Published in final edited form as:

Alcohol. 2015 November ; 49(7): 707–712. doi:10.1016/j.alcohol.2015.04.010.

Anxiety-like behaviors at the end of the nocturnal period in sP rats with a “history” of unpredictable, limited access to alcohol

Giancarlo Colombo^a, Carla Lobina^a, Paola Maccioni^a, Mauro A.M. Carai^a, Irene Lorrai^a, Alessandro Zaru^{a,b}, Andrea Contini^c, Claudia Mugnaini^d, Federico Corelli^d, and Gian Luigi Gessa^{a,b}

^aNeuroscience Institute, Section of Cagliari, National Research Council of Italy, S.S. 554, km. 4,500, I-09042 Monserrato (CA), Italy

^bDepartment of Biomedical Sciences, University of Cagliari, S.S. 554, km. 4,500, I-09042 Monserrato (CA), Italy

^cDepartment of Biomedical Sciences, University of Sassari, Via Muroni 23, I-07100 Sassari (SS), Italy

^dDepartment of Biotechnologies, Chemistry, and Pharmacy, University of Siena, Via Aldo Moro 2, I-53100 Siena (SI), Italy

Abstract

Recent research found that exposure of selectively bred, Sardinian alcohol-preferring (sP) rats to multiple alcohol concentrations (10%, 20%, and 30%, v/v), under the 4-bottle “alcohol vs. water” choice regimen, in daily 1-h drinking sessions with an unpredictable time schedule, promoted high intakes of alcohol (2 g/kg) when the drinking session occurred over the final hours of the dark phase of the light/dark cycle. The present study was aimed at investigating whether these high intakes of alcohol (a) were associated with alterations in rats’ emotional state (Experiment 1) and (b) were pharmacologically manipulable (Experiment 2). In both experiments, over a period of 12 days, sP rats were initially exposed daily to a 1-h drinking session performed during the 12-h duration of the dark phase; time of alcohol exposure was changed each day and was unpredictable to rats. The day after this 12-day drinking phase, rats were (a) exposed to the Social Interaction (SI) test at the 1st or 12th hour of the dark phase with no alcohol available (Experiment 1) or (b) treated acutely with the positive allosteric modulator of the GABA_B receptor, GS39783 (0, 25, 50, and 100 mg/kg, intragastrically [i.g.]), and exposed to a drinking session at the 12th hour of the dark phase (Experiment 2). In Experiment 1, rats exposed to the SI test during the 12th hour spent approximately 35% less time in “social” behaviors than rats exposed to the SI test during the 1st hour. No difference in “social” behaviors was observed between alcohol-naïve sP rats exposed to the SI test at the 1st and 12th hour. In Experiment 2, all doses of GS39783 selectively reduced alcohol intake. These results suggest that (a) expectation of alcohol availability likely exacerbated

Corresponding author: Giancarlo Colombo, Neuroscience Institute, Section of Cagliari, National Research Council of Italy, S.S. 554, km. 4,500, I-09042 Monserrato (CA), Italy, Telephone: +39 070 675 4342, Fax: +39 070 675 4320, colomb@unica.it.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

the anxiety-like state of sP rats and (b) the GABA_B receptor is part of the neural substrate underlying these exceptionally high intakes of alcohol in sP rats.

Keywords

time schedule of alcohol drinking; limited and unpredictable access to alcohol; experimental model of binge drinking; anxiety-like behaviors; positive allosteric modulator of the GABA_B receptor; GS39783; Sardinian alcohol-preferring (sP) rats

Introduction

A recent study found that selectively bred, Sardinian alcohol-preferring (sP) rats were highly sensitive to the time schedule in which alcohol was accessible in daily drinking sessions of limited duration, with the concurrent availability of multiple alcohol concentrations (Colombo et al., 2014). Specifically, alcohol intake increased progressively as the time of alcohol access moved from the earliest to the latest hours of the dark phase of the 12:12-h light/dark cycle. When the drinking session occurred during one of the latest hours of the dark phase (11th and 12th hour), alcohol intake (a) was exceptionally elevated (averaging 2 g/kg), (b) was more than double that recorded over the first hours (1st and 2nd hour) of the dark phase, (c) resulted in blood alcohol levels averaging approximately 100 mg% (meeting the criterion posed for binge drinking in humans [NIAAA, 2004]), and (d) produced clear signs of alcohol intoxication (e.g., impaired performance at a Rota-Rod task) (Colombo et al., 2014). Notably, sensitivity to the time schedule was (1) limited to the dark phase, as it did not extend to the light phase of the light/dark cycle, and (2) specific to alcohol, as it did not generalize to a highly palatable chocolate-flavored beverage (Colombo et al., 2014).

