ETHANOL SELF-ADMINISTRATION CAN INHIBIT DRINKING ELICITED BY EATING IN THE RAT

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Abstract

Ethanol affects meal-related drinking behavior of both humans and rats. The present two-part study investigated the water ingestion of food-deprived Sprague-Dawley rats during a period of unlimited food and water access. Prior to the start of each trial, within-treatment subsets of the rats orally self-administered alcohol-sucrose solutions to completion, containing 0%, 3%, or 6% ethanol composed in a 10% (w/v) sucrose solution. Following ethanol self-administration, the water consumption, latency to drink, and food consumption for each previously weighed rat were recorded. For the first series of trials, rats administered [1 mL ethanol/100 g body-weight] ethanol solutions and the 3% and 6% ethanol subsets each drank significantly less water than the 0% ethanol subset. The ethanol treatments also showed a significantly lower water-to-food ratio (mL water/g eaten) than the 0% ethanol treatment. The 3% ethanol subset significantly consumed more food than both the 0% and 6% ethanol treatments. This finding was further supported by significance being determined regarding the food-to-body weight ratio (g eaten/body weight). For the second series of trials, rats self-administered double the ethanol solution volumes [2 mL ethanol/100 g body-weight] and no statistically significant differences were determined. These findings suggest that the observed ethanol-induced decrease in meal-related water consumption may be selectively applicable to lower volumes of self-administered ethanol.

Introduction

Humans and rats drink water in direct proportion to the amount of food eaten during a meal. In fact at least 70% of drinking in the rat has been linked to food intake with the amount of total water corresponding to the size of the meal eaten **(1)**. In humans, spontaneous fluid intake has been associated with both meal timing and the amount of food consumed **(2,3)**. Food-associated drinking has been described in the framework of homeostatic maintenance **(4,5)** to address decreases in the fluid compartments of cellular and extracellular spaces **(3)**. Cellular dehydration resulting in increased vascular osmolality has been additionally described in terms of meal-related water consumption **(4)**. Water balance maintenance has been defined for the most part by antidiuretic hormone release juxtaposed with controlled thirst mechanisms that direct the compensation of water loss through drinking **(6)**.

The role of ethanol is of interest in the context of meal-related drinking because it has been implicated in directly affecting water intake. For instance, drinking elicited by subcutaneous injection of hypertonic saline in the rat, has been shown to be inhibited by the administration of ethanol **(7)**. It was determined that the inhibitory drinking effect found in this study was dose-dependent with greater concentrations of ethanol proportionally resulting in further decreases in water intake **(7)**. Cellular dehydration resulting from saline infusions in humans has additionally been determined to yield decreased thirst and drinking of water in response to ethanol treatments **(8)**. The finding

that the ingestion of ethanol significantly decreases fluid intake promptly following saline infusion has been suggested to be the result of ethanol inhibition of thirst **(8)**. Both of these studies incorporate the saline infusion paradigm in order to mimic the cellular dehydration associated with the ingestion of a meal.

The current experiment investigated the effect of self-administration of ethanol on meal-related drinking in the rat. The predetermined ethanol solutions of 0%, 3%, or 6% ethanol were self-administered by the rats, prior to the free food-pellet and water exposure **(1)** of each trial. Experimentation was divided into two sets of trials. The first series examined the effects of ethanol on meal-related drinking using [1 mL ethanol/100 g body-weight] ethanol solutions while the second series considered ethanol influence using double volume [2 mL ethanol/100 g body-weight] ethanol solutions. It was hypothesized that the self-administration of ethanol would decrease total food-related drinking during each trial duration, with a prospective effect on drinking latency. The role of ethanol was further believed to express a dose-dependent relationship between the level of inhibition of water drinking and the concentration of ethanol ingested.

Methods

Animals

Twelve adult male Sprague-Dawley rats weighing 276-327 g at the beginning of experimentation were individually housed in standard stainless-steel cages with wire-mesh floors on a 12:12-hr light-dark cycle. The cages were contained within a temperature controlled room (21-23 °C) and the rats had continuous access to pelleted chow (Purina) on the floor of the cage and to fresh tap water available through a stainless-steel spout attached to a graduated glass bottle. Experimental protocols were approved by the institutional animal care and use committee.

Solutions

Alcohol solutions were prepared corresponding to 0%, 3%, and 6% ethanol concentrations. Each solution contained 100 g of reagent-grade sucrose with 0 mL, 30 mL, or 60 mL of 100% ethanol, respectively. The prepared solutions were each brought up to a 1000 mL volume using distilled water and the sucrose was fully dissolved prior to exposure to the rats. Sucrose sweetening of the ethanol solutions was included due to findings indicating sucrose facilitation of ethanol self-administration in the rat **(9)**.

Pretest Preparation

From the date of their arrival, the rats were individually housed in their respective mesh cages with access to both pellet-food and water. Therefore, the animals had the ability to become habituated to their surroundings prior to any testing **(7)**. In order to maximize ethanol self-administration, rats were each exposed to 2%, 4%, and 6% ethanol solutions at two separate intervals to ensure their willingness to ingest the different ethanol concentrations. The pre-loading was carried out over a six-day period in which each rat had two consecutive episodes of exposure to each ethanol concentration. During ethanol pre-loading rats had exposure to pellet-food and water. Throughout the habituation and the experimentation, graduated tubes for both water and ethanol were specifically positioned on the left and right front of each cage, respectively. This was

done to ensure that no extraneous fluid consumption occured due to the rats being conditioned to expect one fluid or the other at a specific location. Rats were habituated to 24-h food deprivation during the ethanol pre-loading exposures.

