resulted in some learned aversion to 3% solutions relative to pharmacologically naive animals that drank 3% solutions. There were no differences in intakes of Kool-Aid+5% consumed by the 0%-5% and the 5%-5% groups. This finding was interpreted as indicating that the positive and negative consequences accompanying the drinking of 5% alcohol did not change differentially as a result of 20 days of drinking sugar water+5%. On the other hand, 20 days of drinking sugar water+7% resulted in a relative increase of positive effects associated with its ingestion as verified by the observation that the 7%-7% group drank more Kool-Aid+7% than the 0%-7% group ($F = 13.34$, $df = 1/18$, $p < .01$). The finding that previous opportunities to drink 7% alcohol solutions increased the subsequent consumption of such solutions, in forced-drinking situations, relative to naive subjects was supported further by comparisons between the two control groups. Specifically, the

Figure 6. Mean volumes of fluids consumed per drinking opportunity during the 10-day conditioning period. Also depicted are the mean dosages of alcohol which resulted from the drinking of alcohol solutions. A particular bar represents data from both subgroups even though they drank their designated fluids on different days.


MEAN AMOUNT CONSUMED (ml)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
0%-3%
0%-5%
0%-7%
0%-7%/C
NAIVE GROUPS
3%-3%
5%-5%
7%-7%
7%-7%/C
EXPERIENCED GROUPS
+ = Alcohol
= Sugar water
= Kool-Aid
= Dosage
DOSAGE (g/kg)
0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 2.4 2.6 2.8

7%-7% control group drank more sugar water+7% than did the 0%-7% control group ($F = 13.81$, $df = 1/18$, $p < .01$).

Relative to pharmacologically naive animals, subjects given previous opportunities to drink alcohol prior to the conditioning period decreased the amounts of 3% alcohol consumed, had no measurable effects at 5%, and increased consumption of alcohol at 7%.

As in the 20-day period, evidence for the development of a conditioned aversion expected to occur during the 10-day conditioning period would be a progressive decrease over days in consumption of solutions which contained alcohol. A progressive decrease in a solution which contained alcohol would be consistent with a learned taste-aversion interpretation in that repeated pairings should enhance the conditioned aversiveness of the alcohol solution and decrease the amount of that fluid consumed during subsequent sessions. However, in no instance was a progressive decrease or increase in consumption of a particular fluid observed to occur over the conditioning period.

Comparisons among groups that drank Kool-Aid+alcohol indicated that consumption varied as an inverse function of concentration (experienced - $F = 4.54$, $df = 2/27$, $p < .05$; naive - $F = 35.83$, $df = 2/27$, $p < .01$) (see Fig. 6). This phenomenon was most pronounced in the naive animals where the 0%-7% group drank less Kool-Aid+alcohol than did either of the other two naive experimental groups (both $ps < .01$), while the 0%-5% group drank less than the 0%-3% group ($p < .05$). The experienced animals also demonstrated decreased consumptions of Kool-Aid+alcohol with increasing concentrations. Follow-up tests substantiated that the 7%-7% group drank less Kool-Aid+alcohol than either the 3%-3%

or the 5%-5% groups (both ps $<$ .05). Even though the 3% animals drank more than the 5% animals, they were not statistically separable. There were no differences in the intakes of sugar water among either the naive or the experienced experimental animals.

The main effect of alcohol concentration was significant in the naive subjects ($F = 28.60$, $df = 3/36$, $p < .01$), with follow-up tests indicating that the daily dosage of 1.16 g/kg administered by the 0%-3% animals was lower than the dosages of the other three groups (all ps $<$ .01). There were no differences between the 1.71 g/kg of the 0%-5% group and the 1.81 g/kg of the 0%-7% group. However, both of these dosages were lower than the 2.08 g/kg of the control group (both ps $<$ .01). The finding that the control group self-administered a larger dosage than any of the experimental groups means that it was a good control for non-associative effects.

The main effect of alcohol concentration was also significant in the experienced subjects ($F = 117.00$, $df = 3/36$, $p < .01$), with follow-up tests indicating that the daily dosage of 1.04 g/kg self-administered by the 3%-3% animals was lower than the dosages of the other three groups (all ps $<$ .01). Although the dosage of 1.71 g/kg resulting in the 5%-5% group was larger than the 3%-3% dosage, it was less than the two 7%-7% groups (both ps $<$ .01). The 2.17 g/kg administered by the experimental 7%-7% group was less than the control 7%-7% group's daily dosage of 2.70 g/kg ($p < .01$). The finding that the experienced control group self-administered a larger dosage than any of the experimental groups means that the control for non-associative effects was probably adequate.

The 0%-3% dosage of 1.16 g/kg/drinking day was larger than the 3%-3% dosage of 1.04 g/kg ($F=6.05$, $df=1/18$, $p < .05$). There were no differences in dosages between the 5% groups, with both groups administering a daily mean dosage of 1.71 g/kg. The 2.17 g/kg of the 7%-7% group was larger than the 1.81 g/kg dosage of the 0%-7% group ($F=13.87$, $df=1/18$, $p < .01$).

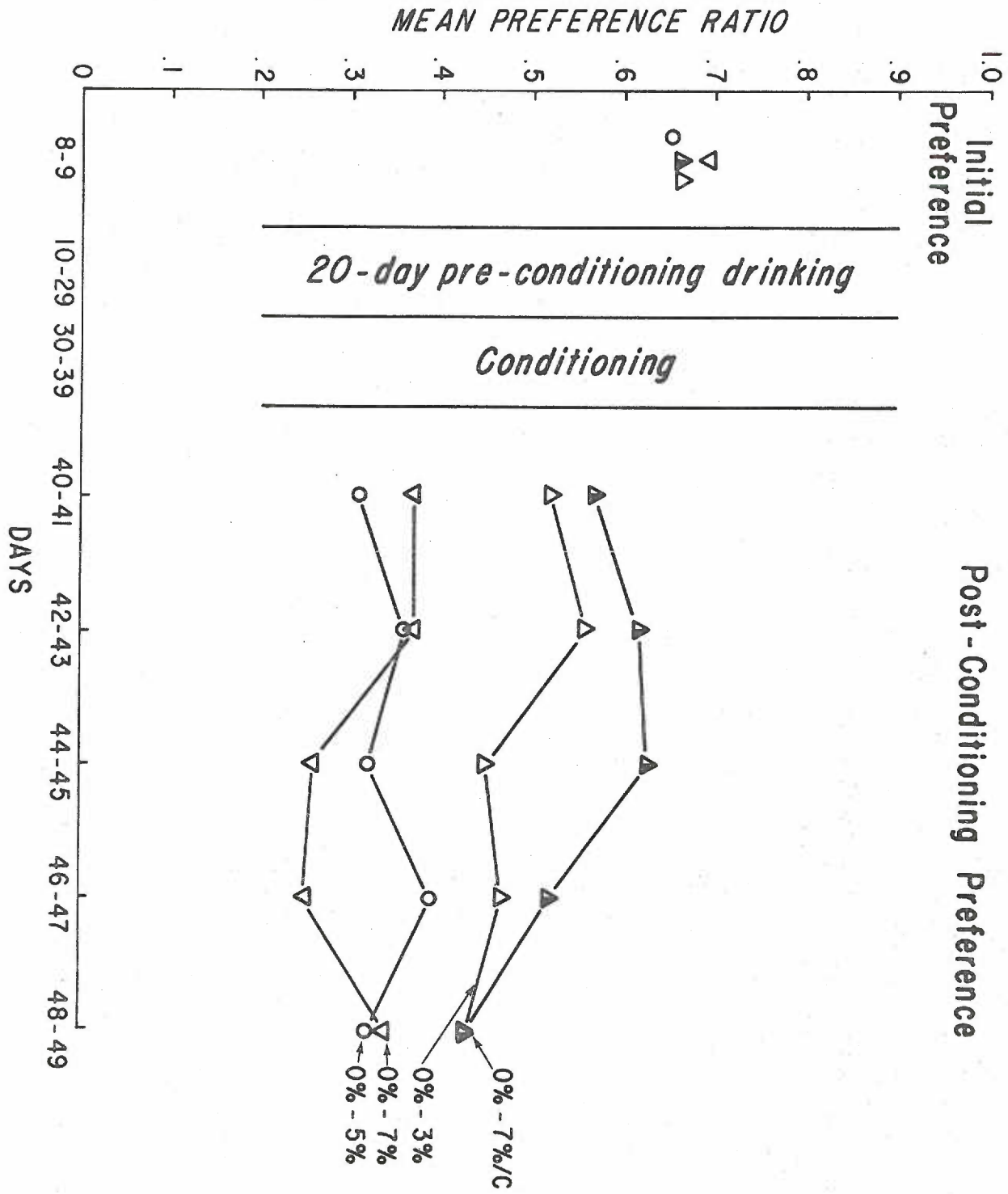
The significant differences in self-administered dosages for different groups cannot be ascribed to weight differences since there were no significant weight differences among the groups throughout the conditioning period (see Appendix K).

Post-conditioning period

The principal measure of conditioned aversion in this experiment involved between-group comparisons of post-conditioning preference ratios, as well as within-group analyses of pre- and post-conditioning ratios. A significant decrease in preference for the preferred flavor of Kool-Aid on post-conditioning tests would suggest the conclusion that the presence of alcohol in the Kool-Aid mixture resulted in that flavor's becoming secondarily aversive.

The results of the pre-conditioning and post-conditioning preference tests for the pharmacologically naive subjects are presented in Fig. 7. Within-group comparisons were conducted between the pre-conditioning preference ratios and the ratios derived from the first 2-day block of post-conditioning preference tests. The 0%-5% group decreased its preference for that flavor of Kool-Aid which had been paired with alcohol during the conditioning period ($F=23.91$, $df=1/9$, $p < .01$) as did the 0%-7% group ($F=27.21$, $df=1/9$, $p < .01$). The preferences of the 0%-3% and 0%-7%/C groups did not change as a result of the procedures they were

Figure 7. Mean preference ratios (proportion of preferred flavor of Kool-Aid to total fluid intake) for each 2-day block during the pre-conditioning and post-conditioning sessions for the pharmacologically naive animals.


MEAN PREFERENCE RATIO
0
.1
.2
.3
.4
.5
.6
.7
.8
.9
1.0
Initial Preference
20-day pre-conditioning drinking
Conditioning
Post-Conditioning Preference
8-9
10-29
30-39
40-41
42-43
44-45
46-47
48-49
DAYS
0%-5%
0%-7%
0%-3%
0%-7%/C

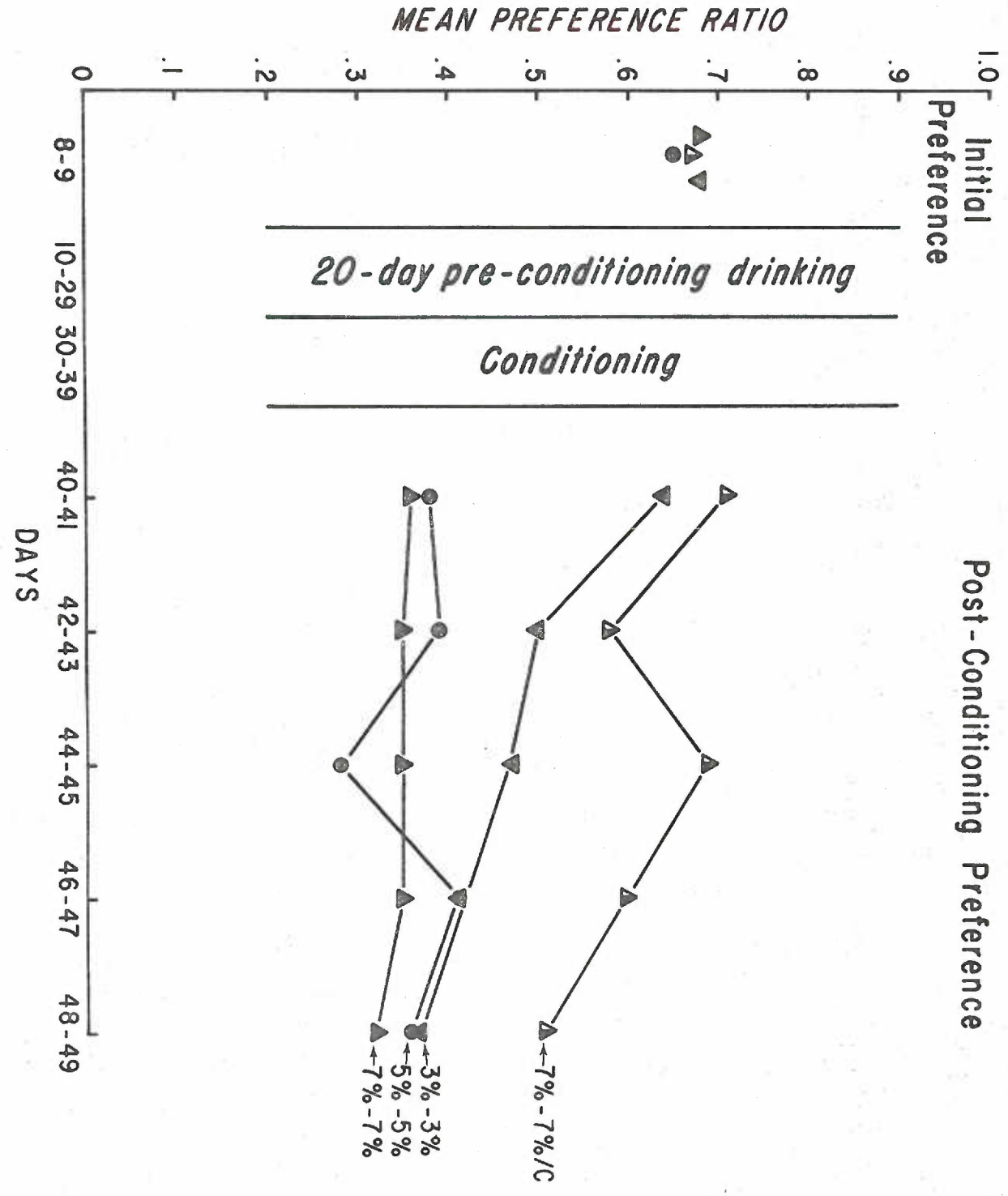
subjected to during the pre-conditioning and conditioning periods.

