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DIFFERENTIAL ALCOHOL CONSUMPTION IN FOOD-DEPRIVED COMPETING RATS

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ABSTRACT

Many studies have been conducted to further the understanding of factors influencing alcohol consumption. The purpose of the current study was to investigate: (1)The competitive behavior of food-deprived rats housed as fixed pairs and competing for food. (2)The correlation between the animal's specific competition score, within its specific dyad, and its individual chronic alcohol consumption, when given a free access to 10% (w/v) alcohol or water. (3)Differential chronic alcohol consumption of winners and losers of rat dyads. (4)The effect of alcohol withdrawal on the competitive behavior of animals.

Forty male rats were used in this study; thirty animals composed 15 fixed dyads, and 10 animals served as a control. Each pair of food-deprived animals was tested in the home cage for food competition. Eleven out of 15 dyads established a stable dominance hierarchy, winners and losers. These animals were then housed individually and had free access to 10% (w/v) alcohol and water for 4 hours a day over a period of 14 days. Daily alcohol consumption was recorded for each subject. It was found that the total amount of alcohol consumed by winners was significantly higher than that of losers (Winners, 39.2±2.3 ml vs Losers, 31.8±2.27ml, $p \leq 0.05$). Further, there was a strong positive correlation between the individual animal's alcohol consumption and its average competition score, that was evident for both winners ($r=0.91$) and losers ($r=0.85$). Alcohol withdrawal failed to significantly change the social status of the animals, only two animals switched rank position. However, chronic alcohol consumption proved deleterious effects on the liver. Animals were sacrificed on the first day of alcohol withdrawal had livers with elevated weight (% of body weight), (Liver weight %, Day-1 Withdrawal, 3.6±0.1 vs Control, 3.2±0.07, $p \leq 0.01$).

It is concluded that animals housed in a social environment could establish a stable dominance hierarchy. Highly competitive animals consumed a significantly higher amount of alcohol than low competitors. Further, the amount of alcohol consumed by the animal, winner or loser, positively correlated with the animal's specific competition score.

INTRODUCTION

Clinical observations of drug-dependent individuals suggest that they are predisposed to addiction because they are unable to cope with stress[1] that might be, in some cases, imposed by a sustained lifestyle of socioeconomic competition. Indeed, for some, drug use may be viewed as self-treatment for internal distress[2]. A given pattern of continued drug use seems to result from an interaction between social, biological, and environmental factors[3]. A great deal of effort and research has been done to develop animal models with which to further the understanding of factors influencing human alcohol consumption.

It has been shown that animals raised over a prolonged period of time in a social environment establish stable dominance hierarchy. In order to assess each individual's social position, investigators have evaluated spontaneous fighting among individual rats in such groups[4], behavioral patterns exhibited towards an intruder[5], competitive behavior for a sexually receptive female[6], or competitive behavior for palatable food or liquid[7]. In addition, Several pharmacological studies have suggested that vulnerability to drug self-administration is determined, at least in part, by the social status (rank order) of the animal in its group. Kudriavtseva and coworkers[8] reported increased alcohol consumption in submissive mice with daily experience of defeat. Similar observations have been reported by Blanchard et al.[9]. It was suggested that the social stress might be a factor in enhancing ethanol consumption. Ellison and coworkers[10] classified rats raised in social colonies into high and low alcohol consumers.

Although the previous studies suggested a relationship between the social status of the animal and its alcohol consumption, data was generally obtained from social colonies. Therefore, the purpose of the present study was to investigate: (1)The competitive behavior of food-deprived rats housed as fixed pairs and competing for food. (2)The correlation between the animal's specific competition score, within its specific dyad, and its individual chronic alcohol consumption, when given a free access to 10% (w/v) alcohol or water. (3)Differential chronic alcohol consumption of winners and losers of rat dyads. (4)The effect of alcohol withdrawal on the competitive behavior of animals.

MATERIALS AND METHODS

Forty male rats, obtained from the National Institute of Drug Control and Research, with initial body weight 150±20 g were used in this study. Animals were housed individually in stainless steel cages measured 35×25×20 cm. Animals were kept at 25±3°C, with illumination on a 12/12 hr light-dark cycle throughout the study. Food and tap water were available ad libitum for one week- accommodation period. Tap water was also available ad libitum during the remainder of the study. However, during that time, the animals were food-deprived to enhance competition for food. The body weight of the animals increased by 10-40 g at the end of the study; that was achieved by restricting the daily access to food to two hours (3:00-5:00 p.m.) after the experimental sessions for that day were concluded.