Procedure

At the completion of the ethanol solution pre-loading, the first experimental series was initiated for the 0%, 3%, and 6% ethanol concentrations. Rats were assigned numbers (#1-12) and these designations remained for the duration of all experimentation. The standard experimental progression consisted of pellet removal and refilling of available tap water at 24-h prior to testing. At 30 minutes remaining in the 24-h food deprivation, the food-deprived body weights were obtained for each rat. When 20 minutes remained in the 24-h deprivation the designated ethanol solutions were made available based on (1 mL/100 g body weight) for each individual rat. The ethanol bottle was lightly shaken without spillage to attract each rat and ensure that the animals maintained interest through completed self-administration. Once the ethanol was completely consumed, a sheet of paper was placed under each cage in order to catch falling food for later meal-weight analysis. A total of 5 weighed pellets were introduced into each cage and the one-hour trial was begun. Latencies to drink were monitored by a stopwatch and water readings were taken at 5-minute intervals to 15 minutes, and then at the 30-, 45-, and 60-minute marks. At the conclusion of the hour duration, food and food spillage was recorded for each rat to determine the relative proportions of the meals consumed. At the conclusion of a trial, food was replaced and water was refilled for a minimum 2-day recovery period from the food deprivation.

Ethanol exposure was carried out using six rats for each experimental treatment to allow for counter-balancing of the order of the experiment. The 0% trial was carried out with rats 1-6 and 7-12 all receiving 0% ethanol for the first trial. For the next trial rats 1-6 received 6% ethanol while rats 7-12 received 3% ethanol. The following trial tested rats 1-6 at 3% ethanol and rats 7-12 at 6% ethanol. The second experimental series followed the same procedure and ethanol distribution as described for the first experiment except for using twice the volume of administered ethanol. This experiment was further defined by its bracketing of the 3%-6% and 6%-3% ethanol trials with 0% ethanol trials. Similar to the first experiment, rats 1-6 and 7-12 were initially exposed to a 0% ethanol trial. In the tests rats 1-6 received 3% ethanol while rats 7-12 received 6% ethanol. The following trial consisted of rats 1-6 receiving 6% ethanol while rats 7-12 received 3% ethanol. The ethanol self-administration defined in the current paradigm was not likely to support an alcohol-dependence **(9)**. The second 0% ethanol bracket following the aforementioned trial allowed for a comparison of the two 0% ethanol trials in order to identify any habituation or other effects that could have occurred as a result of the exposure to the ethanol concentrations.

Data Analysis

Total 60-minute water intakes, food intakes, drinking latency, and 24-h food-deprived body weight measurements were analyzed for within-treatment comparisons using parametric analysis of variance (ANOVA). Corresponding ratios of water-to-food intake (W:F), food-to-body weight (F:BW), and water-to-body weight (W:BW) were similarly analyzed. Significant F-tests were determined for the first experimental series

and were consequentially followed with paired samples t-tests. The α-level was chosen at 0.05.

Results

Experiment 1

The 3% ethanol treatment was determined to selectively show increased food intake compared to the other ethanol treatments ($F(2,22)=10.479$, $p=.001$). The 3% ethanol treatment compared to both the 0% ethanol and 6% ethanol treatments showed increased eating ($t(11)=-3.945$, $p=.002$) and ($t(11)=3.801$, $p=.003$), respectively (see Fig. 1). The increase in food intake was further defined by the food:body weight ratio $F(2,22)=5.897$, $p=.009$) and there was no significant difference in food intake between the 0% and 6% ethanol treatments ($t(11)=-0.933$, $p=.371$).

Throughout the duration of the first experimental series of trials for the varying ethanol concentrations, no significance was determined regarding latency to drink water or body weight ($F(2,22)=2.956$, $p=.073$) and ($F(2,22)=1.897$, $p=.174$), respectively. The latency to drink for the 0% ($\mu=10.48$, $SE=2.79$), 3% ($\mu=10.50$, $SE=4.14$), and 6% ($\mu=12.37$, $SE=2.29$) ethanol treatments and the rat body weights were the only variables that failed to exhibit any level of change during the course of the first set of tests.

Water consumption was initially determined to express a significant decrease ($F(2,22)=6.671$, $p=.005$). Self-administration of 3% and 6% ethanol solutions prior to meal-related drinking each resulted in significant decreases in water consumption compared to the 0% ethanol control ($t(11)=3.362$, $p=.006$) and ($t(11)=3.619$, $p=.004$), respectively (see Fig. 2). There was no statistical difference for drinking observed between the 3% and 6% ethanol treatments ($t(11)=1.407$, $p=.187$).

In support of this decrease in drinking, the water:food ratio exhibited a significant decrease for the ethanol treatments compared to control ($F(2,22)=14.387$, $p=.000$). The 3% ethanol series and 6% ethanol series each compared to the 0% ethanol treatment showed significant drinking decreases ($t(11)=6.629$, $p=.000$) and ($t(11)=4.949$, $p=.000$), respectively (see Fig. 3). Significance was determined for water:body weight ($F(2,22)=5.690$, $p=.010$) and no statistical difference was observed for the water:food ratio between the 3% and 6% ethanol treatments ($t(11)=0.277$, $p=.787$).