A two-factor analysis of variance, with repeated measures over the 2-day blocks of tests indicated differences among the naive groups ($F=7.19$, $df=3/36$, $p < .01$). Follow-up tests revealed that although the 0%-5% and 0%-7% groups did not differ from each other, they were both lower in preference for the originally preferred flavor than the control 0%-7% group (both ps $< .01$). Even though the preference ratios of the 0%-5% and 0%-3% groups were not demonstrably different, the ratios of the 0%-7% group were less than those of the 0%-3% group ($p < .05$). In addition, the 0%-3% group remained statistically inseparable from the 0%-7%/C group. A main effect of 2-day blocks was also shown ($F=2.56$, $df=4/144$, $p < .05$). Follow-up tests substantiated that a decrease in preference over blocks occurred in only the 0%-7%/C group ($p < .05$).

Thus, it is clear that a conditioned aversion occurred in the naive rats subjected to conditioning with 5% and 7%, but not in those that drank 3% solutions. The decrease in preference of the control group over the post-conditioning tests can scarely be due to non-associative effects since such consequences would be most likely to appear on the first 2-day block of post-conditioning tests.

The results of the pre- and post-conditioning preference tests for the experienced animals are shown in Fig. 8. Within-group comparisons of pre-conditioning preference ratios with the first 2-day block of post-conditioning ratios indicated that the preference of neither the 3%-3% group nor of the 7%-7%/C group were altered as a result of the procedures followed during the 20-day pre-conditioning and conditioning periods. However, the 5%-5% group decreased its preference for the preferred

Figure 8. Mean preference ratios (proportion of preferred flavor of Kool-Aid to total fluid intake) for each 2-day block during the pre-conditioning and post-conditioning sessions for the experienced animals.


MEAN PREFERENCE RATIO
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
Initial Preference
20-day pre-conditioning drinking
Conditioning
Post-Conditioning Preference
8-9
10-29
30-39
40-41
42-43
44-45
46-47
48-49
DAYS
7%-7%/C
3%-3%
5%-5%
7%-7%

flavor of Kool-Aid ($F=20.77$, $df=1/9$, $p < .01$) as did the 7%-7% group ($F=28.60$, $df=1/9$, $p < .01$).

A two-factor analysis of variance, with repeated measures over the 2-day blocks of the post-conditioning preference-testing period revealed differences among the groups ($F=11.63$, $df=3/36$, $p < .01$). Follow-up tests demonstrated that although the 5%-5% and 7%-7% groups did not differ from each other, they were both lower in preference for the originally preferred flavor than the control 7%-7% group (both $ps < .01$). In addition, the preference ratios of the 7%-7% and 5%-5% groups were both lower than those of the 3%-3% group (both $ps < .05$). The 3%-3% group was significantly lower in preference than the 7%-7%/C group ($p < .05$); this finding was apparent by the third 2-day block of post-conditioning tests. This last result was not observed in the naive 3% group. A main effect of 2-day blocks was also shown ($F=3.97$, $df=4/144$, $p < .01$). Follow-up tests indicated that the 7%-7%/C group decreased its preference for the preferred flavor to a significant level by the last 2-day block ($p < .05$). In contrast to the results obtained in the pharmacologically naive animals, the 3%-3% group also decreased its preference for the preferred flavor of Kool-Aid during the post-conditioning preference tests ($p < .05$). Neither the 5%-5% nor the 7%-7% group altered its preference over this period.

It is clear from these results that the experienced subjects that drank 5% and 7% developed taste aversions. Similar to the naive control group, the preference ratio of the experienced control group was lower on the last block than on the first block of post-conditioning testing. This finding is also inconsistent with the supposition that non-associative

effects had occurred, since such consequences would be most likely to appear on the first 2-day block. Another unexpected finding was the decrease in preference over blocks exhibited by the 3%-3% group. Again, it would seem that taste aversion produced by the conditioning sessions would be most likely to appear on the first 2-day block.

Post-conditioning performances of pharmacologically naive subjects were compared with those of experienced subjects. In no instance was a significant difference demonstrable.

There were no differences in mean weights among any of the eight groups throughout the post-conditioning preference-testing period (see Appendix L).

Blood alcohol

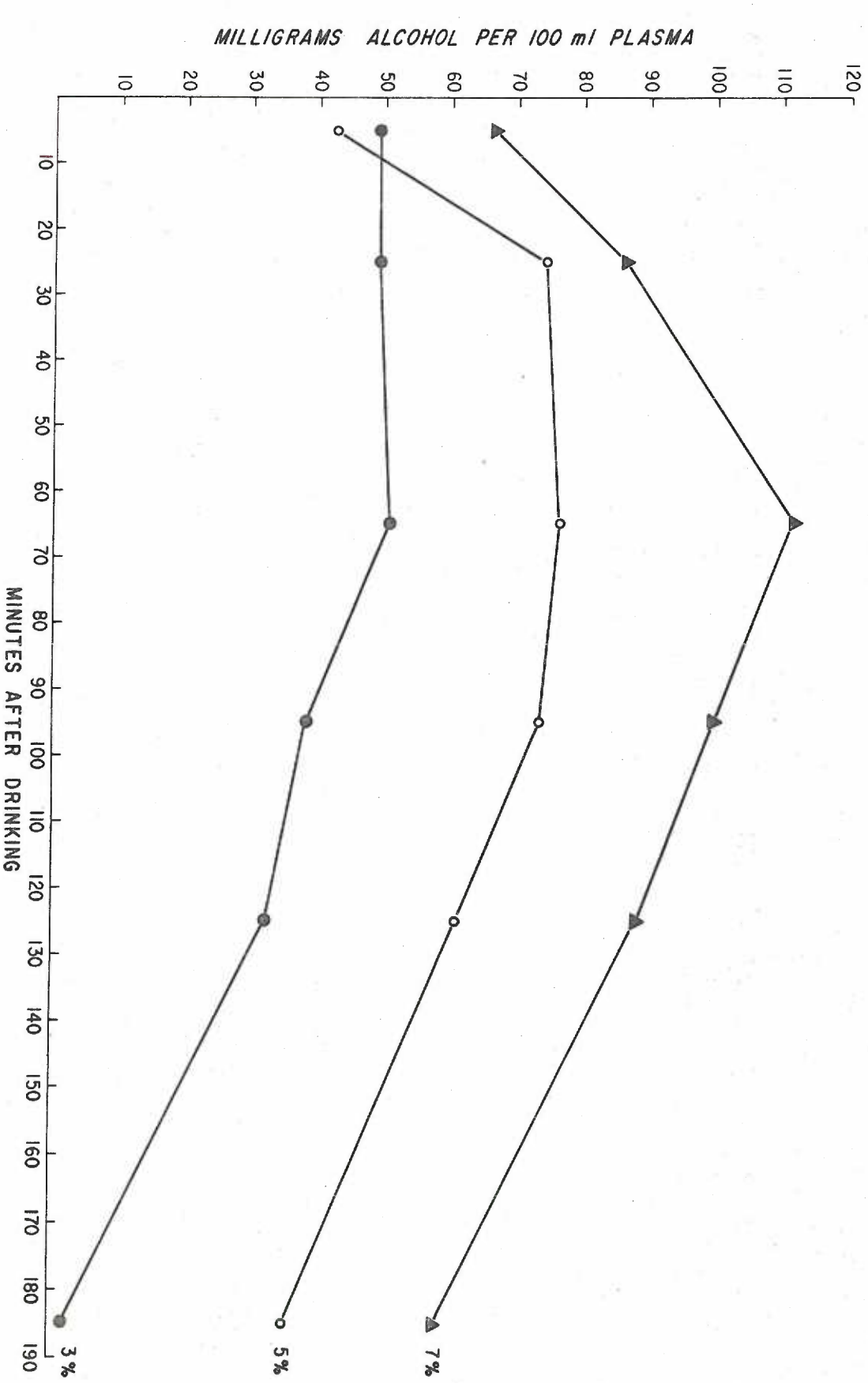
Earlier, it was suggested that the aversive central concomitants of alcohol consumption might be approximated by monitoring blood-alcohol levels. The observation that pharmacologically naive and experienced animals conditioned with the same concentrations of alcohol demonstrated no differences in post-conditioning drinking behavior would lead one to predict that the peak blood-alcohol levels of these groups during the conditioning period would be the same. This expectation was supported by the analysis of the data obtained from the blood-alcohol subjects. The 0%-3% group self-administered a mean dosage of 1.06 g/kg as compared to 1.12 g/kg for the 3%-3% group. This difference was not significant, and the peak blood-alcohol levels for the two groups were also not different. Even though the 0%-5% group administered 1.43 g/kg which was less than the 1.66 g/kg of the 5%-5% group ($p < .05$), there were no differences in the resulting peak blood-alcohol levels for the 5% groups. Finally, the 0%-7% group administered a mean dosage of 1.92 g/kg which was less than

the 2.28 g/kg for the 7%-7% group ($p < .01$). However, there were no differences in peak blood-alcohol levels for the groups. The finding that pharmacologically naive and experienced subjects did not differ in peak blood-alcohol levels after drinking the same concentrations of alcohol is consistent with the observation that their post-conditioning drinking behaviors did not differ. The data derived from pharmacologically naive and experienced subjects were combined for all further analyses and are shown in Fig. 9. All of the data are listed in Appendix M.

If, as implied above, the effective determinant of the conditioned taste aversions demonstrated in this experiment was a centrally mediated aversive state, then the rats that drank 5% and 7% alcohol solutions during the conditioning period should have had higher peak blood-alcohol levels than those that drank 3% solutions. The dosages for the blood-alcohol animals approximated those of the experimental animals. In addition, the direct relation between dosage and concentration noted in the experimental animals was also observed in the blood-alcohol subjects. More specifically, the 3% animals administered less g/kg than the 5% animals, which, in turn administered less than the 7% animals (all $ps < .01$).

A comparison of the peak blood-alcohol levels among the groups revealed differences ($F=8.52$, $df=2/27$, $p < .01$). The peak blood-alcohol levels of the 7% animals were greater than those of the 5% rats ($p < .05$), whereas the levels attained when 3% was consumed were less than at 5% ($p < .05$). Thus, the higher the concentration of alcohol the larger the dose, and the higher the peak blood-alcohol level. However, even though the presumed aversiveness of the central effects, and hence the intensity of the putative UCS in the taste-aversion paradigm, increased from 3% to

Figure 9. Median blood-alcohol curves for fluid-deprived animals given 10-min opportunities to drink Kool-Aid+alcohol solutions (3%, 5%, or 7%). Each of the three groups was composed of 10 subjects, 5 of which had 20 previous opportunities to drink alcohol solutions at the same concentration as the Kool-Aid+alcohol solution and 5 of which were pharmacologically naive.


MILLIGRAMS ALCOHOL PER 100 ml PLASMA
10
20
30
40
50
60
70
80
90
100
110
120
MINUTES AFTER DRINKING
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
3%
5%
7%

7%, the extent of conditioning did not. This statement is supported by the observation that 5% animals self-administered less g/kg than 7% animals during the conditioning period, and hence a reduced peak blood-alcohol level, and yet there were no differences in performance between 5% and 7% animals during post-conditioning testing. The observation that animals drinking 3% did not demonstrate a clear conditioned aversion is consonant with the conclusion that doses of alcohol of 1.04 - 1.16 g/kg are not sufficiently aversive to produce a conditioned aversion with five "pairings."

Discussion

The results obtained during the post-conditioning two-flavor preference tests are consistent with the conclusion that the presence of either 5% or 7% alcohol in the preferred flavor of Kool-Aid during the conditioning period resulted in that flavor's becoming secondarily aversive. In contrast, the presence of 3% alcohol in the preferred flavor did not change the preference for that flavor on post-conditioning tests. These outcomes were not altered by permitting subjects to have 20 opportunities prior to conditioning to drink sugar water+alcohol at the same concentration as that which was used during the conditioning period.

The experimental treatments in this experiment can be viewed as consisting of two distinct components. The first involved 20 days of forced drinking of either sugar water+alcohol or sugar water alone. The second consisted of the conditioning sessions which provided opportunities to drink different concentrations of alcohol with the preferred flavor of Kool-Aid for the experimental animals and with sugar water for the controls. Thus, the failure to obtain post-conditioning differences between naive

and experienced groups given the same concentration of alcohol during the conditioning period could be due to a negligible effect of the first treatment. Another possible explanation would be that the consequences of the first treatment served to alter differentially the amounts of alcohol consumed by the naive and experienced subjects during the conditioning period in such a way as to effectively equate the accompanying aversive consequences for the two groups.

Earlier, it was hypothesized that drinking alcohol solutions for 20 days resulted in animals' experiencing both positive and negative reinforcements. The positive component resulted from the daily reduction of thirst by the drinking of alcohol, whereas the negative component resulted from the aversive consequences accompanying the rapid drinking of alcohol. The observation that the order of decreasing intakes on Day 1 of the 20-day period was 3% > 5% > 7% is consistent with the view that the more aversive the solution the lower the intake (see Fig. 5). Animals that drank small amounts on Day 1 would be expected to have increased thirst on Day 2, which should serve to elicit approach and drinking responses to a greater degree. The increased intakes on Day 2, relative to Day 1, by the 5% and 7% animals support this view. However, one of the consequences of increased consumption would be to increase the g/kg administered and hence increase the magnitude of the aversive central state accompanying alcohol ingestion. A more intense aversive central state should serve to reduce the amount of fluid ingested on the following day. The reduced intakes on Day 3 support this formulation. It therefore seems reasonable to expect the daily intakes of aversive solutions to oscillate initially and then to stabilize at a level which would be

sufficient to maintain body fluids and at the same time prevent highly aversive central states. In other words, this level represented a compromise intake where thirst was partially alleviated and an "intolerable" aversive central state was not produced.

One measure of the effects of the first treatment was the quantity of alcohol consumed during the conditioning period. In that both experienced and naive animals were exposed to the same procedures during conditioning, any differences in alcohol intake during this period can be ascribed to the previous experience of drinking (experienced) or not drinking (naive) alcohol.

