

# Anxiolytic Effect of Voluntarily Consumed Alcohol in Sardinian Alcohol-Preferring Rats Exposed to the Social Interaction Test

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## Abstract

Sardinian alcohol-preferring (sP) rats have been selectively bred for high alcohol preference and consumption. Beside alcohol preference, sP rats display inherent high levels of anxiety-related behaviors (evidenced under multiple experimental procedures). The present study was designed to evaluate whether voluntarily consumed alcohol exerted an anxiolytic effect in adult, male sP rats exposed to the social interaction (SI) test. Alcohol-consuming rats were given alcohol under the homecage 2-bottle “alcohol (10%, v/v) vs water” choice regimen with unlimited access for 15 consecutive days. The SI test was conducted on Day 15 of alcohol drinking, one hour into the dark phase of the daily light/dark cycle. Alcohol intake in alcohol-consuming rats averaged approximately 0.9 g/kg in this first hour of the dark phase. Pairs (n=11) of alcohol-consuming rats displayed approximately 3-fold longer times of SI than pairs (n=10) of alcohol-naive rats. These data (a) suggest that voluntarily alcohol intake exerted an anxiolytic effect in sP rats, (b) extend to a different procedure of experimental “anxiety” previous results obtained using the elevated plus maze test, and (c) strengthen the hypothesis that anxiolysis may represent one of the alcohol effects that drive sP rats to consume high amounts of alcohol.

**Keywords:** Social interaction test; Anxiety-related behaviors; Anxiolytic effect of voluntarily consumed alcohol; Sardinian alcohol-preferring (sP) rats

## Introduction

Sardinian alcohol-preferring (sP) rats have been selectively bred for high alcohol preference and consumption. When exposed to the standard, homecage 2-bottle “alcohol (10%, v/v) vs water” choice regimen with unlimited access for 24 hours/day, sP rats display a clear preference for the alcohol solution and consume daily 6-7 g/kg alcohol [1]. After being temporarily deprived of alcohol, sP rats display relapse-like drinking [1] and loss of control over alcohol [2]. Notably, sP rats meet the fundamental criteria posed to define animal models of alcoholism [1,3].

In addition to the vulnerability to high alcohol preference and consumption, sP rats have been characterized for their unique “emotional” profile comprising (a) high levels of anxiety-related behaviors at the Elevated Plus Maze (EPM) [4-8] and elevated zero maze [9], (b) low levels of spontaneous locomotor activity and high levels of thymotaxis when exposed to an open-field arena [10] and to the multivariate concentric square field [7,8], and (c) low exploratory drive, high risk assessment, and low risk-taking behavior in the multivariate concentric square field [7,8]. Notably, the “anxiety” profile of sP rats has been evidenced in several comparisons with their alcohol-avoiding counterpart (named Sardinian alcohol-non preferring rats) [4-10] as well as in comparison with other selectively bred lines of alcohol-preferring rats [8].

The first study to investigate the “anxiety” profile of sP rats also found that voluntary alcohol drinking, occurring under the 2-bottle choice regimen, reduced some anxiety-related behaviors: the percentage of time spent in and of number of entries into the open arms of an EPM were higher in alcohol-consuming than -naive sP rats [4]. These data were interpreted as anxiolysis representing one of the alcohol effects that likely motivate sP rats to consume alcohol.

In view of the relevance of this research topic, which may contribute towards elucidating the neurobehavioral bases of alcohol preference and drinking in sP rats, the present study was designed to extend to a

different experimental model of “anxiety” the investigation on the ability of voluntary alcohol drinking to ameliorate anxiety-related behaviors in sP rats. To this end, the Social Interaction (SI) test was used; the test is based on the propensity of male adult rats, unknown to each other, to engage in SI when exposed to an environment in which neither has established territory [11-13]. In the SI test, the measured variable is the time spent by pairs of rats in different “social” behaviors, including sniffing, following, walking over, crawling over and under, grooming the partner [14,15]. Importantly, these behaviors have repeatedly been shown to be pharmacologically manipulable with different anxiogenic and anxiolytic (including alcohol) agents [13].

## Materials and Methods

All experimental procedures employed in the present study were in accordance with the Italian law on the “Protection of animals used for experimental and other scientific reasons”.