A critical feature of this experimental design was that time of alcohol exposure changed daily in a semi-random order (meaning that, over 12 consecutive days, rats experienced all 12-h time periods of the dark phase as periods of access to alcohol), becoming unpredictable to rats (Colombo et al., 2014). Unpredictability of time of alcohol availability was an important component of this experimental procedure, as it substantially contributed to the increase in alcohol intake recorded when the drinking session occurred over the final portion of the dark phase.

We hypothesized that the unpredictable schedule of alcohol access, together with the expectation of alcohol availability, may have – as time elapsed – generated progressively increasing emotional “distress”. The observed increase in alcohol intake suggests that rats might have coped with this negative affective state by seeking the anxiolytic effect of alcohol. Experiment 1 of the present study was therefore designed to evaluate whether sP rats with an alcohol “history” comprising repeated, unpredictable exposures to multiple alcohol concentrations in daily drinking sessions of 1 h displayed different levels of anxiety-related behaviors when exposed to the social interaction (SI) test at the first or last hour of the dark phase. The SI test is based on the propensity of adult male rats, unknown to each other, to engage in SI behaviors (e.g., sniffing, following, walking over, crawling over and under, grooming the partner) when exposed to an environment in which neither has established territory; the time spent by pairs of rats in these “social” behaviors provides an

inverse measure of the rat anxiety-like state: the longer the time spent in social behaviors, the lower the “anxiety” of the rats (File, Kenny, & Ouagazzal, 1998; File, Lippa, Beer, & Lippa, 2004).

We were also interested in evaluating whether the exceptionally high intake of alcohol recorded when the drinking session occurred during the latest hours of the dark phase was pharmacologically manipulable. To address this research question, we tested the effect of acute treatment with GS39783 on alcohol intake in sP rats with an alcohol “history” of repeated, unpredictable exposures to multiple alcohol concentrations in daily drinking sessions of 1 h and then exposed to alcohol during the 12th hour of the dark phase of an additional drinking session (Experiment 2). GS39783 is an *in vivo* effective, positive allosteric modulator (PAM) of the GABA_B receptor (GABA_B PAM) (Urwyler et al., 2003). During recent years, several behavioral studies have depicted an interesting pharmacological profile for GS39783: its acute or repeated administration (a) exerted anxiolytic-like effects in some rat and mouse models of anxiety (Cryan et al., 2004; Mombereau et al., 2004; see, however, Paterson & Hanania, 2010; Sweeney, O’Leary, & Cryan, 2013), and (b) suppressed several alcohol-related behaviors, including alcohol drinking in sP rats exposed to the 2-bottle “alcohol vs. water” choice regimen with unlimited access (Orrù et al., 2005) and operant, oral alcohol self-administration in alcohol-preferring sP, Indiana P, and Alko Alcohol (AA) rats (Maccioni et al., 2007, 2008, 2010, 2012, 2015). Notably, with regard to the aims of the present study, a recent investigation (Linsenbardt & Boehm, 2014) found that acute treatment with GS39783 suppressed binge-like drinking in C57BL/6J mice exposed to the “Drinking-in-the-Dark” procedure (i.e., daily exposure to a single alcohol bottle in daily 2- or 4-h drinking sessions, occurring early in the dark phase [see Thiele & Navarro, 2014]).

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 scientific reasons”.