Food-deprived rats were individually trained to consume 50 g regular food pellet whose signaled

delivery occurred at 30 sec. intervals. Food pellets were delivered through a glass tube, 20cm long and 1cm diameter, into a food pot in the home cage. Food pellet delivery occurred 5 sec. after the ringing of a stimulus bell. Nine pellets were delivered per session. Rats were trained daily, one session per day for one week.

Following completion of training, subjects were assigned to dyads (two rats per cage) based on similarity in body weight. Each rat was coded with specific color (red or blue) using food coloring applied to the tail (twice a week) in order to distinguish each rat within its dyad. Following formation of 15 dyads, two feeders were supplied during the two-hour feeding to enhance food availability to all subjects. Excess food was available during the feeding time. The subjects were housed as fixed dyads throughout the competition phases of the study to enhance the establishment of stable dominance ranking in dyads. After a sufficient time has elapsed (one week) since the dyads formation, dominance hierarchy was assessed.

Competition tests were conducted daily (10:00 a.m.-3:00 p.m.) in the home cage of the animals. Competition was scored by direct observation. The animal was given a score of one point for each ingested pellet. The competition score for each animal/session is determined by adding up the number of ingested pellets per session. A rat was ranked as a winner or loser in a specific session based on its composite score within its specific dyad in that session. A final rank order of animals was determined based on the average score of five consecutive daily sessions. Stable dominance ranking was achieved when subjects maintained the same rank position within a given dyad for more than three consecutive daily sessions. Winners and losers were determined in all stable dyads. Unstable dyads were excluded from the study.

Winners and losers of all stable dyads were isolated, each rat was individually housed in a separate cage measured 35×25×20 cm. Each cage was supplied with two 100ml-measuring bottles; one was assigned for water (W) and the other for 10% (w/v) alcohol (A). Each rat was given a free access to both flasks at the same time with free choice to drink water or alcohol. All subjects had the free choice to consume water and 10% (w/v) alcohol for 4 hours a day (10:00 a.m. - 2:00 p.m.) for two weeks. The daily alcohol consumption was determined for each animal at the end of the daily session. Subjects were not exposed to alcohol during the feeding hours. Total alcohol consumption was determined for each subject at the end of the alcohol exposure period (on day 14). The total amount of alcohol consumed by each subject was tested for correlation with the average competition score, during phase I competition, for the same subject. Pearson correlation was applied to analyze both winners' and losers' data.

On the first day of alcohol withdrawal, all winners and losers reunited again to form their original dyads. A second phase of competition test was conducted, as mentioned previously, to investigate the effect of alcohol withdrawal on the competitive behavior of animals. After this competition phase had concluded, 50% of the dyads were sacrificed; the liver was isolated, washed with normal saline (0.9%) and sucrose 0.25 molar solution, and weighed. The liver weight was calculated as a percentage of the body weight. Ten animals of the control group were also sacrificed, the liver was isolated, the liver weight was determined as a percentage of the body weight.

The third phase of competition test was carried out on the remaining 50% of the dyads. Rats were daily tested for their competitive behavior for one week. The average competition score was calculated for each animal. At the end of this phase of competition, animals were sacrificed; the liver was isolated; the liver weight was determined as a % of the body weight; this was compared with the liver weights obtained from animals sacrificed on day-1 alcohol withdrawal and control animals. Table 1 shows a summary of the protocol of the study.

Table 1: A summary of the protocol of the study.

TASK	DURATION
Competition Training	One week
Phase-1 competition test	One week
Alcohol consumption	Two weeks
Alcohol withdrawal, Phase-2 competition test	One day
50% dyads sacrificed, Average % liver weight/body weight	
Remaining 50% dyads, Phase-3 competition test	One week
Animals sacrificed, Average % liver weight/body weight	

Statistical analysis, ANOVA followed by student t-test, was applied for the analysis of winners' and losers' competition scores in different phases of competition. The same statistical procedures were applied for the analysis of total alcohol consumption of winners and losers and the liver weight data. Pearson correlation was used to detect the correlation between alcohol consumption and the competition score for both winners and losers.