It is apparent that the 20-day drinking treatment did not alter alcohol consumption during conditioning in a systematic manner. Rather, this first treatment produced different effects at each of the three concentrations (see Fig. 6). The finding that the experienced 3%-3% subjects drank less Kool-Aid+3% than the naive animals during the conditioning period suggests that the 3% alcohol was more aversive after 20 consecutive days than it was initially. The observation that there were no demonstrable differences in the amounts of Kool-Aid+5% consumed by naive and experienced subjects during the conditioning period is consistent with the notion that there were no differential changes in the strengths of the positive and negative properties as a result of drinking 5% for 20 days. The fact that experienced animals drank more Kool-Aid+7% than naive subjects implies that the drinking of 7% alcohol for 20 days resulted in that concentration's becoming more positively reinforcing than it was initially. However, the failure of the 20-day drinking treatment to produce systematic effects during the conditioning period negates

a simple explanation.

The amount of conditioned aversion to the Kool-Aid flavor mixed with alcohol during conditioning should be a function of the quantity of alcohol consumed during the conditioning period. The effects of the first treatment resulted in different intakes of Kool-Aid+alcohol between naive and experienced animals that drank 3% or 7%. Therefore, it seems plausible to suggest that the experienced and naive animals may have been exposed to different magnitudes of the UCS during conditioning with either 3% or 7%, and hence those animals that administered the most alcohol would have experienced the strongest unconditioned stimulation.

In Experiments I and II, it was concluded that the primary determinant of conditioned taste aversion was an aversive central state produced by the rapid drinking of alcohol solutions. It was also suggested that the onset and magnitude of this central state and its time course could be estimated by measuring peak blood-alcohol levels. Furthermore, it was expected that the peak blood-alcohol level would be closely related to the amount of alcohol ingested (g/kg). Even though the 3%-3% group consumed less Kool-Aid+3%, and hence less g/kg than the 0%-3% group, it probably did not experience a lower central aversive state because the peak blood-alcohol levels obtained in the 0%-3% blood-alcohol subjects were not statistically separable from those of the 3%-3% subjects. The failure to observe differences in peak blood-alcohol levels is not surprising because there are several prominent sources of variability in blood-alcohol determinations. There is variability in the dosages administered within any one group, and there is also great variability in the rate of absorption and consequently in the magnitude of the peak blood-alcohol

level. Thus, it is possible that there were no post-conditioning differences in performance between naive and experienced animals that were conditioned with 3% because the resulting peak blood-alcohol levels, and hence magnitude of the UCS, were the same. Similarly, the amounts of Kool-Aid+5% and the resulting g/kg (peak blood-alcohol levels) were not different in magnitude for the 0%-5% and 5%-5% groups. Consequently, there were no differences in post-conditioning preferences. The 7%-7% group drank more Kool-Aid+7% and therefore administered more g/kg than the 0%-7% group. Although experienced blood-alcohol subjects also drank more Kool-Aid+7% than naive blood-alcohol subjects, there were no differences in peak blood-alcohol levels. These data support the hypothesis that no post-conditioning differences were observed in the drinking performances of the 7% subjects because there were no differences in UCS intensity between the groups during the conditioning period.

It is concluded, therefore, that even though the first treatment significantly altered drinking performance during the subsequent conditioning period, the intensity of the hypothesized primary determinant of conditioned taste aversion was not changed. As a consequence, no differences in preferences between naive and experienced animals conditioned at the same concentration were detectable.

This experiment was also designed to determine if differences in the magnitude of the conditioned aversion resulted from the ingestion of different concentrations of alcohol during the conditioning period. It was expected that the higher the concentration, the more g/kg would be self-administered, and hence the greater the conditioned aversion. On the basis of the findings that the consumption of sugar water+3% was

greater than that of higher concentrations during the 20-day period (Fig. 5), and that more Kool-Aid+3% was drunk during the 10-day conditioning period than any other concentration of alcohol and Kool-Aid (Fig. 6), it is concluded that 3% alcohol was less aversive than either 5% or 7%. The observations that less sugar water+7% was ingested than any other concentration during the 20-day period, and that less Kool-Aid+7% was ingested during conditioning than any other concentration of Kool-Aid+alcohol are consonant with the conclusion that 7% alcohol was more aversive than either 5% or 3%. Thus, as the alcohol concentration of a fluid increased, the aversiveness accompanying its ingestion also apparently increased.

The ingestion of 1.04 g/kg and 1.16 g/kg by the 3%-3% and the 0%-3% groups, respectively, on each of the five days that alcohol was mixed with Kool-Aid was not sufficient to produce conditioned taste aversion. On the other hand, the administration of 1.71 g/kg/alcohol drinking day for both the naive and experienced 5% groups did result in conditioned aversion. The observation that the peak blood-alcohol levels resulting from drinking 5% were greater than those resulting from drinking 3% is consistent with the hypothesis that the centrally mediated aversive state was the primary determinant of the conditioned aversion. This hypothesis was supported further by the finding that even though the experienced animals that drank 3% and 5% during the conditioning period consumed the same amounts of Kool-Aid+alcohol, they differed in dosage, with the 5% animals' administering more g/kg. Similarly, the conditioned taste aversion demonstrated in the 7% experimental animals resulted from the administration of more g/kg and higher peak blood-alcohol levels than 3% subjects. Even though the experienced 7% animals had larger dosages and higher peak blood-alcohol

levels than the experienced 5% animals, there were no differences in post-conditioning preferences. This result is interpreted as indicating that even though the blood-alcohol defined aversive central state accompanying 7% was statistically larger than at 5%, it might not have been more aversive to the subject, or more likely, the maximum aversive central effect, as measured by post-conditioning preference tests, had already been attained at the 5% dosage.

It was mentioned earlier that Amit et al (1973) have suggested that a novel drug state is aversive to animals. Perhaps, the rapid oral ingestion of alcohol solutions results in conditioned taste aversion because the accompanying state of inebriation is novel and therefore aversive to naive animals. The procedure of allowing animals to ingest alcohol solutions for 20 days at the same concentration used during a subsequent conditioning period should have served to "familiarize" them with the central state accompanying alcohol drinking. In fact, more g/kg were administered per day during the 20-day period than during the conditioning period. Therefore, this procedure should have reduced or eliminated the novelty of the state of inebriation. The findings that both 5% and 7% experienced experimental groups demonstrated conditioned taste aversion on post-conditioning preference tests and that their performances were not distinguishable from those of the naive 5% and 7% groups fail to support the Amit et al (1973) hypothesis.

A more pharmacologically based formulation predicts that after an extended period of alcohol drinking, the rate of metabolic destruction of alcohol may be increased thereby decreasing the intensity of the central effect. This possibility relies on the report that chronic exposures to

alcohol results in an increase in the rate of metabolism of alcohol (Hawkins, Kalant, and Khanna, 1966). If peak blood-alcohol levels are correlated with an aversive central state, then any mechanism which served to reduce blood-alcohol levels should concomitantly reduce the aversiveness of the central state. Consequently, a decrease in the conditioned taste-aversion phenomenon in experienced subjects relative to pharmacologically naive animals should result from chronic exposure to alcohol. However, since the peak blood-alcohol levels of the naive and experienced animals were not different, differences in conditioned taste aversion would not be expected, and were not, in fact observed. The observation that the experienced blood-alcohol subjects that drank either 5% or 7% administered more g/kg than their pharmacologically naive counterparts and yet did not display a higher peak blood-alcohol level is consistent with the hypothesis that previous alcohol-drinking sessions serve to increase the metabolic rate of alcohol destruction.

The ingestion of 5% alcohol solutions resulted in different blood-alcohol curves in Experiments II and III. The curve in Experiment III (see Fig. 9) shows a peak blood-alcohol level of approximately 78 mg/100 ml plasma at 65 min, whereas the curve in Experiment II (see Fig. 4) shows a peak level of 105 mg/100 ml plasma at 25 min. In addition, the curve in Experiment II indicates a reduced level of alcohol at 185 min, while substantial levels are still apparent in the Experiment III curve. The animals in Experiment II self-administered 1.40 g/kg which was less than the 1.54 g/kg administered by Experiment III animals. Thus, even though Experiment II animals ingested less alcohol they demonstrated a quicker attainment of higher peak blood-alcohol levels than Experiment III

animals. It seems reasonable to suggest that these differences are most likely owing to different rates of absorption between the groups, but perhaps also to different volumes of distribution. In that the two groups differed in age, in the number of previous opportunities to drink sugar water, and in the number of days on the fluid-deprivation regimen prior to drinking the alcohol, any clear explanation of the differences is difficult.

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APPENDIX A: Daily intakes in ml for Experiment I subjects. (G) indicates grape-flavored Kool-Aid, (O) indicates orange-flavored Kool-Aid, S indicates sugar water, (*) indicates the presence of 5% ethanol, and K indicates the preferred flavor of Kool-Aid.

	water	pref		pref		C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	water	pref	
Day No.	6	9		10		12	13	14	15	16	17	18	19	20	21	22	23	
Experimental Group																		
Subgroup A		O	G	G	O	K*	S	S	K*	S	K*	K*	S	K*	S		G	O
subject 1	15	5	8	2	15	9	19	17	7	17	6	11	19	7	19	19	5	10
3	14	11	2	1	12	9	15	20	4	17	6	7	19	8	18	17	15	3
4	14	6	8	8	9	10	19	18	3	17	8	9	19	8	20	17	9	8
12	11	10	5	3	16	12	14	16	7	16	7	6	17	6	16	16	12	2
15	12	6	7	19	1	10	18	19	5	18	11	5	19	7	20	17	3	12
Subgroup B						S	K*	K*	S	K*	S	S	K*	S	K*			
subject 2	15	5	5	13	1	18	9	7	15	7	15	17	11	16	6	16	3	9
5	15	7	3	12	2	19	9	7	19	8	19	19	9	19	9	17	9	6
11	15	2	14	19	1	19	9	9	18	13	21	18	3	19	12	20	12	3
13	15	3	11	13	4	17	7	8	16	8	15	17	5	17	9	16	2	12
14	19	3	14	2	18	21	12	8	20	13	22	20	6	24	11	22	13	2

	pref		pref		pref		pref		pref		pref		pref		pref		pref		pref	
Day No.	24		25		26		27		28		29		30		31		32		33	
Experimental Group																				
Subgroup A	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O
subject 1	8	8	16	1	13	3	14	3	1	17	17	2	1	17	13	3	1	12	13	2
3	2	18	15	2	1	14	13	3	4	13	13	4	12	4	13	3	10	5	13	1
4	15	3	13	5	4	12	4	12	14	3	14	3	13	3	12	4	1	13	12	4
12	1	13	11	4	6	9	14	2	2	15	9	3	2	12	11	2	5	7	10	3
15	14	3	5	13	12	5	7	6	3	6	10	1	2	10	10	2	11	1	11	1
Subgroup B																				
subject 2	17	1	17	1	17	1	14	3	17	2	17	2	16	1	15	1	14	1	15	0
5	16	1	14	4	3	13	11	6	14	2	13	3	15	1	12	4	4	14	12	5
11	16	2	14	4	17	2	14	4	16	3	12	4	11	4	13	2	13	3	14	3
13	14	2	6	8	10	2	1	10	10	5	1	12	10	5	2	17	13	4	8	7
14	3	13	10	8	6	13	16	2	4	12	5	10	9	8	14	2	9	9	14	1

	pref		pref		pref		pref		pref	
Day No.	34		35		36		37		38	
Experimental Group										
Subgroup A	O	G	G	O	O	G	G	O	O	G
subject 1	3	10	13	1	3	11	3	12	4	13
3	2	13	15	1	18	2	17	1	14	4
4	4	10	9	4	11	4	9	6	9	6
12	4	9	8	6	10	5	8	8	13	1
15	13	6	10	2	9	1	1	13	17	1
Subgroup B										
subject 2	4	13	15	1	13	1	15	1	16	1
5	6	10	17	1	16	1	8	8	16	1
11	18	1	17	2	18	1	15	6	15	3
13	15	2	2	15	13	2	2	16	15	3
14	13	8	12	5	18	2	9	11	12	9

	water	pref		pref		C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	water	pref	
Day No.	6	9		10		12	13	14	15	16	17	18	19	20	21	22	23	
Control Group																		
Subgroup A		O	G	G	O	K	S*	S*	K	S*	K	K	S*	K	S*		G	O
subject 7	14	5	6	12	3	15	8	14	14	5	16	16	14	14	7	18	18	1
9	14	9	4	5	11	15	12	8	13	13	14	15	10	15	10	17	16	2
10	15	11	4	11	6	14	13	9	13	15	16	14	7	15	13	16	11	7
16	13	3	10	6	8	11	17	6	13	9	15	15	9	15	11	15	16	2
18	15	3	14	19	2	17	9	20	16	5	17	17	8	17	15	19	20	2
Subgroup B						S*	K	K	S*	K	S*	S*	K	S*	K			
subject 6	16	8	7	11	5	9	15	18	18	16	4	12	17	14	17	16	18	3
8	15	4	5	9	2	8	11	15	15	13	3	8	14	12	17	19	15	3
17	19	2	11	2	16	15	14	15	8	17	11	16	16	13	18	18	14	6
19	19	1	16	18	2	12	16	20	14	18	13	13	20	14	20	22	19	2
20	16	9	6	11	10	17	14	17	7	17	12	18	15	10	4	16	13	6

	pref		pref		pref		pref		pref		pref		pref		pref		pref		pref	
Day No.	24		25		26		27		28		29		30		31		32		33	
Control Group																				
Subgroup A	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O
subject 7	2	18	19	1	1	17	15	2	3	15	17	2	9	11	16	1	2	14	18	1
9	19	1	16	2	17	1	6	12	16	2	17	2	18	2	14	4	10	7	11	5
10	17	2	8	10	17	2	8	10	16	2	12	5	6	12	1	15	15	1	11	4
16	2	15	17	2	3	15	5	10	3	12	2	15	2	15	15	2	1	14	12	2
18	16	2	18	1	14	5	17	1	12	3	11	2	13	1	15	1	15	1	16	1
Subgroup B																				
subject 6	7	16	16	4	8	12	17	6	7	13	15	4	5	11	13	4	8	9	2	15
8	6	11	17	3	3	16	13	4	5	14	14	4	4	15	16	2	3	15	19	2
17	19	3	5	14	11	11	5	9	4	12	8	9	10	7	1	15	9	9	2	16
19	3	19	17	2	6	13	16	2	6	10	14	2	11	7	17	2	3	17	18	1
20	14	4	12	6	15	5	15	2	6	12	11	3	12	4	11	5	12	4	13	4

	pref		pref		pref		pref		pref	
Day No.	34		35		36		37		38	
Control Group										
Subgroup A	O	G	G	O	O	G	G	O	O	G
subject 7	7	11	14	1	2	13	16	1	2	16
9	10	8	14	4	12	6	17	2	16	2
10	12	1	7	4	11	2	2	13	6	9
16	1	14	16	2	2	14	16	3	2	16
18	11	2	15	2	14	2	12	2	15	2
Subgroup B										
subject 6	9	7	16	2	8	7	11	6	15	1
8	8	9	15	2	4	10	12	2	5	10
17	7	12	2	19	12	8	1	21	11	11
19	2	17	19	3	11	9	19	2	17	4
20	17	2	11	7	13	5	11	7	19	2