Animals

Male sP rats from the 85th generation were used. Independent groups of $n = 34$, $n = 48$, and $n = 32$ rats were used in Experiments 1A, 1B, and 2, respectively. Rats were singly housed in standard plastic cages with wood chip bedding; single housing started at the age of approximately 45 days. The animal facility was under an inverted 12:12-h light/dark cycle (lights on at 8:00 PM) at a constant temperature of 22 ± 2 °C and relative humidity of approximately 60%. Food pellets (2018 Diet; Harlan, San Pietro al Natisone, UD, Italy) and water were always available in the home cage.

Starting from the first day of single-cage housing, rats were extensively habituated to handling. Rats used in Experiments 1A and 1B were also familiarized with the SI-test arena (see below). To this end, they were individually exposed to the test arena in four 10-min sessions occurring on the days preceding the test session; environmental conditions of these familiarization sessions were identical to those of the test session (see below). Rats used in Experiment 2 were extensively habituated to intragastric infusions (by metal gavage).

Experimental procedure

Schematic representations of Experiments 1A and 2 are given in Fig. 1, top and bottom panels, respectively.

At the age of approximately 60 days, rats used in Experiments 1A and 2 were exposed to the home-cage, 4-bottle choice regimen between water and 10%, 20%, and 30% (v/v) alcohol with unlimited access (24 h/day) for 12 consecutive days (Phase 1). A 24-h period of alcohol withdrawal, with water as the sole fluid available, was interposed between Phases 1 and 2.

Rats were then exposed to the above 4-bottle choice regimen, with access limited to 1 h/day, for 13 consecutive days (Phase 2). The initial drinking session of Phase 2 (“acclimatization” session) differed from all subsequent sessions as rats were not yet accustomed to the limited-access regimen; it was predicted that their alcohol drinking could be influenced by this novel condition (namely, abrupt and unexpected removal of the alcohol bottles). Therefore, data from this initial drinking session were excluded from analysis. Time of access to alcohol was established semi-randomly so that, over 12 consecutive days, all 12 hours of the dark phase were tested. The sequence used in Experiment 1A was: 10, 2, 8, 3, 7, 11, 5, 9, 12, 1, 4, and 6; the “acclimatization” session was replicated on the 11th day of the sequence. The sequence used in Experiment 2 was: 10, 4, 2, 8, 12, 6, 7, 11, 1, 9, 3, and 5. The “acclimatization” session was replicated on the 6th day of the sequence. Water was always available (24 h/day).

In both Phases 1 and 2, on a daily basis, bottles were refilled with fresh solution and their positions changed randomly to avoid development of position preference. Alcohol and water intake was expressed in g/kg pure alcohol and mL/kg water, respectively, and monitored by weighing the bottles with a 0.01-g accuracy (a) every day immediately before the start of the dark phase in Phase 1, and (b) immediately before and immediately after each daily drinking session in Phase 2. Possible fluid spillage was calculated by using multiple bottles filled with the different alcohol concentrations and positioned in empty cages interspersed in the cage racks; mean spilled volumes were subtracted before data analysis.

In Experiment 1A, the SI test was conducted on the day immediately after the last drinking session of Phase 2. Rats were divided into 17 pairs, matched for (a) body weight (no more than $\pm 5\%$) and (b) alcohol intake and schedule sensitivity over Phase 2. Eight pairs of rats were exposed to the SI test during the 1st hour of the dark phase (“1st-Hour” rat group); conversely, 9 pairs of rats were exposed to the SI test during the 12th hour of the dark phase (“12th-Hour” rat group). Rats were not exposed to alcohol on the test day. In Experiment 1B, alcohol-naïve rats were divided into 24 pairs, matched for body weight (no more than $\pm 5\%$), and exposed to the SI test at the same exact age of rats of Experiment 1A. Twelve pairs of rats were exposed to the SI test during the 1st hour of the dark phase (“1st-Hour” rat group). Conversely, 12 pairs of rats were exposed to the SI test during the 12th hour of the dark phase (“12th-Hour” rat group). In both Experiments 1A and 1B, pairs were made up of rats unknown to each other (i.e., rats that did not share the same home cage from birth to single housing). The test arena (a) measured $54 \times 54 \times 30$ (height) 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 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 (File et al., 1998, 2004) – was scored and expressed in sec; each rat pair generated one single datum. Behavioral evaluation and recording of SI time were performed by a well-trained observer. At the end of each test session, the test arena was cleaned thoroughly.