RESULTS

The first phase of competition test revealed the ability of the animals to establish a stable dominance rank. Eleven out of fifteen dyads of animals maintained a stable dominance rank order over five consecutive sessions. A stable rank order was demonstrated by comparing the average competition scores across 11 dyads. Student t-test indicated that the average competition scores of winners and losers across 11 dyads over 5 consecutive sessions were significantly different and thus represented two

different populations (Winners, 6.344±1.125 vs Losers, 2.656±1.125, p≤0.001). Values are expressed as the mean ± standard error of the mean.

ANOVA analysis of the competition scores in various competition phases showed that winners and losers maintained their rank position during the three phases, namely, phase I, before alcohol consumption, phase II, 1-day of alcohol withdrawal, and phase III, 1-week after alcohol withdrawal, only two animals switched positions in phase II and III, one animal in each phase. The competition scores of winners were significantly higher than that of losers across the three phases of competition ($F=35.16$, $p\leq0.001$, Fig. 1). In phase II competition, t-test indicated that the average competition score of winners was significantly higher than the average competition score of losers (winners, 5.938±0.9 vs Losers, 3.063±0.9, $p\leq0.001$, Fig. 1).

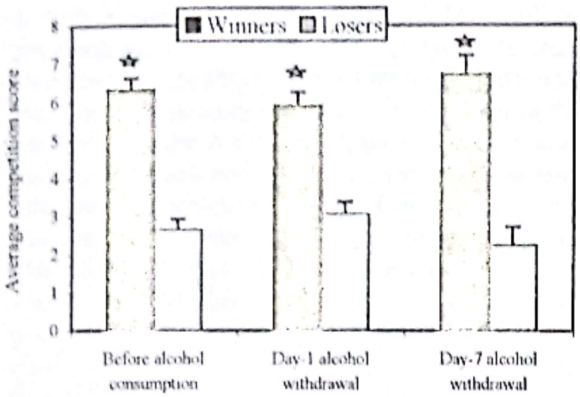

Figure 1: Average competition scores of 5 consecutive sessions of winners and losers across 11 pairs of rats. Competition sessions were conducted before exposure to alcohol, after one day, and one week of alcohol withdrawal.
* Significantly different at $p<0.001$

These animals retained their rank order during the 1-week alcohol withdrawal (winners, 6.738±0.46 vs Losers, 2.263±0.464, $p\leq0.001$, Fig. 1). Therefore, alcohol consumption/withdrawal did not affect the competitive behavior, as measured by the rank position, of the food-deprived animals competing for food.

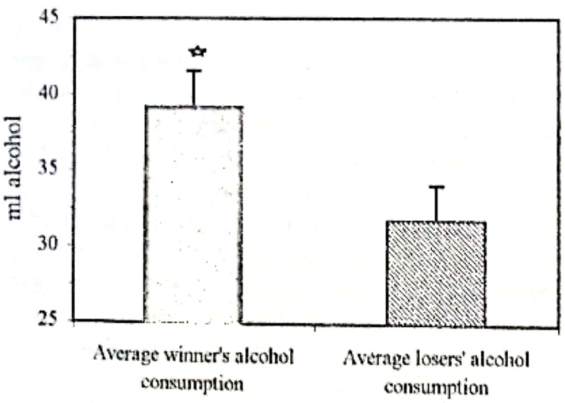

Figure 2: Average alcohol consumption of 11 winners and 11 losers over a period of two weeks of daily alcohol exposure.
* Significantly different at $p<0.05$.

Winners and losers that maintained their rank position, during phase I competition, were tested for differential alcohol consumption in a free choice situation. As indicated by t-test, the total amount of alcohol consumed by winners was significantly higher than that consumed by losers (winners, 39.219±2.329 vs. Losers, 31.808±2.274, $p\leq0.05$, Fig. 2).