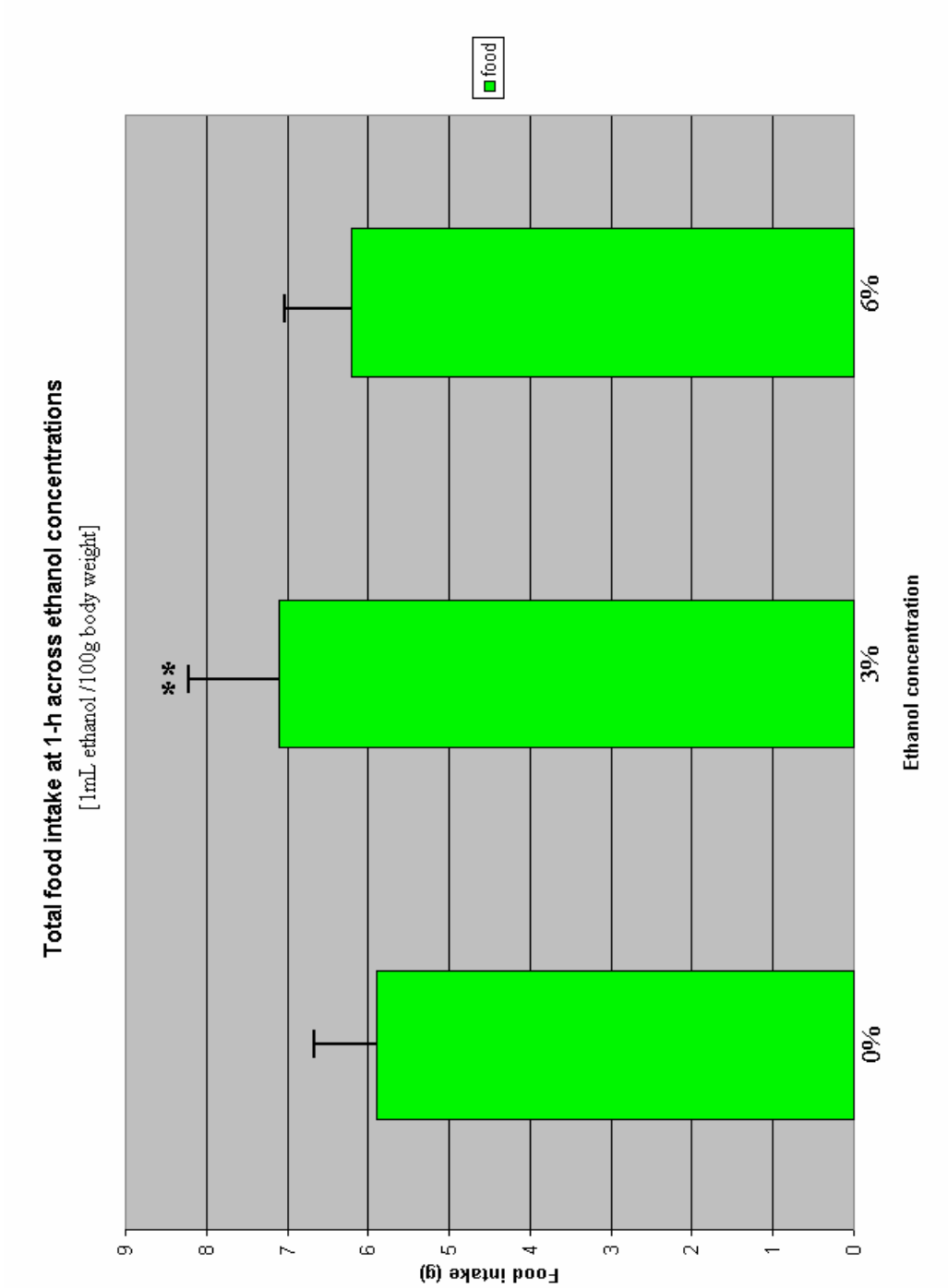

Figure 1.

Figure 1: The mean (±SE) total food intake at 1-h across experimental 0%, 3%, and 6% ethanol treatments. Rats ate significantly more food in the 3% ethanol treatment compared to both the 0% and 6% ethanol trials. ** represents t-test significance below p=.01 between 3% ethanol treatment and other measures.