In Experiment 2, the test was conducted on the day immediately after the last drinking session of Phase 2. Rats were divided into four groups of $n = 8$, matched for (a) body weight and (b) alcohol intake and schedule sensitivity over Phase 2, and exposed to an additional drinking session occurring during the 12th hour of the dark phase. GS39783 (synthesized in-house by CM and FC) was suspended in 2 mL/kg distilled water with a few drops of Tween 80, and administered by gavage at the doses of 0, 25, 50, and 100 mg/kg 60 min before the start of the drinking session. GS39783 dose-range, pretreatment time, and route of administration were identical to those used in several previous studies testing GS39783 on different alcohol-motivated behaviors in sP rats (Maccioni et al., 2007, 2008, 2010, 2012, 2015). Alcohol, water, and food intake were recorded immediately after the test session and expressed in g/kg, mL/kg, and g/kg, respectively.

Statistical analysis

In Experiments 1A and 2, data on alcohol intake over the 12 drinking sessions of Phase 2 were (a) analyzed by 1-way ANOVA with repeated measures and (b) exposed to regression analysis (mean alcohol intake vs. time of the drinking session over the dark phase) and calculation of the Pearson correlation coefficient.

In Experiments 1A and 1B, data on time spent in SI over the first and second 5-min periods of observation were analyzed by a 2-way ANOVA (hour of testing [1st hour; 12th hour]; test interval [first 5-min period; second 5-min period]) with repeated measures on the factor “test interval”, followed by the Newman-Keuls test for *post hoc* comparisons. Data on time spent in SI over the entire 10-min session were analyzed by the unpaired, 2-tailed *t* test.

In Experiment 2, data on alcohol, water, and food intake over the drinking session were analyzed by separate 1-way ANOVAs, followed by the Newman-Keuls test for *post hoc* comparisons.

Results

Experiment 1

Experiment 1A (Social Interaction in rats previously exposed to alcohol)—

During Phase 1, all rats rapidly acquired alcohol-drinking behavior, as indicated by a mean daily intake of alcohol as high as approximately 4.0 g/kg on the initial day of exposure to the 4-bottle choice regimen. Mean daily alcohol intake rose progressively and then stabilized, over the last 5 days, around 6.0 g/kg. Mean daily water intake decreased progressively, compensating for the increase in mean daily alcohol intake (data not shown).

ANOVA indicated highly significant differences in alcohol intake during the 12 drinking sessions of Phase 2 [$F(11,385) = 62.47, p < 0.0001$]. Specifically, mean alcohol intake varied widely, from a minimum value of 0.63 ± 0.05 g/kg, when the drinking session occurred during the 1st hour of the dark phase, to a maximum value of 2.07 ± 0.08 g/kg, when the drinking session occurred during the 12th hour of the dark phase. Mean alcohol intake was highly positively correlated with time of access to alcohol ($r = 0.946$, slope = 0.117, intercept = 0.648, $p < 0.0001$, $n = 34$) (Fig. 2, left panel). In terms of intake from the three different alcohol bottles, 10% concentration was the preferred concentration (approximately 50% of total alcohol intake over entire Phase 2), followed by 20% (approximately 35% of alcohol intake), and 30% (approximately 15% of alcohol intake), with modest differences among the 12 hours of the dark phase (data not shown). Mean water intake was negligible (<1 mL/kg) in each drinking session.

The SI test was conducted the day after completion of Phase 2 and employed two rat groups matched for equal alcohol intake and schedule sensitivity over Phase 2. ANOVA of data collected in the two 5-min intervals of the SI test (0–5 and 6–10 min) indicated a significant effect of hour of testing [$F(1,15) = 9.58, p < 0.01$] and test interval [$F(1,15) = 18.85, p < 0.001$], but not a significant interaction [$F(1,15) = 0.11, p > 0.05$] on time spent in SI. *Post hoc* analysis revealed that, at both time intervals, “12th-Hour” rat pairs spent significantly less time in SI than “1st-Hour” rat pairs. At both time intervals, time spent in SI was approximately 35% lower in “12th-Hour” than “1st-Hour” rat pairs (Fig. 2, right panel). A difference of similar magnitude was also recorded when the time spent in SI over the entire 10-min session was compared (Fig. 2, right panel).