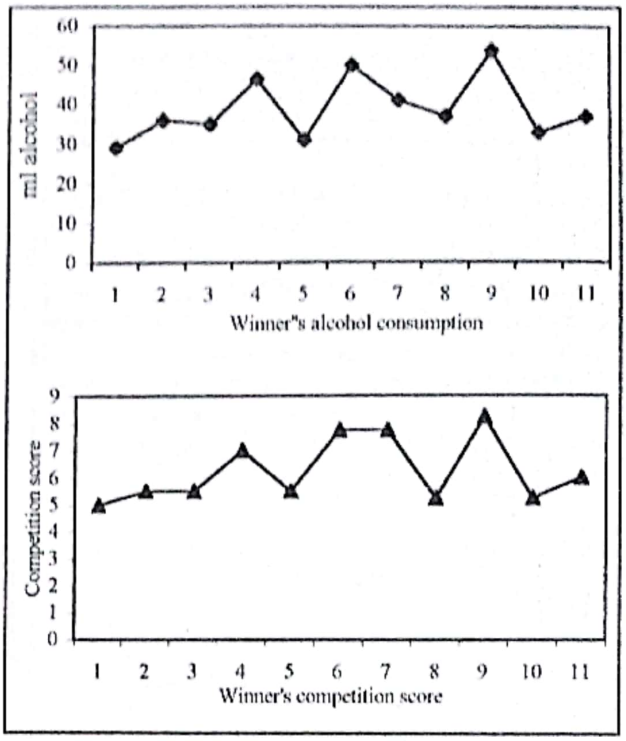

Figure 3: The upper panel shows the total alcohol consumption of each rat of 11 winners over a period of two weeks of daily alcohol exposure. The lower panel shows the average competition score of 5 consecutive daily competition sessions for each winner. There was a positive correlation between the alcohol consumption of each animal and its competition score ($r=0.912$)

More strikingly, Fig. 3 shows an excellent correlation between the individual average competition score of a winner, during phase I competition, and its corresponding total alcohol consumption during the 2-week alcohol exposure period (Pearson correlation, $r=0.912$).

Similarly, Fig. 4 exhibits a very good correlation between the loser's average competition score and its corresponding total alcohol consumption during the 2-week alcohol exposure period (Pearson correlation, $r=0.846$). Therefore, there was a positive correlation between the competition score of the animal and its alcohol consumption in a free choice situation. As the competitive behavior of an animal is enhanced, as measured by its average competition score, its alcohol consumption increases.

ANOVA analysis indicated a significant variation in the liver weight, calculated as a percentage of the body weight, among the three groups, animals sacrificed after one day of alcohol withdrawal, control animals, and animals sacrificed after one week of alcohol withdrawal ($F=5.635$, $p\leq0.01$, Fig. 5). Specific

significant difference in liver weight % was detected by t-test between the liver weight % of animals sacrificed 1-day alcohol withdrawal and the control animals (1-day alcohol withdrawal, 3.633±0.115 vs. Control, 3.221±0.072, $p \leq 0.01$, Fig. 5). Similarly, the liver weight % of animals sacrificed after 1-day alcohol withdrawal was significantly higher than that of animals sacrificed after 1-week alcohol withdrawal (1-day alcohol withdrawal, 3.633±0.115 vs. 1-week alcohol withdrawal, 3.327±0.06, $p \leq 0.05$, Fig. 5).

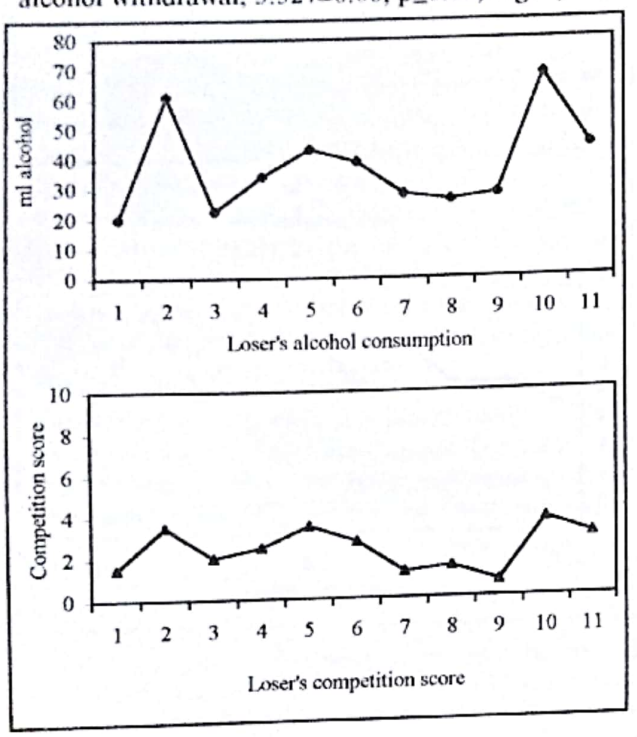

Figure 4: The upper panel shows the total alcohol consumption of each rat of 11 losers over a period of two weeks of daily alcohol exposure. The lower panel shows the average competition score of 5 consecutive daily competition sessions for each loser. There was a positive correlation between the alcohol consumption of each animal and its competition score (r=0.846)