## Animals

Male sP rats (n=42), from the 74th generation, were used. At the age of approximately 60 days, rats were singly housed in standard plastic cages with wood chip bedding. The animal facility was under an inverted 12:12 hour light/dark cycle (lights on at 8:00 p.m.) at a constant temperature of 22 ± 2°C and relative humidity of approximately 60%. Regular food pellets (Mucedola, Settimo Milanese, MI, Italy) were always available in the home cage. Starting from the day of single-

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cage housing, rats were extensively habituated to handling. Rats were also made familiar to the test arena (see below); to this end, they were singly exposed to the test arena in four 10-min sessions, the first two occurring on the two days preceding the start of the free-choice period (see below) and the last two occurring on the two days preceding the test session. Environmental conditions of these familiarization sessions were identical to those of the test session (see below).

### Experimental procedure

Rats were divided into two groups: alcohol-consuming (n=22) and -naive (n=20). Starting from the age of approximately 75 days, rats from the alcohol-consuming group with exposed to the homecage, 2-bottle “alcohol (10%, v/v) vs. water” choice regimen with unlimited access (24 hours/day) for 15 consecutive days. Conversely, rats in the alcohol-naive group had water as the sole fluid available (in two bottles). On a daily basis, bottles were refilled with fresh solution and their position changed randomly to avoid development of position preference. Daily alcohol and water intake was expressed in g/kg pure alcohol and ml/kg water, respectively, and monitored by weighing the bottles (with a 0.1-g accuracy) every day immediately before the start of the dark phase. Possible fluid spillage was calculated by using multiple bottles filled with 10% (v/v) alcohol and water and positioned in empty cages interspersed in the cage racks; mean spilt volumes were subtracted before data analysis.

On the test day (corresponding to Day 15 of the free-choice period), rats of the alcohol-consuming and -naive groups were divided into 11 and 10 pairs, respectively. These rat pairs were weight-matched (no more than ±5%) and made up of rats unknown to each other (i.e. rats that did not share the same homecage from birth to single housing). The test arena (a) measured 54×54×30 (h) cm, (b) was made of white Plexiglas, and (c) was located in a soundproof room, adjacent to the housing room and lit only by a red light. The test session (a) was performed at 9:00 a.m., i.e. immediately after the end of the first hour of the dark phase of the daily light/dark cycle, and (b) lasted 10 min. The time spent by each rat pair in active SI – both aggressive (i.e., kicking, boxing, wrestling, and submitting) and nonaggressive (i.e., sniffing, following, and grooming the partner), according to the definitions posed by File and co-workers [14,15] – was scored and expressed in ‘s’. Behavioral evaluation and recording of SI time were performed by an observer unaware of the group allocation of each rat pair. At the end of each test, the test arena was cleaned thoroughly. Alcohol and water intake over the first hour of the dark phase of Day 15 was recorded and expressed as described above.

### Statistical analysis

Data on daily alcohol and water intake in the alcohol-consuming rat group over the first 14 days of the free-choice period were analyzed by separate 1-way ANOVAs. Data on time spent in SI over the first and second 5-min periods of observation were analyzed by a 2-way [condition (alcohol-exposure; alcohol-naivety); time (first 5-min period; second 5-min period)] ANOVA with repeated measures on the factor “time”, followed by the Least Significant Difference (LSD) test for post hoc comparisons. Data on time spent in SI over the entire 10-min session were analyzed by the unpaired, 2-tailed Mann-Whitney test.

### Results

When exposed to the 2-bottle “alcohol vs. water” choice regimen, with unlimited access for 24 hours/day, rats in the alcohol-consuming group rapidly acquired alcohol drinking behavior, as indicated by daily alcohol intakes higher than 4 g/kg (i.e., the selection criterion

adopted in the breeding program of sP rats [1]) by Day 5 in each rat. Daily alcohol intake averaged around 5 g/kg over the first 4 days and then rose progressively to mean values varying between 6.5 and 7.1 g/kg over Days 11-14 [F(13,273)=13.78, P<0.0001] (Figure 1, top panel). These data were highly similar to those repeatedly recorded in sP rats exposed to alcohol and water under the 2-bottle choice regimen [1]. Conversely, daily water intake decreased progressively over the 14-day period [F(13,273)=12.77, P<0.0001] (Figure 1, bottom panel).

On the test day (Day 15), alcohol and water intake over the first hour of the dark phase averaged  $0.89 \pm 0.05$  g/kg and  $2.12 \pm 0.40$  ml/kg, respectively; daily alcohol and water intake averaged  $7.12 \pm 0.38$  g/kg and  $14.60 \pm 2.13$  ml/kg, respectively. ANOVA revealed a significant effect of condition (alcohol-exposure or alcohol-naivety) [F(1,19)=10.74, P<0.005] and a significant effect of time (first or second 5-min period of observation) [F(1,19)=8.77, P<0.01], but not a significant interaction [F(1,19)=2.41, P>0.05], on time spent in SI. Post hoc analysis revealed that time spent in SI was approximately 3-fold longer in alcohol-consuming than -naive rat pairs at both time intervals (Figure 2). A significant, approximately 3-fold large difference between alcohol-consuming and -naive rat pairs was also found in total time spent in SI (i.e., over the entire 10-min session) (P<0.005, Mann-Whitney test) (Figure 2).