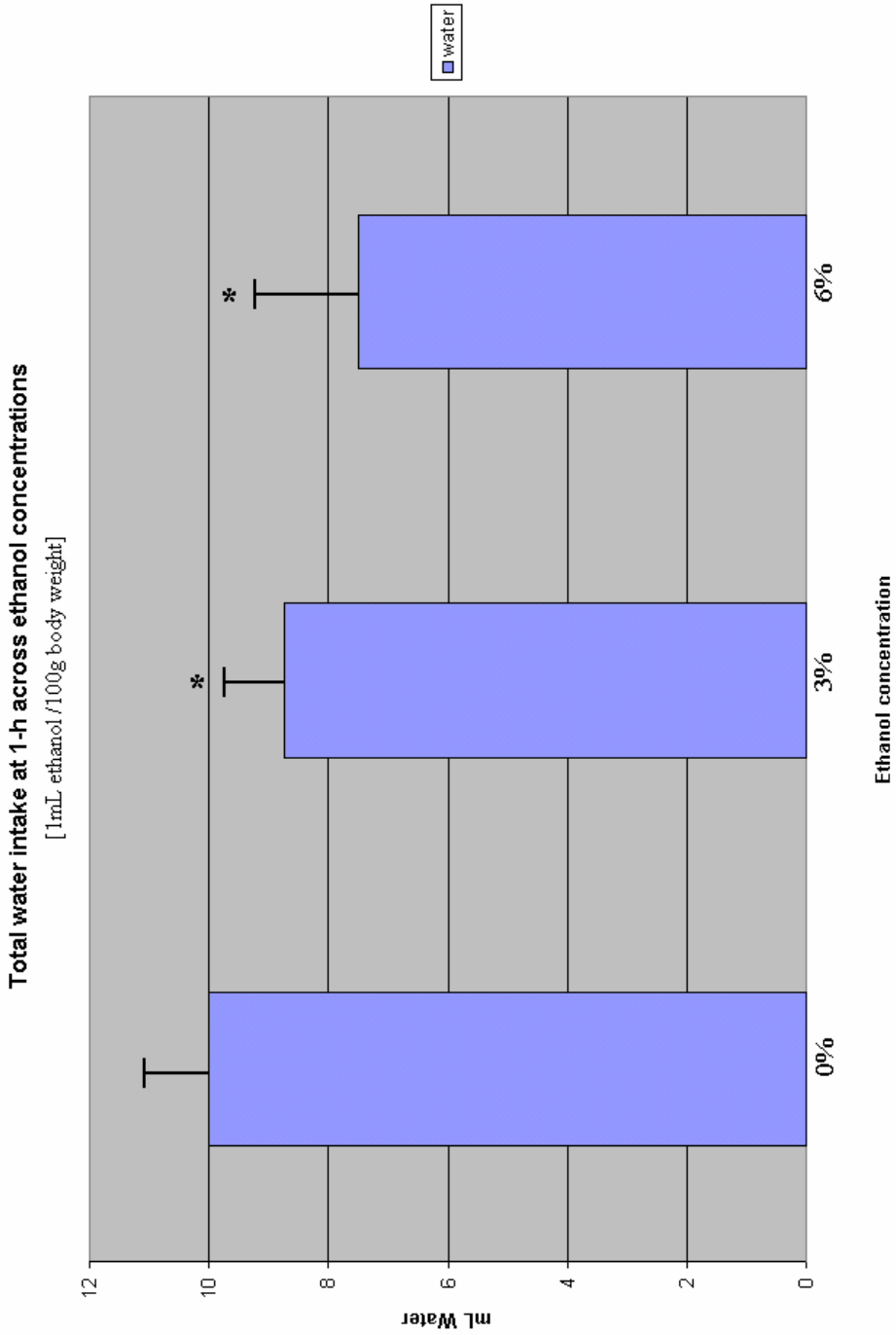

Figure 2.

Figure 2: The mean (±SE) total water intake at 1-h across experimental 0%, 3%, and 6% ethanol treatments. Ethanol treatments display significant drinking decrease compared to control yet yielded no difference between ethanol concentrations. * represents t-test significance below p=.05 between control and ethanol measures.