APPENDIX B: Daily weights in g for Experiment I subjects.

	initial weight	water	pref	pref	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Day No.		6	9	10	12	13	14	15	16	17	18	19	20	21
Experimental Group														
Subgroup A														
subject 1	242	222	208	210	212	210	214	214	212	216	210	206	216	206
3	216	204	196	198	196	194	198	200	192	200	196	192	200	192
4	236	224	212	214	216	210	218	218	212	216	212	210	216	210
12	250	216	210	212	216	212	214	216	210	218	212	206	214	208
15	252	218	216	216	218	218	220	224	214	222	220	214	221	214
Subgroup B														
subject 2	236	216	208	208	208	210	208	202	204	204	206	212	210	210
5	252	230	220	220	222	220	216	210	220	218	222	226	220	222
11	234	200	196	196	196	200	192	190	194	198	200	200	192	196
13	256	224	220	220	220	226	218	214	216	216	220	218	214	216
14	230	202	200	202	204	210	202	196	200	202	206	208	200	206

	water	pref	pref	pref	pref
Day No.	22	23	24	37	38
Experimental Group					
Subgroup A					
subject 1	212	212	208	214	210
3	196	194	196	204	200
4	216	216	216	218	216
12	214	216	218	214	220
15	222	222	222	188	190
Subgroup B					
subject 2	204	206	204	218	216
5	216	218	218	226	220
11	196	196	198	194	200
13	214	214	216	198	200
14	204	206	206	200	204

	initial weight	water	pref	pref	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Day No.		6	9	10	12	13	14	15	16	17	18	19	20	21
Control Group														
Subgroup A														
subject 7	234	238	226	226	226	224	220	220	222	214	220	220	222	220
9	236	212	200	200	200	200	200	194	198	198	200	200	196	200
10	222	198	192	190	194	192	194	186	192	194	194	192	188	190
16	234	210	208	206	206	206	206	200	200	200	202	206	204	204
18	240	212	216	214	218	222	216	218	214	208	214	214	212	212
Subgroup B														
subject 6	248	234	224	226	224	218	222	224	228	224	216	216	220	218
8	240	216	206	202	208	204	206	206	210	210	200	198	204	204
17	262	228	226	224	230	230	226	228	222	228	226	228	232	228
19	248	220	220	220	222	220	220	224	222	228	224	222	228	224
20	256	228	226	224	228	230	230	230	224	228	230	230	232	224

	water	pref	pref	pref	pref
Day No.	22	23	24	37	38
Control Group					
Subgroup A					
subject 7	214	216	216	230	230
9	196	198	200	204	202
10	190	190	192	192	190
16	204	202	208	202	206
18	214	216	224	204	206
Subgroup B					
subject 6	220	218	222	224	218
8	208	210	212	220	216
17	234	234	234	236	236
19	230	232	236	220	226
20	220	220	226	220	222

APPENDIX C: Blood-alcohol determination

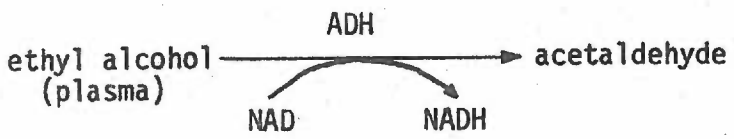
Upon completion of the drinking session, the subject was removed from its cage and lightly anesthetized with ether. Approximately one inch from the base of the tail, a mid-ventral cut (about two inches in length) was made through the full thickness of the integument. The integument was reflected and the connective tissue and caudal vein were exposed. Procaine (10 mg procaine hydrochloride/ml) was applied topically to the area as a local anesthetic, with repeated applications every hr. The animal was then placed into a restrainer which was designed to prevent gross body movements without physically injuring the subject. The tail vein was severed, and an initial .75 ml sample of blood was taken in a heparinized micro-hematocrit glass capillary tube. The time after completion of the drinking bout to the collection of the first blood sample was 5 min. Small clamps, hemostats, or gauze strips were applied directly to the vein or to the general severed area to prevent continued blood flow. The restrictive device was removed and then reapplied to permit additional blood samples to be taken at 25, 65, 95, 125, and 185 min periods.

All blood samples were stored in a refrigerator until analyzed, with all analyses being conducted within 24 hr after being collected. The samples were centrifuged in a micro-hematocrit centrifuge for 5 min, and then a .02 ml aliquot of plasma was removed and added to 1 ml of 0.9 N NaCl. Exactly .1 ml of the plasma-NaCl mixture was then added to a previously prepared mixture at room temperature of 1 ml NAD (nicotinamide-adenine dinucleotide), 1.5 ml phosphate buffer

(pH = 8.7), and .4 ml of ADH (alcohol dehydrogenase) prepared from a yeast extract.

The phosphate buffer was made by combining 1 g sodium pyrophosphate, .25 g semicarbazide·HCl, .05 g glycine in 25 ml water, 2 ml 1N NaOH; this mixture was diluted with water to 30 ml. This solution was stored at 4° C. New buffer solutions were mixed every 14 days. The NAD was also stored at 4° C and was made anew every 14 days at the concentration of 14.2 mg NAD/10 ml phosphate buffer. The ADH was made-up each day at the concentration of 1.5 mg ADH/10 ml phosphate buffer.

The resulting mixture of plasma-NaCl, NAD, ADH, and phosphate buffer was maintained for 1 hr at room temperature to insure completion of the following reaction:



All acetaldehyde formed in this reaction was prevented from undergoing a reverse reaction to form ethanol by the semicarbazide·HCl contained in the phosphate buffer.

A spectrophotometer was set at 340 nanometers and the indicator was set at zero absorbance by using a plasma blank which consisted of plasma from an animal not given alcohol to drink; its plasma sample was treated in an identical manner as the samples obtained from experimental animals. The experimental samples were then evaluated for absorbance relative to the plasma blank. The readings were taken at 340 nm because the values obtained at this setting are specific for NADH, relative to the other

chemicals used in this reaction. Originally, there is no NADH present in the mixture, but as alcohol is converted to acetaldehyde, the coupled reaction of NAD ⟶ NADH also occurs. Thus, the amount of NADH present in a sample after completion of the above procedures is a direct and quantitatively accurate measure of the amount of ethanol present in the plasma sample.

The formula used to relate the absorbance value of the experimental sample (Δ A) to mg of alcohol per 100 ml sample is:

$$\frac{\Delta A}{2.07} \times \frac{50}{.1} \times \frac{46.07}{106} \times 100 = \text{g alcohol/100 ml plasma}$$

where Δ A is the absorbance of the sample relative to the plasma blank. The 2.07 value in the above equation was determined as follows: the absorbance of one micromole of NADH/1 ml solution in a one cm light path at 340 nm is 6.22. Thus, the conversion of one micromole of NADH in the 3.0 ml of reaction mixture of alcohol corresponds to an absorbance change of $\frac{6.22}{3.0} = 2.07$. Because the .1 ml sample was composed of 1 part plasma to 50 parts NaCl, the total number of micromoles in a 1 ml plasma sample is $\frac{A}{2.07} \times \frac{50}{.1}$ micromoles. In order to convert micromoles of NADH to moles of alcohol (g), the last formula is multiplied by the molecular weight of ethyl alcohol (46.07) and divided by 10^6 (μg to g). Multiplying the result by 100 converts this last value into g of ethanol/100 ml plasma.

APPENDIX D: Daily intakes in ml for Experiment II subjects. (G) indicates grape-flavored Kool-Aid, (O) indicates orange-flavored Kool-Aid, S indicates sugar water, (*) indicates the presence of 5% ethanol, and K indicates the preferred flavor of Kool-Aid.

	pref		pref		C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	pref		pref		pref		pref	
Day no.	6		7		8	9	10	11	12	13	14	15	16	17	18		19		20		21	
Massed Group																						
Subgroup A	G	O	O	G	K*	S	S	K*	S	K*	K*	S	K*	S	O	G	G	O	O	G	G	O
subject 1	9	4	10	7	6	12	10	9	11	8	8	11	8	12	9	8	9	10	14	3	10	7
2	8	7	5	12	8	12	10	10	11	9	10	11	10	12	11	6	2	14	9	6	7	10
3	3	14	13	4	10	12	10	10	11	10	11	11	10	12	11	4	13	2	11	7	17	2
4	11	8	5	14	12	12	10	7	11	12	4	11	8	12	7	13	4	15	16	3	10	11
5	8	5	6	10	6	12	10	10	11	7	9	11	9	12	12	2	9	8	13	4	3	15
Subgroup B					S	K*	K*	S	K*	S	S	K*	S	K*								
subject 6	4	11	5	12	12	11	6	10	10	12	11	9	10	10	12	2	2	14	3	15	2	15
7	10	5	2	16	12	12	5	10	11	12	11	7	10	12	12	3	12	5	16	1	2	15
8	8	8	3	16	12	9	8	10	10	12	11	9	10	12	14	3	2	14	14	4	3	15
9	9	6	11	6	12	8	10	10	11	12	11	10	10	9	5	13	16	2	6	13	17	2
10	2	15	4	13	12	7	10	10	11	12	11	11	10	10	2	18	6	10	2	17	10	8

	pref		pref		C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	pref		pref		pref		pref	
Day No.	6		7		8	9	10	11	12	13	14	15	16	17	18		19		20		21	
Massed Control Group																						
Subgroup A	G	O	O	G	K	S*	S*	K	S*	K	K	S*	K	S*	O	G	G	O	O	G	G	O
subject 11	8	8	15	2	12	10	10	10	11	12	11	11	10	12	17	2	12	1	17	2	16	2
12	8	4	14	4	12	12	10	10	11	12	11	11	10	12	15	3	15	1	16	1	1	14
13	9	7	6	11	12	11	10	10	11	12	11	11	10	12	9	8	13	5	10	9	6	15
14	2	12	4	9	12	6	10	10	11	12	11	11	10	12	9	8	4	12	3	14	2	14
15	10	2	12	3	12	8	10	10	11	12	11	11	10	12	3	14	4	10	12	3	2	13
Subgroup B					S*	K	K	S*	K	S*	S*	K	S*	K								
subject 16	2	13	2	14	12	12	10	10	11	12	11	11	10	12	1	18	16	2	10	7	14	6
17	5	8	11	2	12	12	10	10	11	12	11	11	10	12	11	3	14	2	11	3	3	13
18	7	5	3	8	12	8	10	10	11	12	11	11	10	12	10	3	1	10	3	9	8	4
19	6	5	2	15	10	12	10	10	11	12	11	11	10	12	11	5	6	13	13	6	16	1
20	8	5	1	11	12	12	10	10	11	12	11	11	10	12	1	15	5	9	1	14	9	5

	pref		pref		C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	pref		pref		pref		pref	
Day No.	6		7		8	9	10	11	12	13	14	15	16	17	18		19		20		21	
Distributed Group																						
Subgroup A	G	O	O	G	K*	S	S	K*	S	K*	K*	S	K*	S	O	G	G	O	O	G	G	O
subject 21	10	6	14	3	12	12	10	10	11	12	11	11	10	12	16	2	13	6	5	14	1	18
22	2	12	6	11	12	12	10	10	11	12	11	11	10	12	12	7	12	7	8	11	5	14
23	6	9	12	4	12	12	10	10	11	12	11	11	10	12	17	3	15	4	16	4	15	5
24	12	3	5	12	12	12	10	10	11	12	11	11	10	12	2	16	14	4	1	17	16	3
25	2	12	14	2	12	12	10	10	11	12	11	11	10	12	17	1	9	6	15	1	17	2
Subgroup B					S	K*	K*	S	K*	S	S	K*	S	K*								
subject 26	3	11	9	9	12	12	10	10	11	12	11	11	10	12	5	14	4	14	2	15	2	17
27	12	2	10	5	12	12	10	10	11	12	11	11	10	12	16	1	15	2	1	15	14	3
28	7	6	5	10	12	12	10	10	11	12	11	11	10	12	7	10	12	3	2	13	4	12
29	2	14	5	10	12	12	10	10	11	12	11	11	10	12	15	3	12	5	14	2	12	5
30	6	6	8	7	12	12	10	10	11	12	11	11	10	12	15	1	15	1	2	14	4	11

	pref		pref		C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	pref		pref		pref		pref	
Day No.	6		7		8	9	10	11	12	13	14	15	16	17	18		19		20		21	
Distributed Control																						
Subgroup A	G	O	O	G	K	S*	S*	K	S*	K	K	S*	K	S*	O	G	G	O	O	G	G	O
subject 31	2	15	13	5	12	12	10	10	11	12	11	11	10	12	18	1	4	13	14	2	3	13
32	6	6	8	9	12	12	10	10	11	12	11	11	10	12	15	1	1	16	1	14	4	12
33	9	9	14	4	12	12	10	10	11	12	11	11	10	12	19	1	17	4	5	13	2	15
34	1	10	1	14	12	12	10	10	11	12	11	11	10	12	1	16	1	15	2	13	1	14
35	1	13	6	9	12	12	10	10	11	12	11	11	10	12	14	2	11	5	15	1	10	8
Subgroup B					S*	K	K	S*	K	S*	S*	K	S*	K								
subject 36	2	13	5	10	12	12	10	10	11	12	11	11	10	12	11	5	5	10	8	8	1	17
37	2	12	8	5	12	12	10	10	11	12	11	11	10	12	2	12	3	11	1	12	1	15
38	1	15	4	10	12	12	10	10	11	12	11	11	10	12	16	1	6	12	9	6	1	16
39	1	12	10	3	12	12	10	10	11	12	11	11	10	12	16	1	1	16	16	1	1	16
40	1	14	3	12	12	12	10	10	11	12	11	11	10	12	13	3	8	10	2	16	3	13