Experiment 1B (Social Interaction in alcohol-naïve rats)—ANOVA of data collected in the two 5-min intervals of the SI test (0–5 and 6–10 min) indicated a significant effect of test interval [$F(1,22) = 12.89, p < 0.005$] but not of hour of testing [$F(1,22) = 0.03, p > 0.05$], and no significant interaction [$F(1,22) = 0.45, p > 0.05$], on time spent in SI. Time spent in SI was virtually identical, at each time interval, between “1st-Hour” and “12th-Hour” rat pairs (Fig. 3).

Experiment 2

During Phase 1, all rats rapidly acquired alcohol-drinking behavior, as indicated by a mean daily intake of alcohol as high as approximately 4.5 g/kg on the initial day of exposure to the 4-bottle choice regimen; mean daily alcohol intake rose progressively and then stabilized, over the last 5 days, to approximately 6.0 g/kg. Mean daily water intake decreased progressively, compensating for the increase in mean daily alcohol intake (data not shown).

ANOVA indicated highly significant differences in alcohol intake during the 12 drinking sessions of Phase 2 [$F(11,385) = 45.43, p < 0.0001$]. Specifically, mean alcohol intake varied largely, from a minimum value of 0.82 ± 0.06 g/kg, when the drinking session occurred during the 1st hour of the dark phase, to a maximum value of 2.24 ± 0.09 g/kg, when the drinking session occurred during the 12th hour of the dark phase. Mean alcohol intake was highly positively correlated with time of access to alcohol ($r = 0.914$, slope = 0.101, intercept = 0.838, $p < 0.0001$, $n = 32$) (Fig. 4, left panel). In terms of intake from the

three different alcohol bottles, 10% concentration was the preferred concentration (approximately 45% of total alcohol intake over entire Phase 2), followed by 20% (approximately 35% of alcohol intake), and 30% (approximately 20% of alcohol intake) with modest differences among the 12 hours of the dark phase (data not shown). Mean water intake was negligible (<0.5 mL/kg) in each drinking session.

The test with GS39783 was conducted the day after completion of Phase 2, when an additional drinking session took place at the 12th hour of the dark phase. Before the session, rats were divided into groups matched for equal alcohol intake and schedule sensitivity over Phase 2. ANOVA indicated a significant effect of acute treatment with GS39783 on alcohol intake [$F(1,28) = 19.84, p < 0.0001$]. Alcohol intake in vehicle-treated rats averaged 2.00 ± 0.10 g/kg, an amount similar to that recorded on the 5th day of Phase 2 (when the drinking session occurred at the 12th hour of the dark phase). Treatment with 25, 50, and 100 mg/kg GS39783 reduced mean alcohol intake by 45%, 45%, and 62%, respectively, in comparison to vehicle-treated rats, reaching high statistical significance at the *post hoc* analysis at each dose (Fig. 4, right panel).

Conversely, treatment with GS39783 failed to alter both water [$F(1,28) = 1.62, p > 0.05$] and food [$F(1,28) = 0.86, p > 0.05$] intake during the drinking session (Table 1).

Discussion

Phase 2 of both Experiments 1A and 2 confirmed the sensitivity to time schedule of alcohol drinking in sP rats exposed to daily drinking sessions of 1 h, during the dark phase of the light/dark cycle, with multiple alcohol concentrations, and unpredictable access to alcohol. Indeed, in both experiments, alcohol intake positively correlated with the time of alcohol access, increasing progressively from an average of 0.6–0.8 g/kg, when the drinking session occurred during the 1st hour, to an average of more than 2 g/kg, when the drinking session occurred during the 12th hour. These data closely replicate those collected in the initial study (Colombo et al., 2014) and confirm that this experimental procedure may lead sP rats to binge-like drinking.