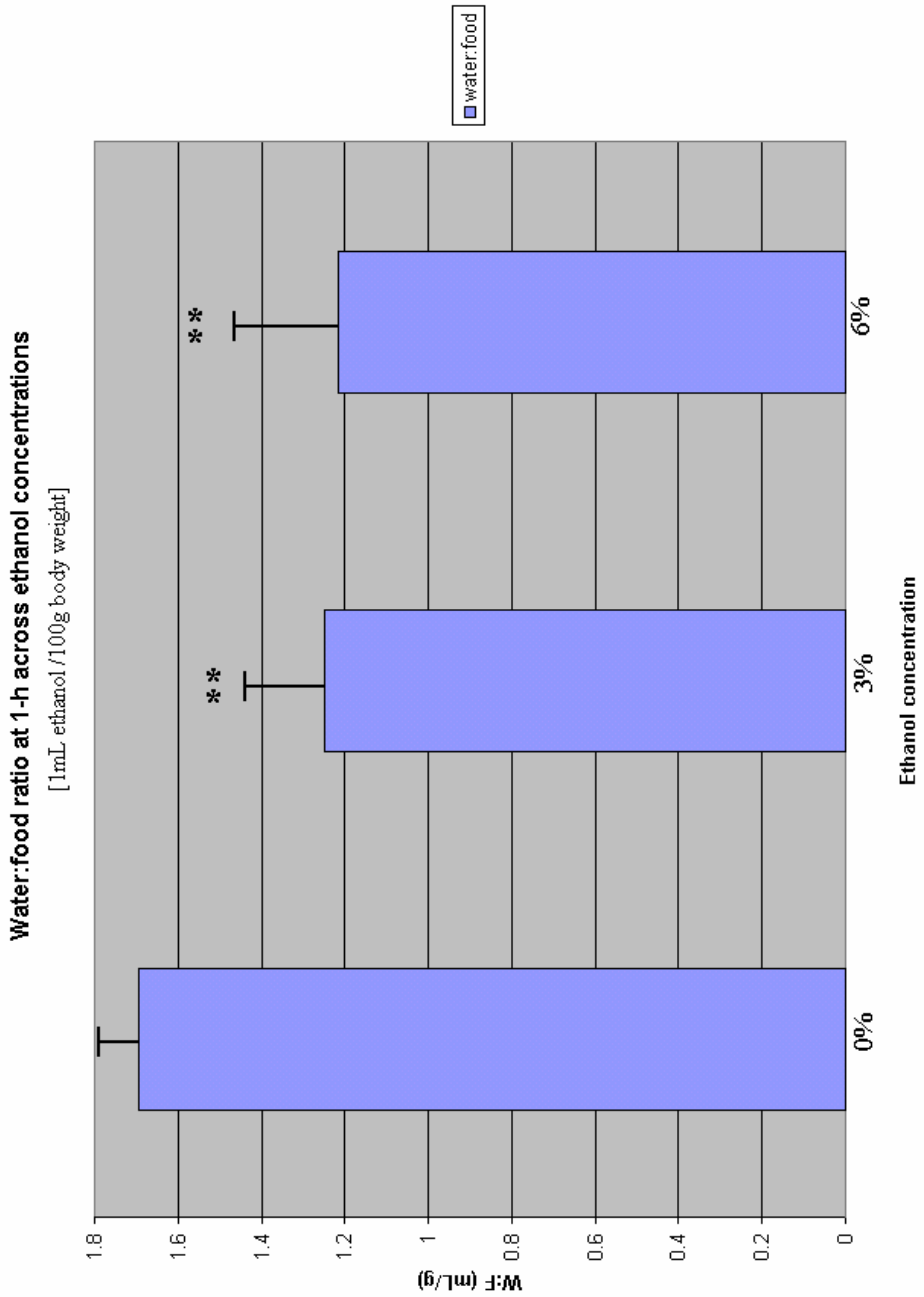

Figure 3.

Figure 3: The mean (±SE) water:food ratio at 1-h across experimental 0%, 3%, and 6% ethanol treatments. Significantly less water was consumed per gram of food eaten in the ethanol treatments compared to control yet no difference was determined between ethanol concentrations. ** represents t-test significance below p=.01 between control and ethanol measures.

Experiment 2

In comparing the two 0% ethanol treatments from the second experimental series, significance was exclusively determined for body weight ($t(11)=3.303$, $p=.007$). This significant difference was established as a result of body weight assessment spanning the extent of the entire second experiment. All remaining measures for the two 0% ethanol treatments lacked significance (data not shown). Since the two 0% ethanol treatments only differed in body weight values, their subsequent dependent measures were averaged for comparison to the 3% and 6% ethanol treatments.

Food intake analysis resulted in no significance across the experimental treatments ($F(2,22)=0.007$, $p=.993$) (see Fig. 4). Analysis of latency to drink for 0% ($\mu=17.55$, $SE=3.79$), 3% ($\mu=18.48$, $SE=5.55$), and 6% ($\mu=20.97$, $SE=5.95$) ethanol yielded an absence of significance ($F(2,22)=1.521$, $p=.241$). Water intake yielded no significant difference across treatments ($F(2,22)=2.239$, $p=.130$) (see Fig. 5) and analysis of the water:food ratio similarly yielded no significant changes across the ethanol concentrations and control ($F(2,22)=2.284$, $p=.126$) (see Fig. 6). Non-significant differences across the varied 0%, 3%, and 6% ethanol concentrations for the second experiment were additionally determined for body weight, food:body weight ratio, and water:body weight ratio ($F(2,22)=0.802$, $p=.461$), ($F(2,22)=0.010$, $p=.990$), ($F(2,22)=2.222$, $p=.132$), respectively.