APPENDIX E: Daily weights in g for Experiment II subjects.

	pref	pref	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	pref	pref
Day No.	6	7	8	9	10	11	12	13	14	15	16	17	18	21
Massed Group														
Subgroup A														
subject 1	216	214	214	210	214	214	212	202	204	204	208	208	212	226
2	204	202	204	202	204	202	204	192	194	194	198	196	200	208
3	228	228	226	224	224	224	222	212	214	212	214	212	214	222
4	210	216	220	218	220	216	216	206	210	204	208	208	210	218
5	222	224	220	212	216	212	210	200	200	196	200	198	200	212
Subgroup B														
subject 6	226	226	228	226	224	222	222	218	220	220	220	222	220	232
7	228	226	228	230	228	222	224	222	224	224	222	222	224	232
8	218	220	222	220	216	216	214	212	214	214	212	212	214	230
9	230	228	226	226	226	220	218	216	220	222	220	218	220	230
10	218	220	222	222	216	216	216	212	214	220	216	218	218	234

	pref	pref	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	pref	pref
Day No.	6	7	8	9	10	11	12	13	14	15	16	17	18	21
Massed Control Group														
Subgroup A														
subject 11	208	208	212	208	208	208	206	206	206	206	208	206	210	222
12	220	220	224	224	224	222	222	222	222	224	226	224	228	238
13	200	200	200	200	196	198	196	196	200	200	200	196	200	210
14	242	244	242	238	232	232	232	230	232	234	236	236	236	248
15	208	206	208	206	204	204	202	204	206	208	212	210	214	216
Subgroup B														
subject 16	220	224	226	222	222	222	220	210	214	214	216	216	216	230
17	222	220	220	216	218	216	216	208	208	210	212	210	214	218
18	190	190	190	188	188	186	190	180	182	184	184	184	190	192
19	224	224	226	222	222	224	224	214	216	216	220	220	222	234
20	204	206	206	202	204	202	200	192	196	196	198	198	202	212

	pref	pref	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	pref	pref
Day No.	6	7	8	9	10	11	12	13	14	15	16	17	18	21
Distributed Group														
Subgroup A														
subject 21	216	218	220	218	224	218	220	216	214	216	214	212	212	218
22	226	226	230	228	228	224	224	224	222	220	220	216	216	232
23	214	216	218	216	220	218	216	214	214	214	214	212	212	226
24	206	204	204	204	204	204	204	200	200	202	202	198	200	212
25	224	224	226	226	224	222	222	216	214	214	212	210	212	222
Subgroup B														
subject 26	214	212	218	214	214	216	216	214	216	216	214	212	214	228
27	216	216	218	216	212	208	208	206	206	208	206	204	206	214
28	206	204	204	206	206	204	204	204	208	204	206	202	206	216
29	236	238	238	236	234	232	228	230	230	232	230	228	228	236
30	202	202	206	206	208	206	204	206	206	206	210	208	210	220

	pref	pref	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	pref	pref
Day No.	6	7	8	9	10	11	12	13	14	15	16	17	18	21
Distributed Control														
Subgroup A														
subject 31	212	212	214	214	210	212	208	210	208	208	206	208	210	214
32	208	210	212	212	208	208	208	208	210	210	210	206	210	218
33	228	228	228	228	228	226	224	224	222	220	220	216	220	232
34	204	204	204	206	204	204	202	206	204	204	202	200	204	212
35	230	230	232	230	228	224	224	224	224	220	218	218	220	230
Subgroup B														
subject 36	218	216	220	220	216	218	216	212	216	212	210	210	212	222
37	194	196	198	196	196	196	192	196	198	198	198	198	202	202
38	224	228	226	224	228	224	224	222	226	226	222	224	224	226
39	224	224	222	224	226	226	224	224	224	222	226	226	224	234
40	212	216	216	218	216	216	218	216	216	216	218	218	218	226

APPENDIX F: Data for blood-alcohol subjects, which were subjected to the same procedure as Experiment II subjects.

	Kool-Aid+5% (ml)	Weight (g)	Blood alcohol (g/100 ml plasma) 5 min	25 min	65 min	95 min	125 min	185 min
Massed Group								
subject 1	8	228	53.4	56.8	44.5	66.8	43.4	11.1
3	8	226	113.5	133.6	116.9	120.2	121.3	86.8
5	7	217	103.5	105.7	75.7	62.3	14.5	8.9
13	9	207	83.5	87.9	91.3	56.8	22.3	0.0
15	9	220	66.8	122.4	113.5	94.6	63.4	27.8
2-ml Group								
subject 2	2	223	22.3	39.0	23.4	21.2	2.2	1.1
4	2	203	16.7	22.3	0.0	0.0	0.0	0.0
6	2	206	10.0	20.0	5.6	1.1	0.0	0.0
7	2	216	24.5	21.2	25.6	4.4	3.3	12.2
14	2	246	16.7	20.0	2.2	1.1	0.0	0.0

	Kool-Aid+5% (ml)	Weight (g)	Blood alcohol (g/100 ml plasma) 5 min	25 min	65 min	95 min	125 min	185 min
Distributed Group								
subject 8	12	208	12.2	17.8	10.0	2.2	1.1	0.0
9	12	218	10.0	3.3	3.3	0.0	0.0	0.0
10	12	222	16.7	11.1	10.0	0.0	0.0	0.0
11	12	204	13.4	30.0	8.9	11.1	0.0	0.0
12	12	215	10.0	2.2	0.0	0.0	0.0	0.0

APPENDIX G: Daily consumptions in ml for Experiment III subjects during the 20-day pre-conditioning treatment.

Day No.	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
0%-3% Group																				
Subgroup A																				
subject 1	15	16	17	18	19	16	17	17	15	16	15	16	17	16	16	13	13	15	15	17
2	15	19	19	19	17	17	20	19	18	17	15	14	15	13	17	12	11	15	15	16
3	13	19	16	18	13	18	19	16	14	20	17	17	17	19	20	14	14	18	19	20
4	16	19	19	19	18	18	16	16	16	17	17	16	18	18	17	17	16	19	18	19
5	20	19	20	21	22	19	21	20	17	20	16	19	15	16	19	19	14	20	20	20
Subgroup B																				
subject 6	18	17	16	18	13	15	15	15	17	14	14	15	16	17	15	16	17	16	17	15
7	19	20	19	13	20	19	15	21	17	17	18	18	17	17	18	19	20	17	16	20
8	19	19	19	20	19	18	17	20	16	19	18	16	17	17	20	17	18	16	20	17
9	13	18	16	17	14	13	15	15	15	16	14	17	18	16	17	18	16	16	16	16
10	17	19	16	16	17	14	16	16	17	15	18	17	14	14	15	15	14	17	15	15

Day No.	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
0%-5% Group																				
Subgroup A																				
subject 11	17	19	18	18	17	18	16	19	18	18	19	19	16	17	19	18	18	17	21	17
12	18	15	20	20	22	17	17	17	17	18	15	17	14	17	15	13	17	20	19	18
13	19	20	20	20	20	20	17	19	17	21	21	20	19	16	18	17	18	19	21	20
14	19	22	22	20	20	20	20	21	21	18	17	15	21	16	19	19	22	20	20	18
15	15	16	16	13	16	14	13	15	14	16	17	17	12	12	17	16	17	12	12	14
Subgroup B																				
subject 16	13	17	20	15	19	15	19	19	18	18	20	21	17	15	17	19	18	16	15	16
17	18	15	18	18	18	16	14	19	19	17	14	15	16	17	18	18	15	17	13	16
18	18	18	20	17	20	19	20	21	20	17	20	15	20	15	20	18	20	18	20	21
19	18	16	18	16	17	15	17	16	17	17	15	16	14	15	14	17	17	17	15	14
20	13	16	19	16	15	14	12	15	17	17	15	15	13	15	13	14	13	15	16	16

Day No.	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
0%-7% Group																				
Subgroup A																				
subject 21	14	17	19	14	16	18	19	16	12	17	13	18	16	13	17	14	15	17	19	16
22	16	18	18	13	15	17	16	12	16	14	13	14	13	12	14	12	14	13	12	14
23	20	15	20	19	17	17	17	17	18	17	18	16	18	18	19	16	15	17	18	18
24	18	14	18	17	15	15	15	15	16	11	15	15	16	15	15	15	17	15	15	13
25	18	18	21	21	21	21	20	16	20	18	17	18	17	17	17	15	15	18	17	19
Subgroup B																				
subject 26	16	18	17	14	15	19	15	18	13	15	11	17	16	13	16	15	18	15	15	14
27	15	17	17	18	17	14	18	18	15	15	16	18	17	16	16	12	16	16	19	16
28	17	18	14	15	18	17	15	14	14	17	15	12	12	16	17	13	14	15	15	15
29	19	17	20	19	18	18	17	16	18	17	18	18	15	15	15	15	17	20	20	18
30	15	17	16	13	18	14	15	13	16	15	13	15	15	14	16	12	13	17	14	15

Day No.	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
0%-7%/C Group																				
Subgroup A																				
subject 31	19	20	20	18	20	20	22	22	19	17	15	22	20	17	17	16	17	16	17	17
32	14	15	16	17	20	17	13	17	16	16	16	15	12	12	16	15	18	18	15	15
33	13	15	15	18	19	18	18	19	17	20	17	17	19	17	19	19	18	19	19	19
34	18	19	21	17	16	20	16	18	19	18	20	19	20	15	16	14	20	17	16	17
35	13	13	17	15	17	14	14	16	14	15	15	13	11	13	15	13	14	14	17	16
Subgroup B																				
subject 36	16	19	18	18	18	20	19	19	16	17	17	15	17	16	18	15	16	19	17	19
37	13	16	18	16	17	19	19	19	15	17	15	18	15	14	18	14	18	17	20	18
38	17	17	17	17	18	16	16	18	19	16	15	15	18	14	15	13	15	15	15	16
39	15	15	19	17	16	16	15	17	16	15	14	18	16	13	15	14	16	16	15	14
40	19	16	18	18	20	19	17	18	18	18	16	17	15	15	18	13	15	18	17	17

Day No.	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
3%-3% Group																				
Subgroup A																				
subject 41	14	18	17	16	17	20	19	19	19	19	18	20	20	19	19	20	18	21	18	19
42	16	18	17	17	16	18	15	18	18	14	17	13	15	17	17	17	14	15	17	19
43	17	17	14	13	15	14	17	15	17	16	13	15	15	13	16	10	15	17	17	15
44	13	17	15	15	17	13	14	16	18	17	16	16	15	14	17	15	13	15	15	14
45	16	16	16	17	17	16	18	15	14	10	16	14	15	16	17	16	17	15	16	15
Subgroup B																				
subject 46	15	17	15	16	15	18	17	15	16	13	13	17	17	14	17	17	16	15	15	14
47	17	17	17	18	15	19	16	17	15	13	18	16	17	17	18	15	14	13	16	15
48	15	19	20	18	20	18	19	20	19	19	16	18	18	18	18	16	15	19	17	18
49	10	16	17	17	13	14	20	17	19	11	14	18	20	16	15	16	16	20	18	15
50	15	15	13	17	16	17	10	16	16	15	13	13	15	15	17	12	10	13	11	17

Day No.	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
5%-5% Group																				
Subgroup A																				
subject 51	13	11	9	15	16	13	14	14	15	15	17	17	14	17	20	12	13	14	14	12
52	11	13	13	16	12	16	12	13	15	15	16	17	15	12	12	15	10	14	16	15
53	11	16	14	17	4	10	16	17	14	16	17	16	9	13	16	13	15	13	14	15
54	12	16	7	11	15	17	17	17	14	15	16	18	19	17	17	16	19	19	19	19
55	13	20	9	15	15	16	20	16	13	14	16	15	17	15	14	15	12	13	16	16
Subgroup B																				
subject 56	11	12	11	15	16	14	16	14	13	13	12	15	13	10	17	17	13	16	13	11
57	9	17	14	13	13	18	12	13	16	16	17	17	18	13	18	17	16	17	17	20
58	10	14	9	15	8	17	10	15	12	14	12	16	12	13	12	14	14	19	15	14
59	9	12	16	10	10	15	14	15	10	8	14	15	16	15	14	15	15	12	16	18
60	10	17	6	11	13	16	11	16	12	15	10	12	16	16	14	14	13	15	13	13

Day No.	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
7%-7% Group																				
Subgroup A																				
subject 61	7	13	5	9	10	12	12	12	14	11	12	11	11	13	13	10	12	14	11	12
62	6	10	6	8	11	7	9	11	13	11	12	15	12	14	13	15	12	12	10	11
63	11	10	14	11	12	14	11	13	11	12	12	13	11	15	10	12	13	14	12	12
64	9	12	7	13	13	12	13	13	9	14	13	14	15	13	14	14	14	13	11	15
65	6	15	5	10	12	12	14	17	15	13	13	14	16	15	12	12	13	12	12	13
Subgroup B																				
subject 66	8	11	14	8	11	10	12	11	14	8	13	15	14	13	8	9	9	13	9	9
67	6	12	4	8	9	10	13	12	13	13	12	15	13	13	11	11	11	13	12	12
68	8	18	3	12	11	10	12	11	14	15	16	16	12	18	16	13	13	16	16	12
69	8	11	5	9	15	11	15	13	12	12	12	10	13	13	15	16	13	10	11	15
70	6	12	6	10	13	13	12	10	19	6	8	9	9	11	11	12	12	16	20	13

Day No.	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
7%-7%/C Group																				
Subgroup A																				
subject 71	8	14	5	8	9	11	11	17	12	14	12	12	13	12	15	12	14	10	15	13
72	6	9	5	8	9	9	11	10	12	9	12	10	13	15	12	12	10	10	13	10
73	8	12	4	8	11	10	12	10	12	11	11	12	10	12	13	12	15	13	10	13
74	5	15	2	6	7	9	13	7	10	11	10	9	14	10	7	13	12	15	15	10
75	5	11	9	12	12	13	10	15	14	13	17	15	10	10	14	15	14	17	17	15
Subgroup B																				
subject 76	3	13	3	7	10	7	10	11	13	15	14	15	16	11	13	16	17	7	8	8
77	8	10	7	9	11	9	12	10	11	9	11	11	13	12	17	14	10	9	9	9
78	11	10	11	8	11	11	10	12	10	9	9	12	15	9	10	10	10	12	14	8
79	13	9	6	9	13	11	12	13	10	13	11	13	10	10	11	11	9	12	10	13
80	7	14	5	8	13	10	10	12	10	14	12	13	14	13	16	12	13	11	14	14

APPENDIX H: Amounts, in ml, of fluids consumed during the conditioning period for Experiment III subjects. (K) indicates the preferred flavor of Kool-Aid, (S) indicates sugar water, and (*) indicates the presence of 5% ethanol.