### Discussion

The present study was designed to evaluate whether voluntarily consumed alcohol produced an anxiolytic effect in selectively bred, alcohol-preferring sP rats exposed to the SI test. To this end, a group of rats was initially exposed to a 2-week period of unlimited access to alcohol under the homecage, 2-bottle “alcohol (10%, v/v) vs. water” choice regimen, i.e. the standard procedure under which sP rats have been selectively bred [1]; as expected, rats rapidly acquired alcohol

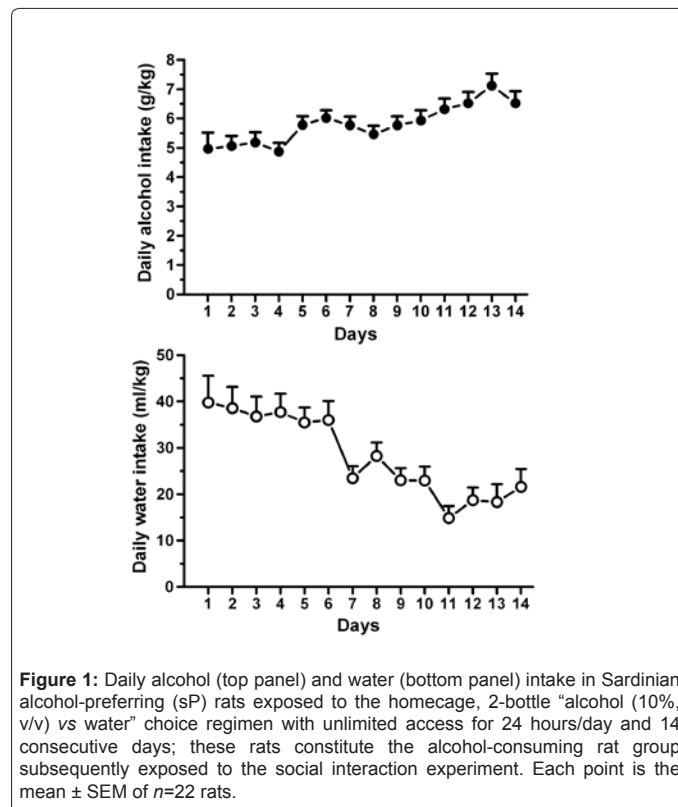
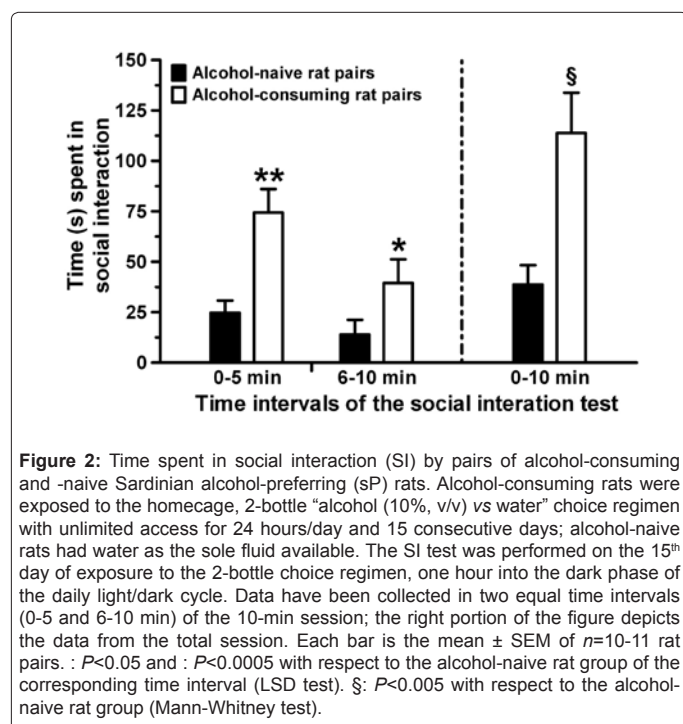