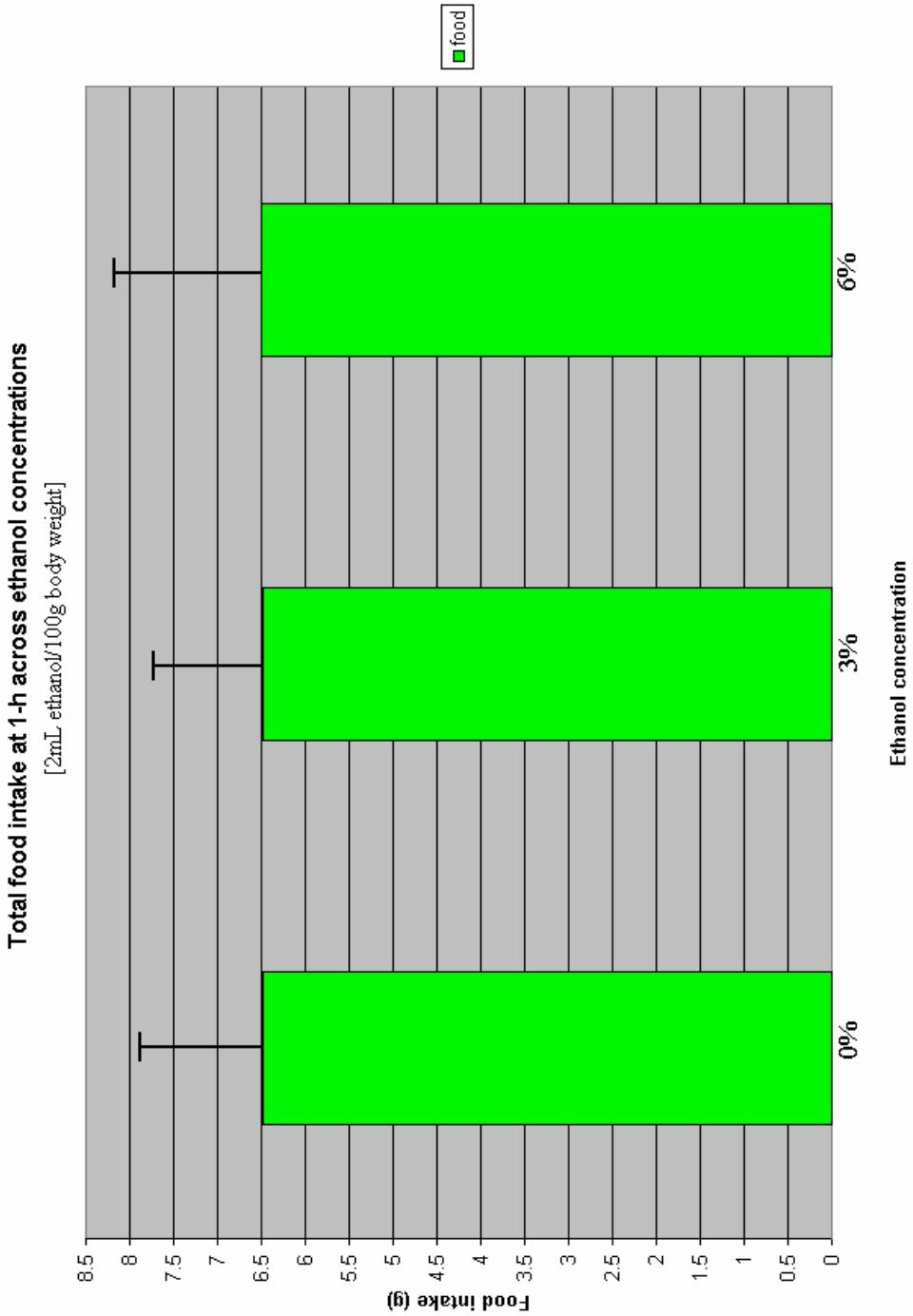

Figure 4.

Figure 4: The mean (±SE) total food intake at 1-h across experimental 0%, 3%, and 6% ethanol treatments under double volume solution administration yielded lack of significant difference across treatments. Note 0% ethanol treatment represents mean score from both experiment 2 trials.

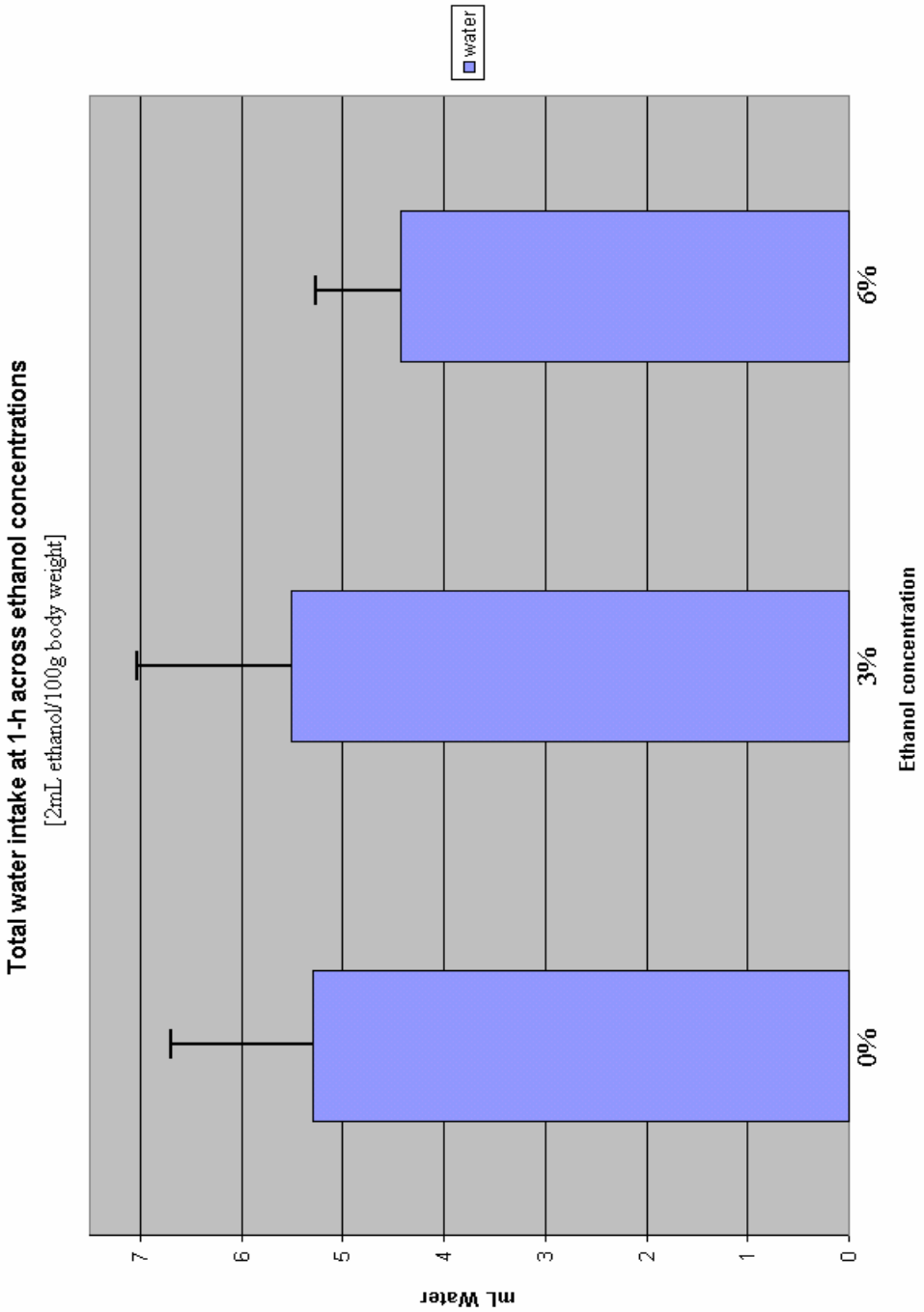

Figure 5.

Figure 5: The mean (±SE) total water intake at 1-h across experimental 0%, 3%, and 6% ethanol treatments under double volume solution administration yielded lack of significant difference across treatments. Note 0% ethanol treatment represents mean score from both experiment 2 trials.