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Day No.	30	31	32	33	34	35	36	37	38	39
0%-3% Group										
Subgroup A	K*	S	S	K*	S	K*	K*	S	K*	S
subject 1	12	18	18	12	18	13	14	17	12	17
2	12	20	17	13	16	12	16	19	11	18
3	14	18	17	14	17	13	15	18	11	21
4	11	18	17	11	16	11	13	16	9	19
5	15	22	17	13	21	11	12	18	12	20
Subgroup B	S	K*	K*	S	K*	S	S	K*	S	K*
6	15	11	10	18	7	17	12	8	15	11
7	19	12	13	19	12	17	21	9	20	12
8	19	13	13	22	12	19	18	13	20	13
9	17	11	9	16	11	15	14	11	15	14
10	20	9	10	16	11	15	15	10	17	11

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Day No.	30	31	32	33	34	35	36	37	38	39
0%-5% Group										
Subgroup A	K*	S	S	K*	S	K*	K*	S	K*	S
subject 11	11	18	18	12	13	10	12	18	10	17
12	12	18	19	9	19	13	9	18	12	20
13	11	18	19	13	20	12	13	22	12	21
14	10	22	21	10	19	11	6	21	11	20
15	14	17	17	7	14	9	11	15	9	13
Subgroup B	S	K*	K*	S	K*	S	S	K*	S	K*
subject 16	18	11	11	19	10	16	17	8	15	11
17	19	9	10	20	10	18	16	9	17	11
18	15	11	11	20	11	18	19	10	17	11
19	17	10	10	15	9	16	14	8	17	11
20	14	9	9	15	10	16	17	10	18	11
0%-7% Group										
Subgroup A	K*	S	S	K*	S	K*	K*	S	K*	S
subject 21	11	22	19	6	18	9	9	19	9	19
22	8	20	18	8	18	11	7	20	11	20
23	8	18	21	6	21	9	8	19	10	18
24	7	16	16	5	17	5	6	17	6	16
25	7	21	19	8	18	8	8	21	9	20
Subgroup B	S	K*	K*	S	K*	S	S	K*	S	K*
subject 26	18	6	5	18	6	16	15	7	15	11
27	16	8	8	18	7	17	17	6	16	7
28	15	8	7	17	7	16	17	10	19	12
29	17	8	4	20	9	19	20	9	20	10
30	16	10	4	17	5	15	15	7	17	8

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Day No.	30	31	32	33	34	35	36	37	38	39
0%-7%/C Group										
Subgroup A	K	S*	S*	K	S*	K	K	S*	K	S*
subject 31	13	14	13	16	10	17	15	11	18	11
32	11	7	7	13	5	12	12	7	13	6
33	14	8	8	13	8	16	15	11	16	10
34	9	7	7	11	10	14	16	11	16	8
35	9	8	9	12	5	13	13	12	14	5
Subgroup B	S*	K	K	S*	K	S*	S*	K	S*	K
subject 36	11	13	17	6	19	8	8	18	6	17
37	14	11	15	7	15	7	11	18	9	13
38	12	13	14	5	14	12	6	16	10	16
39	7	11	14	9	15	5	6	14	10	17
40	12	9	14	10	13	8	13	15	12	14
3%-3% Group										
Subgroup A	K*	S	S	K*	S	K*	K*	S	K*	S
subject 41	11	17	16	10	18	11	14	18	13	21
42	9	13	14	11	18	10	11	16	10	19
43	10	15	17	11	15	11	13	16	12	17
44	12	18	18	10	17	10	11	15	12	18
45	9	19	17	10	17	10	11	17	10	17
Subgroup B	S	K*	K*	S	K*	S	S	K*	S	K*
subject 46	16	10	11	16	8	16	15	10	18	8
47	17	11	9	16	11	16	18	8	17	10
48	16	10	11	18	10	18	18	10	18	12
49	16	9	9	18	8	16	19	9	18	9
50	14	11	10	16	13	12	12	11	15	11

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Day No.	30	31	32	33	34	35	36	37	38	39
5%-5% Group										
Subgroup A	K*	S	S	K*	S	K*	K*	S	K*	S
subject 51	11	17	12	9	17	9	12	22	9	21
52	10	17	16	11	16	8	8	19	12	20
53	10	15	15	12	19	9	11	20	11	20
54	15	17	20	13	15	12	13	17	13	16
55	13	17	19	9	20	13	10	17	9	17
Subgroup B	S	K*	K*	S	K*	S	S	K*	S	K*
subject 56	12	10	10	15	8	17	16	9	15	10
57	20	8	11	19	12	20	19	12	22	11
58	17	8	10	18	8	18	17	7	20	13
59	15	8	9	14	9	18	19	12	17	8
60	14	8	9	20	10	19	18	10	17	10
7%-7% Group										
Subgroup A	K*	S	S	K*	S	K*	K*	S	K*	S
subject 61	9	16	16	8	17	9	9	15	10	17
62	9	15	17	7	17	9	9	18	8	20
63	11	16	18	8	15	10	11	20	10	20
64	9	18	13	8	15	9	8	15	10	18
65	12	16	19	8	17	10	9	19	11	16
Subgroup B	S	K*	K*	S	K*	S	S	K*	S	K*
subject 66	13	9	7	15	9	17	18	8	19	8
67	17	12	10	18	11	15	16	7	20	10
68	16	9	8	15	10	22	17	12	20	10
69	17	7	12	16	9	19	17	7	18	9
70	18	8	13	18	6	21	18	8	19	10

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Day No.	30	31	32	33	34	35	36	37	38	39
7%-7%/C Group										
Subgroup A	K	S*	S*	K	S*	K	K	S*	K	S*
subject 71	10	10	11	16	15	13	14	12	13	10
72	12	10	12	13	10	13	14	12	16	14
73	13	9	8	15	11	14	16	10	15	12
74	14	9	12	13	12	16	15	9	16	9
75	14	13	8	14	9	15	13	12	15	12
Subgroup B	S*	K	K	S*	K	S*	S*	K	S*	K
subject 76	9	11	16	12	15	7	11	16	8	17
77	11	12	12	11	17	13	15	17	14	19
78	12	15	15	10	17	10	14	17	11	17
79	14	14	15	12	18	8	12	19	12	17
80	10	11	14	10	13	12	16	13	10	12

APPENDIX I: Amounts, in ml, of Kool-Aid consumed by Experiment III subjects. (G) indicates grape-flavored Kool-Aid, and (O) indicates orange-flavored Kool-Aid.

	Pre-Conditioning										Post-Conditioning													
	pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref	
Day No.	8		9		40		41		42		43		44		45		46		47		48		49	
0%-3% Group																								
Subgroup A	G	O	O	G	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O
subject 1	9	4	3	13	2	11	9	6	2	14	5	8	2	15	13	5	3	17	15	3	16	3	3	17
2	4	10	9	7	2	11	8	4	2	10	2	13	3	12	10	2	3	12	12	3	3	12	10	5
3	8	6	12	3	6	5	7	6	1	11	12	1	2	16	12	1	2	14	12	3	7	9	2	22
4	13	3	8	6	9	3	13	3	3	12	12	2	15	3	14	2	14	2	16	2	3	14	13	4
5	12	4	2	16	9	5	3	15	6	12	1	15	5	11	5	10	12	4	2	19	5	13	3	15
Subgroup B																								
subject 6	5	6	3	12	2	10	3	9	3	9	11	2	6	7	4	10	4	9	9	9	7	8	3	14
7	5	13	9	6	7	8	13	5	4	14	14	2	3	16	17	2	2	15	15	2	6	12	13	5
8	4	12	3	12	11	2	15	2	14	2	13	2	9	8	2	12	12	3	6	12	16	3	10	9
9	11	3	11	4	9	4	12	2	2	12	12	3	4	11	12	3	9	6	13	4	14	3	8	10
10	10	2	2	10	1	10	11	2	2	11	11	2	2	10	12	2	3	10	12	2	5	9	10	3

	Pre-Conditioning										Post-Conditioning													
	pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref	
Day No.	8		9		40		41		42		43		44		45		46		47		48		49	
0%-5% Group																								
Subgroup A	G	O	O	G	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O
subject 11	3	11	4	11	5	8	15	2	1	14	16	3	2	11	15	2	12	5	13	3	12	5	13	4
12	7	9	2	14	7	5	5	9	4	10	10	7	4	12	2	17	2	13	2	13	5	13	2	16
13	2	12	12	2	2	8	13	1	2	11	6	6	1	12	12	5	2	13	15	2	2	14	15	2
14	9	6	7	8	9	2	2	11	14	1	8	9	10	5	5	11	12	3	8	7	15	2	2	15
15	11	2	12	3	2	11	12	1	6	9	11	2	3	9	13	2	3	10	13	2	6	8	11	6
Subgroup B																								
subject 16	10	4	2	14	7	5	2	11	11	3	1	14	13	3	2	13	11	5	2	15	13	6	2	15
17	9	5	5	7	4	8	6	7	9	3	8	5	8	5	7	6	12	2	7	8	16	1	11	7
18	3	12	11	2	2	9	4	7	1	10	3	8	2	12	4	11	2	14	7	7	2	14	2	14
19	8	4	6	9	7	2	5	6	10	3	11	2	10	3	11	4	10	4	14	1	11	2	2	14
20	5	9	4	9	3	9	4	8	3	9	2	10	6	8	4	10	2	12	10	2	7	6	8	6

	Pre-Conditioning										Post-Conditioning													
	pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref	
Day No.	8		9		40		41		42		43		44		45		46		47		48		49	
0%-7% Group																								
Subgroup A	G	O	O	G	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O
subject 21	12	2	11	2	8	2	12	4	13	2	10	3	13	2	9	5	4	11	10	4	13	1	8	6
22	1	14	1	13	1	16	9	7	6	8	4	12	1	14	13	1	2	12	9	6	1	12	3	12
23	7	5	2	12	9	3	11	7	14	1	6	7	15	1	9	8	13	1	9	10	16	2	1	17
24	2	9	3	8	1	9	5	6	1	12	7	6	1	12	5	9	1	12	12	1	3	10	1	13
25	3	9	12	3	3	10	16	1	12	4	13	2	5	9	13	1	9	5	16	2	13	3	14	4
Subgroup B																								
subject 26	11	1	1	10	1	8	3	7	5	7	2	10	7	7	1	14	6	10	1	13	9	6	1	15
27	9	3	1	13	11	1	11	4	17	1	9	8	16	1	6	9	15	1	1	18	16	1	1	16
28	10	2	1	12	2	10	8	6	8	3	10	3	11	2	8	6	13	1	1	13	12	3	9	6
29	9	4	9	3	5	7	13	1	9	5	13	1	4	11	15	1	6	10	15	1	9	7	11	6
30	6	7	9	1	1	11	14	2	3	13	12	3	6	7	13	1	1	13	13	2	4	10	9	5

	Pre-Conditioning										Post-Conditioning													
	pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref	
Day No.	8		9		40		41		42		43		44		45		46		47		48		49	
0%-7%/C Group																								
Subgroup A	G	0	0	G	0	G	G	0	0	G	G	0	0	G	G	0	0	G	G	0	0	G	G	0
subject 31	10	6	2	14	4	12	11	6	16	3	16	2	14	3	17	1	13	2	14	2	14	3	16	1
32	4	7	2	9	2	12	2	10	2	12	2	13	2	11	2	11	2	13	1	12	4	11	1	14
33	8	3	5	8	12	5	15	2	12	5	11	6	14	4	14	4	7	10	12	4	11	4	7	11
34	7	6	2	10	1	15	4	10	1	15	3	14	1	14	15	1	1	13	2	12	4	11	5	13
35	10	2	2	10	12	2	14	1	13	2	14	1	11	2	7	7	12	2	10	4	14	1	12	1
Subgroup B																								
subject 36	2	11	3	9	1	15	3	12	8	6	1	10	10	4	6	10	2	12	1	13	6	6	5	12
37	8	3	10	3	10	7	15	1	10	5	4	11	3	13	4	10	8	7	2	14	16	1	14	3
38	4	6	2	11	1	14	1	13	1	12	12	2	1	13	2	11	1	12	3	12	5	8	1	13
39	9	3	6	8	1	13	15	1	2	14	15	1	1	15	15	2	13	2	15	1	14	1	14	3
40	4	10	12	3	4	10	3	10	4	12	3	13	12	1	2	14	2	15	8	5	1	11	13	2