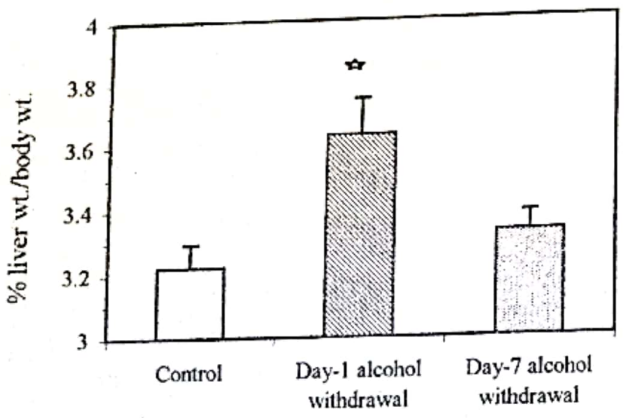

Figure 5: The average liver weight, as a percentage of the body weight, was calculated for rats sacrificed one day after alcohol withdrawal (6 pairs), one week after alcohol withdrawal (5 pairs), and the control group (5 pairs).
* Significantly different at $p<0.05$.

Thus, the increase in liver weight % that was found in animals sacrificed shortly after cessation of alcohol consumption has dissipated after one week of alcohol withdrawal, and the liver weight % returned to normal.

DISCUSSION

In the present study, the animals were housed as dyads (pairs), eleven out of fifteen dyads could establish stable dominance hierarchy. These results are consistent with previous results reported by other investigators[7]. Several competition studies have been conducted using dyads of rats[11] in which dominant and subordinate animals were identified. In the early days of competition test, animals tended to fight and actively compete, a feature that did fade during the late competition sessions. The subordinate subject seems to become either satisfied by its social position or overwhelmed by its competition partner. When a well-defined dominance hierarchy exists, fighting is generally minimal. Schaub[12] showed that dominant animals often could suppress the competitive behavior of subordinates by overt aggression or by their mere presence. It is possible that the home-cage fighting that preceded competition sessions had an impact on the competitive behavior of animals during the competition test. Therefore, housing animals as fixed pairs might have contributed in establishing stable hierarchy within these animal dyads.

Rats in the current study were food-deprived to further enhance competition and development of dominance hierarchy. Hunger motivated each subject in a given dyad to compete for the food pellet. However, the animal's motivation to obtain the food is counterbalanced by possible attacks from the competition partner. Therefore, the procedure might be interpreted as a conflict procedure that may generate stress and anxiety. In agreement with this concept, it has been shown that rats housed individually after a social defeat experience exhibited higher levels of stress and anxiety[13].

In the present study, both the individually housed winners and losers may have chronically consumed alcohol as a response to the anxiety and stress imposed by food deprivation and competition for food. Highly competitive animals consumed larger amounts of alcohol, that was clearly demonstrated by the positive correlation between the animal's competition score and amount of alcohol consumed by the animal. In line with this observation, the winners had significantly higher average competition scores than losers, again the average total alcohol consumption of winners was significantly higher than that of losers. Several investigators studied the mediators of alcohol self-administration in rats and man. Chronic ethanol exposure has been recently shown to increase Gamma-Aminobutyric acid type (A) (GABA-A) receptors in rat brain (amygdula), which may contribute to alcohol drinking behavior and development of ethanol dependence[14]. In addition, it is known that several anxiolytic agents, e.g. benzodiazepines, produce their antianxiety effects

via GABA enhancement[15]. Therefore, it is not unlikely that animals consumed alcohol for its pharmacological reinforcing effects.

There has been growing acceptance for the notion that alcohol self-administration in humans is a distinct set of responses governed primarily by the reinforcing properties of alcohol[16]. As with other areas of psychopharmacology, many animal models of drug self-administration exist. One important consideration in the selection of a particular methodology is the route of administration employed for the drug. Alcohol is taken by humans via the oral route, therefore, there is an exclusive utilization of this route for alcohol consumption; thus, in the present study, animals had free access to orally consume alcohol or water.

Laster and Freed[17] suggested that animals consumed ethanol for its caloric value and not for its reinforcing psychopharmacological effects. While this is a relevant argument, it does not seem to be defensible since it has been demonstrated that food-satiated animals would drink alcohol solutions greater than 8% concentration in preference to water[18]. In addition, animals have been shown to work for ethanol despite free access to food and water[19]. Therefore, animals drinking alcohol in situations where food and competing fluids are continuously available seem likely to be doing so for reasons other than hunger and thirst, and it would appear then that animals orally consumed alcohol for its pharmacologically reinforcing effects.