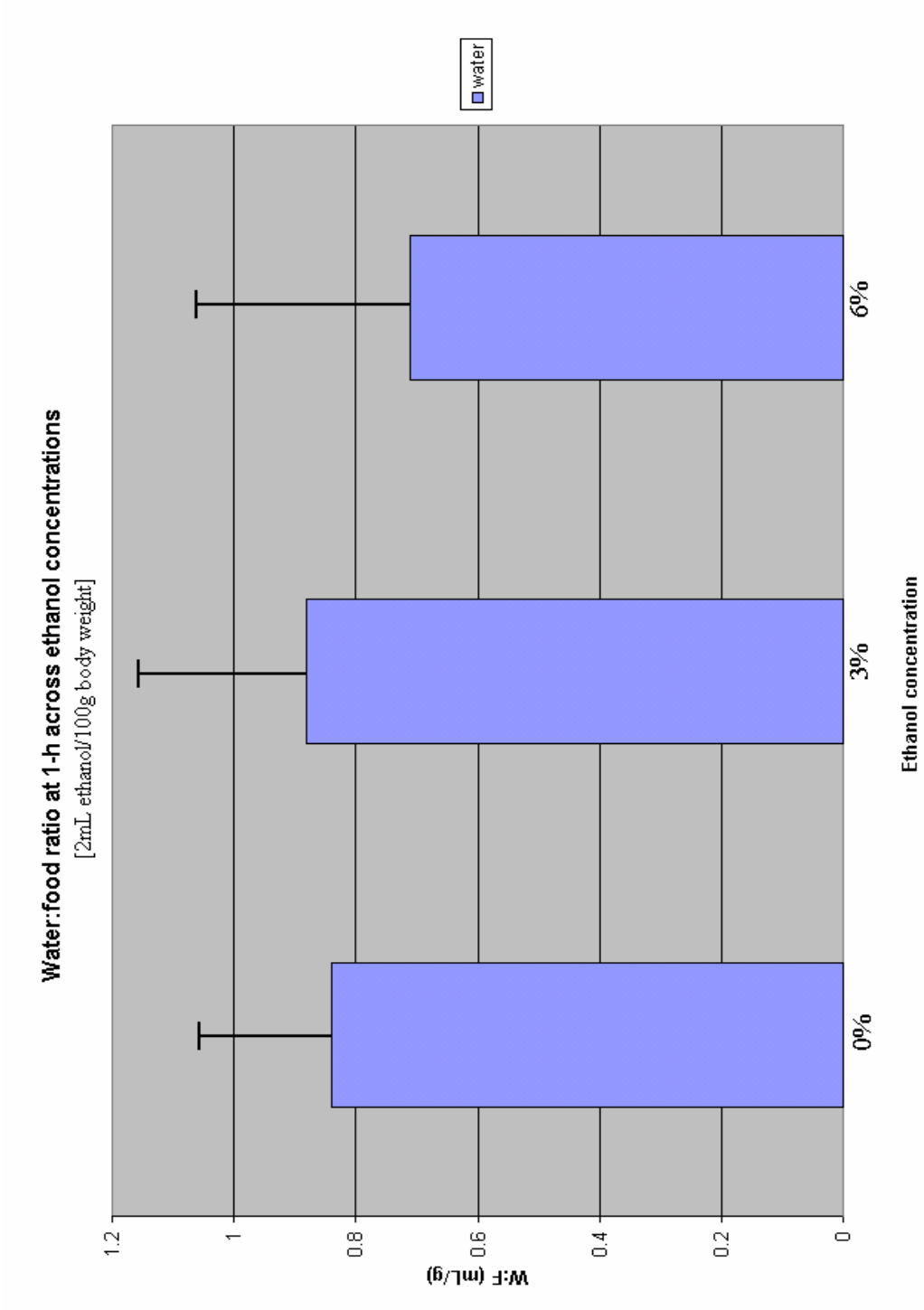

Figure 6.

Figure 6: The mean (±SE) water:food ratio at 1-h across experimental 0%, 3%, and 6% ethanol treatments under double volume solution administration yielded lack of significant difference across treatments. Note 0% ethanol treatment represents mean score from both experiment 2 trials.

Discussion

The results yield novel insight into the influence of ethanol on food-related drinking. The findings of the first experiment exhibited that the self-administration of ethanol in the rat indeed decreased the water intake associated with meal ingestion. This is represented by the drinking responses of both the 3% ethanol and 6% ethanol treatments in comparison to the 0% treatment. This is consistent with work in both humans and rats regarding the ability of alcohol to decrease the fluid intake associated with simulated food ingestion **(7,8)**. The finding that the water:food ratios of the 3% and 6% ethanol trials paralleled the identified decreased overall drinking patterns, makes sense regarding the amount of food that was consumed relative to each animal. What these results show is that food-related drinking was significantly decreased for the ethanol treatments proportionately based on the amount of food that was eaten and in proportion to body weight. Human meal-related drinking patterns have similarly implicated both the timing and amount of eating with water intake **(2)**. Although an effect of ethanol on drinking latency was not determined in either of the current experiments, similar experimental paradigms incorporating saline infusion have been able to show that ethanol can display inhibitory effects on latency to drink in the rat **(10)**.