	Pre-Conditioning										Post-Conditioning													
	pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref	
Day No.	8		9		40		41		42		43		44		45		46		47		48		49	
3%-3% Group																								
Subgroup A	G	O	O	G	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O
subject 41	12	4	2	11	2	12	13	3	12	4	12	5	9	6	10	5	2	11	14	3	15	2	7	9
42	3	11	3	12	3	10	2	12	5	9	7	7	7	6	10	4	3	10	1	14	5	9	1	12
43	8	4	5	7	2	11	5	8	12	2	10	7	7	7	10	6	8	5	6	10	14	1	1	14
44	6	6	3	11	4	8	1	13	7	9	1	16	12	2	2	13	5	10	1	17	9	7	1	15
45	11	1	8	4	8	3	11	3	10	3	7	8	11	5	10	5	12	1	6	10	14	1	7	8
Subgroup B																								
subject 46	6	6	7	6	7	3	7	4	8	1	11	2	3	16	13	2	1	12	12	1	15	1	1	14
47	10	1	1	10	1	14	11	2	2	12	8	6	3	11	10	4	2	11	10	7	6	9	5	10
48	11	3	3	12	10	2	3	13	9	6	5	11	8	5	10	4	10	2	13	4	9	5	9	10
49	8	4	2	10	11	2	9	4	12	1	15	2	12	2	9	4	10	3	16	1	10	5	13	1
50	5	6	2	11	2	12	11	3	2	12	6	8	12	2	10	2	10	2	10	4	13	1	4	9

	Pre-Conditioning												Post-Conditioning											
	pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref	
Day No.	8		9		40		41		42		43		44		45		46		47		48		49	
5%-5% Group																								
Subgroup A	G	O	O	G	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O
subject 51	3	11	9	5	11	4	14	2	9	7	14	4	10	7	12	4	10	5	13	3	13	2	11	7
52	5	7	4	12	7	5	2	14	15	1	1	15	12	3	2	14	3	13	2	15	14	2	10	7
53	6	7	13	2	7	4	13	1	8	4	13	2	12	3	13	1	8	6	10	5	9	3	10	4
54	5	12	9	9	2	12	9	7	4	11	15	2	1	16	15	1	3	14	11	7	5	10	11	6
55	4	8	2	13	3	11	1	14	5	10	1	16	10	3	1	13	3	9	11	5	14	1	9	8
Subgroup B																								
subject 56	4	11	10	4	1	11	5	9	4	9	4	11	4	10	3	10	2	14	5	9	5	10	8	8
57	5	9	2	14	11	3	17	1	3	14	16	1	14	4	1	17	2	13	5	13	1	15	2	15
58	8	3	5	9	13	1	1	16	8	10	1	17	7	8	1	16	5	10	4	14	18	1	1	16
59	9	3	6	6	4	10	8	6	7	8	2	14	15	1	8	7	11	2	4	11	13	2	8	9
60	9	7	5	10	5	9	3	11	5	10	7	9	11	3	8	6	7	7	1	16	11	6	1	16

	Pre-Conditioning										Post-Conditioning													
	pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref	
Day No.	8		9		40		41		42		43		44		45		46		47		48		49	
7%-7% Group																								
Subgroup A	G	O	O	G	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O
subject 61	11	3	9	6	15	2	2	14	14	1	12	5	13	2	9	7	9	7	2	13	15	2	2	15
62	11	3	2	11	5	8	3	12	4	11	2	15	5	9	2	10	1	10	2	12	1	11	2	13
63	9	5	7	7	5	9	2	14	11	5	5	12	14	1	1	15	14	2	1	15	17	2	1	16
64	7	4	2	13	1	10	2	11	2	11	2	11	1	12	1	12	1	12	2	14	2	12	1	14
65	13	4	8	8	6	6	7	6	13	3	9	7	11	1	10	5	10	5	10	7	12	7	2	16
Subgroup B																								
subject 66	13	1	1	12	2	11	2	14	3	12	2	15	4	10	2	12	3	9	1	14	4	10	2	16
67	10	7	11	4	14	2	16	1	11	5	14	1	13	1	13	2	13	3	11	2	11	4	15	1
68	2	14	1	15	7	7	3	14	16	2	2	12	11	2	2	12	13	2	1	18	12	5	1	20
69	6	6	8	7	9	6	14	1	9	6	15	1	11	4	12	3	10	4	13	2	11	2	12	5
70	10	1	2	12	10	5	1	13	6	8	1	14	15	1	2	12	7	7	1	12	6	5	5	9

	Pre-Conditioning												Post-Conditioning											
	pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref		pref	
Day No.	8		9		40		41		42		43		44		45		46		47		48		49	
7%-7%/C Group																								
Subgroup A	G	O	O	G	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O	O	G	G	O
subject 71	3	9	12	3	13	3	5	10	9	7	5	13	11	7	6	9	6	8	1	10	9	8	1	16
72	3	10	3	11	1	15	5	10	4	13	2	15	2	14	5	10	2	13	1	13	9	8	1	18
73	14	1	10	4	3	15	15	2	14	4	14	4	11	5	13	2	12	2	13	3	16	1	15	2
74	12	1	2	15	1	15	6	11	3	14	4	11	7	10	3	12	5	10	3	13	8	7	2	12
75	12	1	4	12	3	15	18	1	10	5	13	3	8	6	14	3	13	2	14	2	14	2	8	10
Subgroup B																								
subject 76	2	10	2	12	1	16	9	9	12	8	9	8	5	13	9	8	2	13	12	3	9	7	2	16
77	12	1	1	14	1	15	15	2	1	17	15	1	1	17	15	1	1	15	10	6	1	16	16	1
78	6	6	5	7	1	17	8	9	3	14	8	9	4	13	12	4	1	16	9	7	10	8	17	1
79	12	4	15	2	14	5	18	1	16	1	17	1	16	1	1	13	12	2	13	1	1	13	8	9
80	8	5	8	8	2	12	5	9	2	12	5	10	3	12	11	2	6	5	10	2	13	1	9	8

APPENDIX J: Weights in g of Experiment III subjects during 20-day period.

	initial weight	20-day period								
Day No.		10	11	12	13	14	15	16	17	18	19
0%-3% Group											
Subgroup A											
subject 1	258	224	224	218	218	229	236	236	236	234	234
2	260	225	225	230	241	241	241	248	248	254	252
3	276	226	221	226	222	226	233	236	238	240	243
4	200	217	214	210	221	224	226	226	228	228	226
5	260	240	240	236	246	246	251	254	254	247	247
Subgroup B											
subject 6	234	215	214	216	226	230	228	230	232	230	233
7	244	209	208	212	215	216	216	217	216	223	218
8	264	226	228	222	234	235	237	235	237	240	237
9	244	216	216	214	222	226	226	226	227	231	228
10	250	227	233	230	236	236	239	237	238	243	239
0%-5% Group											
Subgroup A											
subject 11	254	226	229	230	241	242	245	246	248	254	252
12	261	222	226	218	230	234	236	232	228	234	234
13	264	220	224	218	224	227	229	226	226	228	226
14	252	236	239	236	239	242	242	240	243	248	244
15	252	224	223	218	226	226	228	226	226	229	228
Subgroup B											
subject 16	264	236	236	233	244	246	244	244	245	238	246
17	260	220	220	216	224	227	230	228	226	234	234
18	246	222	226	220	226	228	232	232	232	233	231
19	250	230	236	230	240	242	243	239	239	246	244
20	255	223	224	220	230	233	232	228	227	234	236

Day No.	20	21	22	23	24	25	26	27	28	29
0%-3% Group										
Subgroup A										
subject 1	235	234	234	236	236	240	239	232	231	231
2	249	248	244	246	246	246	244	242	244	242
3	246	248	246	246	249	251	248	244	247	248
4	228	228	228	229	230	232	232	228	229	229
5	251	253	253	254	254	259	258	256	256	255
Subgroup B										
subject 6	231	233	233	234	236	238	237	237	239	239
7	219	221	224	222	224	222	224	223	224	221
8	240	241	238	238	243	244	243	238	243	239
9	231	233	233	236	236	236	236	234	235	235
10	241	245	242	243	240	242	240	241	243	240
0%-5% Group										
Subgroup A										
subject 11	255	256	260	258	258	262	264	264	266	266
12	234	232	228	232	232	234	230	233	236	237
13	229	230	227	228	226	226	223	227	227	227
14	246	246	246	242	238	242	245	248	249	249
15	234	237	231	231	229	230	234	234	234	230
Subgroup B										
subject 16	246	248	251	250	249	251	250	254	255	252
17	236	236	231	233	234	240	240	238	238	236
18	234	234	228	230	230	234	236	236	237	240
19	245	245	245	246	246	248	249	250	251	249
20	236	236	234	236	236	236	237	236	235	237

	initial weight	20-day period									
Day No.		10	11	12	13	14	15	16	17	18	19
0%-7% Group											
Subgroup A											
subject 21	242	218	220	216	226	226	228	230	228	230	228
22	239	222	224	218	232	228	230	228	229	231	230
23	235	222	226	220	236	240	246	237	242	243	242
24	247	210	215	212	221	224	226	225	230	232	230
25	258	222	230	224	233	233	236	238	238	236	236
Subgroup B											
subject 26	252	216	219	224	232	232	232	234	238	240	240
27	233	213	214	208	221	225	226	223	228	233	229
28	245	223	225	222	230	232	236	237	234	236	236
29	254	231	234	230	244	247	246	246	246	254	252
30	260	223	226	224	232	232	238	238	238	241	243
0%-7%/C Group											
Subgroup A											
subject 31	243	240	240	232	240	240	242	245	246	250	246
32	261	222	218	214	226	226	228	225	226	232	230
33	237	210	210	207	216	223	226	224	227	226	227
34	256	232	236	232	244	248	252	250	250	252	254
35	240	210	207	204	214	216	218	216	218	222	221
Subgroup B											
subject 36	250	234	233	228	234	234	237	240	242	240	240
37	261	223	224	217	218	216	224	223	228	224	223
38	258	216	220	214	224	226	229	223	222	227	226
39	232	202	204	200	214	214	216	218	219	223	223
40	261	255	256	252	263	266	276	271	274	277	278

Day No.	20	21	22	23	24	25	26	27	28	29
0%-7% Group										
Subgroup A										
subject 21	229	228	231	233	232	230	227	228	230	232
22	230	226	224	223	226	225	224	226	226	221
23	249	244	244	248	251	254	251	250	254	251
24	210	215	212	221	224	226	225	230	232	230
25	238	241	240	245	246	246	245	246	251	250
Subgroup B										
subject 26	242	240	241	243	243	244	243	246	242	246
27	233	233	234	238	239	236	230	232	229	232
28	240	238	238	236	244	243	241	243	241	245
29	255	254	257	254	252	249	249	252	249	252
30	248	244	245	247	247	252	249	250	250	252
0%-7%/C Group										
Subgroup A										
subject 31	248	244	248	251	246	250	248	252	249	249
32	234	232	230	228	227	231	230	230	230	227
33	230	226	224	227	228	228	230	229	232	233
34	256	258	254	256	253	256	257	258	258	258
35	224	224	218	218	218	222	224	223	224	226
Subgroup B										
subject 36	242	244	238	242	239	242	241	241	242	239
37	224	225	226	226	227	227	227	226	230	231
38	221	222	222	226	222	218	221	221	220	221
39	224	227	225	227	227	226	229	230	230	228
40	279	278	276	276	278	283	277	282	286	285

	initial weight	20-day period								
Day No.		10	11	12	13	14	15	16	17	18	19
3%-3% Group											
Subgroup A											
subject 41	246	224	226	226	232	234	236	240	240	241	242
42	276	237	241	232	238	241	244	245	247	253	254
43	238	224	228	220	230	230	232	228	232	229	232
44	254	234	236	237	240	241	245	241	242	248	247
45	237	218	226	218	224	226	228	228	232	235	234
Subgroup B											
subject 46	233	214	224	215	227	226	228	226	232	228	226
47	246	218	225	222	224	228	226	228	232	228	226
48	244	218	221	216	226	228	230	229	225	232	230
49	268	228	226	226	238	236	234	236	241	243	241
50	238	216	218	213	224	226	228	226	229	231	234
5%-5% Group											
Subgroup A											
subject 51	235	216	214	208	212	219	220	220	220	225	228
52	253	228	226	222	227	233	232	238	236	238	238
53	244	232	228	224	229	232	226	224	230	235	237
54	234	218	214	208	210	210	214	214	216	219	219
55	254	227	228	227	233	234	234	239	241	239	237
Subgroup B											
subject 56	232	218	217	212	218	223	224	224	225	235	228
57	238	218	216	210	220	221	224	227	226	230	232
58	254	237	233	230	232	236	236	236	235	242	239
59	250	226	224	220	228	230	226	232	234	235	230
60	217	204	204	203	202	204	206	210	209	218	215

Day No.	20	21	22	23	24	25	26	27	28	29
3%-3% Group										
Subgroup A										
subject 41	244	245	242	242	244	246	246	245	245	246
42	254	251	247	252	253	256	258	254	254	257
43	234	231	228	228	229	232	228	230	229	227
44	250	244	243	244	246	249	251	245	248	245
45	232	232	230	232	229	232	235	240	237	234
Subgroup B										
subject 46	233	230	232	237	232	235	236	239	236	236
47	230	230	232	236	234	232	234	237	233	234
48	236	229	229	234	234	236	233	233	235	235
49	238	238	240	244	242	240	241	241	246	246
50	232	230	228	234	233	239	236	232	230	229
5%-5% Group										
Subgroup A										
subject 51	230	231	230	229	230	234	230	229	229	229
52	245	247	242	246	242	243	246	240	246	247
53	242	240	241	238	234	239	241	240	240	242
54	218	220	220	223	224	222	224	226	227	230
55	237	239	238	242	244	244	240	236	235	237
Subgroup B										
subject 56	226	225	223	225	226	229	228	228	229	231
57	234	236	236	242	234	239	238	238	236	237
58	244	242	243	247	244	246	242	244	248	248
59	230	234	238	236	234	237	236	238	235	237
60	218	216	214	222	220	220	220	222	227	225

	initial weight	20-day period								
Day No.		10	11	12	13	14	15	16	17	18	19
7%-7% Group											
Subgroup A											
subject 61	251	214	209	204	204	204	204	206	206	210	210
62	233	220	213	206	208	206	208	204	204	208	208
63	244	218	216	208	221	222	221	221	218	225	226
64	234	216	211	206	215	218	217	217	218	222	221
65	244	226	217	214	219	216	217	219	221	232	227
Subgroup B											
subject 66	258	230	225	220	228	229	224	226	227	232	234
67	245	222	216	212	217	216	214	213	216	224	226
68	268	238	233	227	226	229	228	226	228	230	236
69	266	233	226	220	218	223	228	226	229	234	236
70	240	228	221	218	218	222	225	224	224	230	237
7%-7%/C Group											
Subgroup A											
subject 71	244	230	223	218	218	224	220	221	221	230	228
72	243	225	216	210	212	214	212	210	212	215	218
73	240	219	216	210	208	208	210	208	208	210	214
74	251	226	220	215	213	214	214	208	212	212	216
75	286	244	236	232	236	240	240	241	242	244	246
Subgroup B											
subject 76	265	219	211	208	208	210	208	204	206	214	213
77	238	212	206	202	203	205	204	205	208	212	210
78	235	212	212	208	212	216	216	213	214	218	215
79	250	234	226	220	217	224	223	222	222	226	224
80	230	207	202	200	199	204	206	202	202	210	207