Figure 1: Daily alcohol (top panel) and water (bottom panel) intake in Sardinian alcohol-preferring (sP) rats exposed to the homecage, 2-bottle “alcohol (10%, v/v) vs water” choice regimen with unlimited access for 24 hours/day and 14 consecutive days; these rats constitute the alcohol-consuming rat group subsequently exposed to the social interaction experiment. Each point is the mean ± SEM of n=22 rats.

drinking behavior and displayed stable and high levels of daily alcohol intake. On the test day, rat exposure to the SI test occurred one hour into the dark phase of the daily light/dark cycle; this time schedule was conceived on the basis of previous data demonstrating that sP rats exposed to the unlimited access to alcohol under the 2-bottle choice regimen tend to distribute their alcohol drinking in 3-4 bouts interspersed over the dark phase, with the first bout invariably occurring during the first hour [16]. Thus, performing the SI test at the end of the first hour of the dark phase would likely have captured the effect of alcohol consumed in the first daily bout. Apparently this was the case, as alcohol intake in alcohol-consuming rats averaged  $0.89 \pm 0.05$  g/kg over the first hour of the dark phase of the test day; variability among the 22 rats was also relatively limited, suggesting that virtually all rats consumed pharmacologically relevant amounts of alcohol.

Comparison with pairs of alcohol-naive rats (kept under identical environmental conditions, with the sole exception of the complete unavailability of alcohol) demonstrated that alcohol-consuming rats spent significantly more time engaged in SI. This difference was (a) rather large, as time spent in SI by alcohol-consuming rats was approximately 3-fold longer than that spent by alcohol-naive rats, and (b) evident at both time intervals (the first and second 5-min periods of the 10-min session) as well as when the total time of the session was taken into account. These results are in agreement with the working hypothesis of the present study and suggest that voluntarily consumed alcohol by sP rats exerted an anxiolytic effect.

These results extend to the SI test previous data indicating that voluntarily consumed alcohol produced an anxiolytic effect in sP rats exposed to the EPM test [4]. The results of the present study, together with those of the EPM study [4], suggest that anxiolysis produced by voluntarily consumed alcohol likely (a) reverses, at least in part, the genetically-determined, high degree of anxiety-related behaviors of sP rats [4-10], and (b) likely constitutes one of the alcohol effects capable of motivating alcohol drinking behavior in sP rats.



**Figure 2:** Time spent in social interaction (SI) by pairs of alcohol-consuming and -naive Sardinian alcohol-preferring (sP) rats. Alcohol-consuming rats were exposed to the homecage, 2-bottle "alcohol (10%, v/v) vs water" choice regimen with unlimited access for 24 hours/day and 15 consecutive days; alcohol-naive rats had water as the sole fluid available. The SI test was performed on the 15<sup>th</sup> day of exposure to the 2-bottle choice regimen, one hour into the dark phase of the daily light/dark cycle. Data have been collected in two equal time intervals (0-5 and 6-10 min) of the 10-min session; the right portion of the figure depicts the data from the total session. Each bar is the mean  $\pm$  SEM of  $n=10-11$  rat pairs. :  $P<0.05$  and :  $P<0.0005$  with respect to the alcohol-naive rat group of the corresponding time interval (LSD test). §:  $P<0.005$  with respect to the alcohol-naive rat group (Mann-Whitney test).

Notably, similar data have been collected in selectively bred, Indiana alcohol-preferring P rats: alcohol intake, occurring under the 2-bottle choice paradigm with unlimited access for 9 consecutive days, reduced the innate high levels of anxiety-related behaviors of P rats tested at the EPM [17]. Together, these results may be interpreted as P and sP rats "self-medicating" their inherent "anxiety" by voluntary alcohol drinking, modeling-to some extent-the relatively common clinical situation in which alcoholics are predisposed to alcohol drinking because of anxiety (the so-called "tension-reducing" hypothesis of etiology of excessive alcohol drinking) [18]. Because of their large variability in a number of alcohol-related traits and behaviors, selectively bred alcohol-preferring rat lines are often seen as possible experimental models of the different types, or typologies, of human alcoholics [1,3,8,19]. According to this hypothesis and taking into account the results of the EPM [4,17] and SI [present study] experiments, alcohol-preferring P and sP rats might represent proper models for those typologies of alcoholics in whom alcohol drinking is primarily driven by a search for anxiety relief [20,21].

To summarize, the results of the present study indicate that voluntarily alcohol intake exerted a clear anxiolytic effect in alcohol-preferring sP rats exposed to the SI test. These results (a) extend previous results obtained using the EPM test to a different procedure of experimental "anxiety" and (b) strengthen the hypothesis that anxiolysis may represent one of the alcohol effects that sustain alcohol drinking behavior in sP rats.

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