The first experiment was able to establish a significant distinction between the water intake of both the ethanol treatments compared to the control, yet the slight concentration difference between 3% and 6% ethanol solutions may not have been large enough to elicit an observable dose-dependent difference from the first series of tests. Taken together, experiments 1 and 2 suggest that a dose-dependent phenomenon **(10)** may be present within the overall experimental findings. When the observation that no significant differences were established for the second experiment is related to the significance of decreased drinking in the first experiment, it becomes plausible that the inhibition of drinking due to ethanol may only be occurring at low alcohol concentrations and also at low solution volumes. This possibility leads to the deduction that increasing the ethanol volume in the second experiment and thus in effect doubling the amount of ingested ethanol, may have negated effects of ethanol on drinking that are associated with low doses. From this perspective a dose-dependent phenomenon can be suggested by the unified comparison of both self-administration experiments.

An unexpected finding was discovered in the framework of the actual amount of consumed food during the trial periods of the 3% ethanol treatment. The determination that the 3% ethanol testing resulted in a selective increase in pellet eating, suggests that there may be a connection between very low levels of ingested ethanol and associated appetite. This result was further strengthened by similar significant findings of increased food ingestion in proportion to body weight. Alcohol studies in humans have suggested that ethanol ingestion essentially supplements caloric intake instead of replacing it **(11)**. In contrast to this observation in humans, rats exposed to ethanol as their sole fluid source tend to sustain their normal daily energy intake by decreasing their food intake to compensate the ingested calories associated with the alcohol **(12)**. Ethanol has been further implicated in the inhibition of oxytocin pathways and influencing increases in solute ingestion **(13)**. Since the current experimentally observed eating phenomenon was not found in the second increased ethanol volume trials, this significant finding for food

increase may be of further interest regarding particularly low concentration testing of ethanol effects during meal-related drinking and also specifically in the eating paradigm.

The next stages of investigation concerning the determined decrease in food-related drinking resulting from self-administered ethanol, should attempt to further address the mechanisms for ethanol's effect on meal-related drinking. Physiological systems such as the rat abdominal vagus have been described in terms of food-related drinking **(4,14,15)**, as well as the activation of gastric osmoreceptors from ingested diet **(1,16)**. It has been suggested that the elicitation of drinking following food ingestion in the rat may occur after an increase in plasma osmolality has been detected by osmoreceptors, and that the rate in which intestinal osmoreceptors are activated could depend on the gastric emptying rate of solutions **(14)**. Osmolality has been related to cellular dehydration **(4,6,8)** as well as release of histamine and serotonin **(3,4,17)** in relationship to drinking patterns **(18,19,20)**. Osmoreceptors and their associated signaling pathways are further implicated in terms of vasopressin and oxytocin secretion related to water intake in the rat **(19)**. Oxytocin has previously been described as having an important role in both regulating fluid balance and in affecting salt appetite **(21)**. The expansive connection of oxytocin and vasopressin to both food desirability **(21,22)** and drinking **(23,24,25)** warrant serious consideration of these pathways as particular points of interest regarding expansion of the current findings of ethanol effects on selectively increasing eating and its association with decreased meal-related drinking.

Other essential components of further mechanistic investigation involve but are not limited to dopaminergic pathways influenced by ethanol intake and ingestion patterns **(26,27,28)**, serotonin receptor subtypes in the context of ethanol consumption and self-administration **(29,30,31),** and serotonin influence specifically in the drinking response (**32,33**). The roles of cholecystokinin **(34,35)** and the opioid pathways **(36,37)** must also be appreciated for their described relevance to both reinforcement systems and water ingestion. Histaminergic signaling furthermore shares integral aspects of influence in meal-related drinking. Gastric mucosa release of histamine in response to eating for instance, has been defined in terms of supporting meal-related drinking in the rat **(38,39,40)**. The mediation of food-related drinking has been specifically described based on the influences of particular histaminergic receptor subtypes **(41)**. Selective antagonism of the H_3 histamine receptor subtype has exemplified the significance of peripheral and central H_3 receptors in meal-related drinking in the rat **(42)**. Peripheral and brain H_1 and H_2 histamine receptor subtypes in the rat have been described as being part of the drinking response resulting from food ingestion **(17,43)**. In describing eating inhibition due to food intake, H_2 histamine receptor antagonism has been shown to weaken saline infusion inhibition of eating **(44)**. The implications of these results suggest roles for histamine beyond meal-related drinking to additionally include influence in the mechanistic pathways related to food consumption.

The mechanisms underlying food-related drinking have been further illustrated in terms of histamine and its relation to the renin-angiotensin system **(3,45,46)**. The release of renal renin has been depicted in influencing increases of angiotensin II in rat plasma with associated effects on thirst stimulation **(6,47,48)**. The value of angiotensin in meal-related drinking in the rat has also been distinguished by the specific influence of angiotensin AT_1 and AT_2 receptor subtypes **(49)**. Central and peripheral nervous system implications for angiotensin have been described regarding ethanol intake **(50,51)** and

specific blockage of angiotensin II synthesis has also been shown to enhance dilute ethanol intake in the rat **(52).** The renin angiotensin system is of particular interest regarding the physiological implications of ethanol ingestion because its stimulation has been suggested in relation to chronic alcohol abuse **(53)**. Similar to the present experimental findings that self-administration of ethanol decreases food-related drinking, it has been shown that the inhibition of meal-related drinking induced by angiotensin converting enzyme blockage in the rat brain and periphery can be counteracted by supplemental injection of angiotensin II **(54)**. In the specific context of the current study there is therefore a possibility of angiotensin II influence in repairing the decreased food-related drinking observed under ethanol self-administration, if the mechanism behind the ethanol-mediated drinking decrease involves the angiotensin system. The possible research directions remain abundant yet the currently established ethanol self-administration experimental construct must be expanded in order to categorize and collectively understand the biological implications of the effects of alcohol on eating and decreased drinking associated with meal intake.

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