Day No.	20	21	22	23	24	25	26	27	28	29
7%-7% Group										
Subgroup A										
subject 61	210	212	208	209	214	214	213	210	211	210
62	210	214	215	216	220	222	224	223	224	220
63	228	228	226	229	230	229	231	233	232	231
64	224	227	225	229	230	230	232	230	227	226
65	227	232	230	230	231	232	232	232	232	234
Subgroup B										
subject 66	232	237	240	240	242	238	236	233	236	235
67	228	232	232	233	233	233	234	231	230	234
68	241	242	240	241	245	247	245	243	250	248
69	237	234	230	236	236	244	246	243	242	240
70	235	228	225	224	224	224	228	228	231	235
7%-7%/C Group										
Subgroup A										
subject 71	236	236	231	235	236	240	240	238	236	242
72	220	219	217	218	221	223	224	222	223	224
73	214	216	212	215	215	217	218	218	218	217
74	215	214	209	210	210	208	214	211	216	214
75	249	253	251	255	254	258	264	261	263	263
Subgroup B										
subject 76	216	218	220	226	224	224	228	226	220	217
77	210	213	208	212	214	219	220	218	216	215
78	213	216	216	218	218	218	216	216	217	219
79	226	226	227	228	228	228	228	226	228	226
80	210	210	214	218	219	221	221	220	223	222

APPENDIX K: Weights in g of Experiment III subjects during the conditioning period.

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Day No.	30	31	32	33	34	35	36	37	38	39
0%-3% Group										
Subgroup A										
subject 1	232	231	233	234	232	236	230	230	230	228
2	244	244	248	250	248	249	247	245	245	240
3	252	248	247	251	251	251	245	244	243	242
4	231	228	227	231	229	233	226	224	225	222
5	258	252	258	261	258	261	254	256	251	250
Subgroup B										
subject 6	238	237	236	238	244	240	235	234	228	231
7	223	224	222	222	224	223	222	224	219	226
8	237	236	237	237	243	240	237	238	234	237
9	235	236	233	230	235	233	232	232	227	229
10	241	243	240	239	242	237	238	238	233	238
0%-5% Group										
Subgroup A										
subject 11	265	259	261	264	256	256	250	247	249	243
12	240	231	232	240	232	238	234	228	229	228
13	227	221	227	233	228	234	225	222	228	233
14	249	244	251	256	250	254	246	240	246	242
15	230	237	229	232	226	227	221	219	221	216
Subgroup B										
subject 16	254	253	250	248	254	252	248	251	245	250
17	237	238	234	232	239	237	235	234	228	231
18	240	232	230	230	237	235	232	233	226	229
19	251	250	247	244	248	245	244	244	238	240
20	237	234	230	232	244	233	230	233	230	234

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Day No.	30	31	32	33	34	35	36	37	38	39
0%-7% Group										
Subgroup A										
subject 21	234	230	235	236	226	230	224	220	226	223
22	222	218	224	226	220	224	218	215	223	220
23	255	247	251	255	246	250	240	236	242	237
24	233	228	230	235	226	230	217	216	220	214
25	251	244	247	254	246	252	238	235	243	239
Subgroup B										
subject 26	245	248	241	236	241	234	236	238	230	232
27	233	230	226	224	229	225	222	228	220	224
28	244	242	236	234	239	234	233	237	232	236
29	253	250	245	238	250	247	247	251	245	250
30	255	251	247	242	246	240	238	243	236	240
0%-7%/C Group										
Subgroup A										
subject 31	250	247	247	246	247	242	240	242	242	243
32	229	227	224	221	223	220	220	219	217	220
33	236	232	227	224	225	224	224	224	221	224
34	259	252	249	246	246	244	245	251	246	248
35	226	222	218	219	222	214	213	216	216	217
Subgroup B										
subject 36	245	239	239	243	234	241	231	229	233	232
37	235	242	221	226	222	224	214	218	220	219
38	222	219	219	223	216	218	215	209	213	214
39	228	222	224	224	220	224	213	210	211	209
40	286	282	277	281	276	277	269	270	269	268

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Day No.	30	31	32	33	34	35	36	37	38	39
3%-3% Group										
Subgroup A										
subject 41	253	245	246	248	244	250	240	238	242	239
42	258	250	251	254	250	255	247	245	249	245
43	228	224	225	232	227	228	222	222	224	223
44	246	245	244	248	245	249	242	239	240	243
45	234	232	236	241	235	237	230	228	228	226
Subgroup B										
subject 46	236	236	232	232	234	232	230	233	232	232
47	232	232	231	228	229	230	230	234	227	229
48	236	232	229	229	232	229	226	230	226	229
49	245	242	238	237	240	236	235	240	233	236
50	234	233	228	228	230	231	225	228	224	228
5%-5% Group										
Subgroup A										
subject 51	233	230	231	230	227	232	224	226	233	229
52	247	246	245	246	244	248	239	236	240	238
53	242	240	245	245	240	244	234	238	243	240
54	230	225	229	234	228	229	223	226	223	221
55	238	234	237	240	235	242	234	232	234	230
Subgroup B										
subject 56	229	227	226	227	229	226	225	229	224	226
57	237	239	233	233	237	234	235	239	234	237
58	251	252	247	247	252	248	250	252	247	252
59	240	238	235	232	235	234	235	240	239	237
60	222	222	222	219	225	224	225	225	223	223

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
Day No.	30	31	32	33	34	35	36	37	38	39
7%-7% Group										
Subgroup A										
subject 61	211	207	212	217	212	216	209	207	210	207
62	221	219	224	228	224	226	222	220	226	220
63	232	233	236	242	235	237	231	229	240	232
64	229	226	232	232	228	230	226	224	224	223
65	233	230	237	243	240	238	236	229	235	229
Subgroup B										
subject 66	234	236	236	236	238	236	237	244	240	244
67	234	236	235	235	241	237	234	236	230	238
68	247	246	244	240	246	242	245	248	245	250
69	245	247	242	244	246	244	247	248	242	246
70	236	240	238	240	244	237	241	242	238	240
7%-7%/C Group										
Subgroup A										
subject 71	244	243	238	240	246	246	242	242	238	238
72	224	225	225	226	226	223	223	224	226	224
73	218	218	217	216	218	218	216	218	214	217
74	211	212	213	215	218	217	216	221	216	220
75	264	264	263	261	260	259	257	260	259	260
Subgroup B										
subject 76	215	214	216	221	220	222	214	218	218	214
77	214	215	218	216	214	218	214	221	218	221
78	218	218	220	224	220	224	217	219	220	217
79	229	230	231	236	233	238	230	230	236	233
80	226	223	222	224	224	227	220	223	222	219

APPENDIX L: Weights in g for Experiment III subjects during pre- and post-conditioning sessions.

	Pre-Conditioning		Post-Conditioning		
Day No.	8	9	40	41	49
0%-3% Group					
Subgroup A					
subject 1	217	220	232	230	235
2	221	224	244	241	245
3	217	222	248	243	248
4	210	214	229	227	238
5	231	235	254	254	263
Subgroup B					
subject 6	210	212	229	230	235
7	207	206	231	222	230
8	218	224	234	234	240
9	210	214	232	228	232
10	226	226	236	234	226
0%-5% Group					
Subgroup A					
subject 11	222	224	249	250	252
12	216	218	235	231	242
13	218	217	226	221	224
14	228	230	249	244	247
15	210	215	220	216	226
Subgroup B					
subject 16	232	233	245	246	259
17	218	218	230	230	235
18	218	220	230	230	232
19	226	229	240	241	244
20	219	222	234	230	231

	Pre-Conditioning		Post-Conditioning		
Day No.	8	9	40	41	49
0%-7%					
Subgroup A					
subject 21	212	215	229	224	229
22	213	218	229	224	226
23	213	218	241	235	244
24	208	210	222	217	221
25	222	222	246	243	249
Subgroup B					
subject 26	213	214	231	228	237
27	208	210	221	219	237
28	217	222	235	233	241
29	228	229	247	244	255
30	220	222	238	236	249
0%-7%/C Group					
Subgroup A					
subject 31	236	240	238	240	250
32	217	220	214	219	227
33	207	208	221	224	229
34	229	229	245	247	258
35	200	203	210	214	220
Subgroup B					
subject 36	228	230	234	234	236
37	217	218	222	222	230
38	210	211	217	218	224
39	198	202	216	216	232
40	250	254	272	270	275

	Pre-Conditioning		Post-Conditioning		
Day No.	8	9	40	41	49
3%-3% Group					
Subgroup A					
subject 41	224	225	245	241	249
42	233	235	255	254	259
43	222	222	227	225	230
44	234	235	248	246	254
45	222	222	232	231	243
Subgroup B					
subject 46	216	213	230	233	236
47	219	218	225	228	235
48	215	218	229	228	230
49	230	230	237	235	246
50	212	215	229	226	236
5%-5% Group					
Subgroup A					
subject 51	207	213	235	236	237
52	220	223	245	241	250
53	224	226	248	246	251
54	214	220	222	220	224
55	220	224	236	235	240
Subgroup B					
subject 56	212	217	224	224	232
57	212	210	235	234	245
58	233	234	250	250	260
59	224	226	237	236	242
60	196	200	219	222	228

	Pre-Conditioning		Post-Conditioning		
Day No.	8	9	40	41	49
7%-7% Group					
Subgroup A					
subject 61	208	212	213	214	220
62	216	218	227	227	229
63	214	216	236	240	246
64	213	212	229	226	236
65	219	222	234	232	240
Subgroup B					
subject 66	226	230	242	243	254
67	214	217	237	239	244
68	232	236	247	246	253
69	225	230	246	246	250
70	224	226	244	238	243
7%-7%/C Group					
Subgroup A					
subject 71	220	224	238	242	247
72	216	220	226	228	230
73	213	216	218	219	225
74	220	224	217	219	229
75	238	241	260	263	270
Subgroup B					
subject 76	218	218	220	220	228
77	205	208	222	220	228
78	210	212	222	222	230
79	230	231	237	240	249
80	200	202	222	222	227

APPENDIX M: Data for blood-alcohol animals which were subjected to the same procedures as Experiment III subjects.

	Kool-Aid+alcohol (ml)	Weight (g)	Blood alcohol (g/100 ml plasma)					
			5 min	25 min	65 min	95 min	125 min	185 min
0%-3% Group								
subject 4	8	228	44.5	41.2	23.4	7.8	1.1	0.0
7	10.5	216	32.3	45.6	54.5	34.5	45.6	5.6
13	11	230	26.7	45.6	50.0	46.7	31.2	2.2
19	10.5	232	31.2	27.8	34.5	21.1	8.9	0.0
25	11	232	56.8	69.0	63.4	56.8	32.3	0.0
3%-3% Group								
subject 1	7	235	65.5	59.0	11.1	6.7	1.1	0.0
10	11	220	53.4	57.9	57.9	46.7	33.4	5.6
16	12	228	34.5	39.0	37.8	36.7	34.5	22.3
22	12.5	223	56.8	83.5	64.6	40.1	36.7	7.8
28	10	214	61.2	52.3	51.2	44.5	52.3	44.5

	Kool-Aid+alcohol (ml)	Weight (g)	Blood alcohol (g/100 ml plasma)					
			5 min	25 min	65 min	95 min	125 min	185 min
0%-5% Group								
subject 5	7	219	47.9	53.4	34.5	25.6	12.2	10.0
8	9	223	66.8	102.4	115.8	105.7	83.5	50.1
14	10.5	237	42.3	75.7	85.7	75.7	65.7	39.0
20	5	223	50.1	66.8	44.5	33.4	22.3	10.0
26	10	227	25.6	109.1	136.9	135.8	112.4	96.8
5%-5% Group								
subject 2	9	228	35.6	72.3	66.8	70.1	54.5	23.4
11	10.5	237	43.4	77.9	121.3	134.7	108.0	97.9
17	10	217	53.4	82.4	94.6	94.6	91.3	72.3
23	9	210	26.7	69.0	39.0	59.0	53.4	31.2
29	9	241	16.7	44.5	24.5	24.5	11.1	2.2

	Kool-Aid+alcohol (ml)	Weight (g)	Blood alcohol (g/100 plasma) 5 min	25 min	65 min	95 min	125 min	185 min
0%-7% Group								
subject 6	7	248	44.5	74.6	75.7	51.2	28.9	0.0
9	10	221	72.3	97.9	111.3	86.8	75.7	55.6
15	8	221	77.9	100.2	142.5	111.3	100.2	65.7
21	10	234	61.2	148.0	194.8	188.1	187.0	153.6
27	8	256	76.8	66.8	61.2	59.0	51.2	24.5
7%-7% Group								
subject 3	8	208	57.9	51.2	33.4	44.5	23.4	24.5
12	9	232	22.3	43.4	60.1	66.8	66.8	53.4
18	9	202	79.0	177.0	184.8	213.7	189.2	174.7
24	9	208	136.9	122.4	145.8	161.4	165.8	148.0
30	9	220	16.7	51.2	111.3	178.1	179.2	123.5