In agreement with this view, the assumption that food deprivation may increase the reinforcing efficacy of alcohol through a stress factor. This is consistent with the demonstrations that food deprivation increases the consumption of drugs, such as cocaine[20] and PCP[21] that do not contain any calories. Further, it has been recently shown that the corticosterone secretion produced by the stress of food deprivation plays a key role in the enhancement of ethanol intake[22].

In the current study, both food-deprived winners and losers consumed moderate amounts of alcohol. However, alcohol intoxication was not evident, and consequently withdrawal symptoms were not observed. Nevertheless, the competition scores of winners tended to decrease on day-1 withdrawal that was accompanied by a trend of increase in the losers' competition scores. In line with this view, it has been demonstrated that withdrawal from chronic morphine administration was associated with an increase in competition[23] and fighting behavior in rats[24].

In the current investigation, the amounts of alcohol consumed by rats were high enough to increase the weight of the liver of animals sacrificed on day-1 of alcohol withdrawal; this change in liver weight dissipated after one week of alcohol withdrawal. Parallel to the observed time course of change in liver weight, Navasumrit et al.[25] showed that during chronic exposure of rats to ethanol (5%), free radical generation increased significantly after one week and then declined again to remain at a low level over the next 2 weeks. The authors claimed that this transient increase corresponded closely with the induction of the enzyme cytochrome P-450 E1 in response to chronic ethanol consumption. Further, during chronic alcohol exposure, an increase in the frequency of DNA breaks was seen at 3 days, reached a peak at 1 week and then declined slowly over the next 5 weeks[25].

It is concluded that animals housed in a social environment could establish a stable dominance hierarchy. Highly competitive animals consumed a significantly higher amount of alcohol than low competitors. Further, the amount of alcohol consumed by the animal, winner or loser, positively correlated with the animal's specific competition score.

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الاستهلاك النوعى للكحول فى الجرذان المحرومة من الطعام والمتنافسة

د / السيد العوضى

قسم الأدوية والسموم - كلية الصيدلة - جامعة قناة السويس - الإسماعيلية

لقد أجريت العديد من الأبحاث العلمية لدراسة العوامل المؤثرة على شرب الكحولات. وكان الغرض من الدراسة الحالية هو بحث :

١- السلوك التنافسى لدى الجرذان المحرومة من الطعام والمتنافسة عليه.

٢- العلاقة بين الدرجة التنافسية للجرذان واستهلاك الحيوان للكحول إذا أعطى الاختيار الحر بين الكحول ١٠% والماء.

٣- الفرق فى استهلاك الكحول لكل من الفائزين والخاسرين فى التنافس.

٤- تأثير غياب (سحب) الكحول على السلوك التنافسى للحيوان.

استخدم فى هذه الدراسة أربعون من الجرذان الذكور ، كون ثلاثون منهم خمسة عشر زوجاً ثابتاً للتنافس على الطعام ، وعشرة جرذان كونوا مجموعة ضابطة. تم اختبار كل زوج على حدة فى التنافس على الطعام. استطاع إحدى عشر زوجاً من إجمالى خمسة عشر أن يحافظوا على ترتيب ثابت فى التنافس من فائزين وخاسرين. تم تسكين هذه الحيوانات كل حيوان بمفرده وأتيح لكل منهم فرصة شرب الكحول ١٠% أو الماء لمدة أربع ساعات يومياً على مدى ١٤ يوم ، وتم تسجيل الاستهلاك اليومى للكحول لكل حيوان.

وجد أن الفائزين فى التنافس استهلكوا كمية من الكحول أكبر من التى استهلكها الخاسرين ، ٣٩,٢ملل مقابل ٣١,٨ ملل على التوالى. هذا بالإضافة إلى أنه يوجد علاقة طردية بين الكمية المستهلكة من الكحول لكل حيوان ودرجته التنافسية ، ذلك بالنسبة لكل من الفائزين (عامل ارتباط =٠,٩١) والخاسرين (عامل ارتباط =٠,٨٥).

بالرغم من أن سحب الكحول لم يؤثر على درجة التنافس فى الحيوانات إلا أن شرب الحيوانات للكحول قد أثبت تأثيراً ضاراً على الكبد. ظهر ذلك من خلال ارتفاع وزن الكبد بعد يوم واحد من سحب الكحول.