The results of the SI test, performed at the end of Experiment 1A, indicate that sP rats with a “history” of repeated, unpredictable exposures to daily alcohol drinking sessions displayed different levels of anxiety-related behaviors at the 1st and 12th hour of the dark phase. Time spent in SI behaviors was indeed markedly reduced at the 12th compared to the 1st hour, suggestive of higher anxiety-like states in rats exposed to the SI test at the 12th rather than 1st hour. These results may be interpreted as the “history” of unpredictable availability of alcohol producing an emotional “distress”, or anxiety-like state, the intensity of which increased progressively over the dark phase and resulted – when alcohol was finally available – in proportionally larger intakes of alcohol. The results of Experiment 1B – in which anxiety-related behaviors did not differ, even minimally, between alcohol-naïve sP rats exposed to the SI test at the 1st and 12th hour of the dark phase – suggest that the differences observed in Experiment 1A were indeed due to the rats’ alcohol “history”, rather than merely to time, over the dark phase, of exposure to the test.

Several previous studies have unanimously demonstrated that sP rats display a unique emotional profile comprising high levels of anxiety-related behaviors (Agabio et al., 2001; Cagiano et al., 2002; Colombo et al., 1995; Leggio et al., 2003; Richter, Zorrilla, Basso, Koob, & Weiss, 2000; Roman & Colombo, 2009; Roman et al., 2012). Additionally, voluntarily consumed alcohol markedly reduced these anxiety-related behaviors (Colombo et al., 1995; Lobina, Gessa, & Colombo, 2013), suggesting that anxiolysis is likely one of the alcohol effects that drive sP rats to seek and consume alcohol. Together, these results (a) indicate the suitability of sP rats as an animal model for studies aimed at bridging excessive alcohol drinking and anxiety-like states, and (b) lead to the hypothesis that changes in alcohol demand and intake in sP rats are secondary to alterations in their anxiety-like state. The procedure of alcohol availability used in the present study has likely produced an exacerbation of the innate anxiety-like state of sP rats: the uncertainty of time of alcohol access and expectation of alcohol availability would have added an emotional “distress” to the basal, “anxiety” levels of sP rats, contributing to the exceptionally high alcohol intakes recorded when the drinking session took place during the last hours of the dark phase. In other words, we hypothesize that sP rats may have coped with the negative affective state generated by this procedure of uncertain availability of alcohol with the observed increase in alcohol intake, seeking the anxiolytic effect of alcohol. This negative affective state, as well as the amount of alcohol consumed to cope with it, increased progressively together with alcohol expectation. It would therefore be of interest to test whether alcohol drinking of other lines of selectively bred alcohol-preferring rats, known not to be phenotypically characterized by high levels of anxiety-like states, is less sensitive than that of sP rats to the unpredictable time schedule of alcohol availability used in the present study. Additional studies will also be undertaken to investigate possible changes at the level of the neuroendocrine and neurotransmitter systems known to underlie stress and anxiety responses.

The results of Experiment 2 indicate that acute treatment with the GABA_B PAM, GS39783, markedly reduced the intake of alcohol in sP rats with a “history” of unpredictable access to alcohol and tested with GS39783 when the drinking session occurred at the 12th hour of the dark phase. Notably, the reduction produced by GS39783, with minimal dose-related differences, was so large that alcohol intake dropped to values (approximately 0.8 g/kg) similar to those usually collected in untreated rats during the first hours of the dark phase. This leads us to hypothesize that the anxiolytic effects of GS39783 (Cryan et al., 2004; Mombereau et al., 2004; see, however, Paterson & Hanania, 2010; Sweeney et al., 2013) may have substituted for those of alcohol, resulting in the virtually complete abolishment of (a) the extra amount of alcohol (due to the late access to alcohol during the dark phase) and, likely, (b) the increased anxiety-like state produced by the time schedule of alcohol access.

In order to assess the selectivity of GS39783 effect on alcohol intake, data on water and food intake during the drinking session were also recorded. Water intake was extremely low in all four rat groups, making its analysis virtually meaningless in terms of evaluation of the selectivity of GS39783 action. The reason for these low levels of water intake is likely that water was always available (24 h/day) and not restricted solely to the time of alcohol access; thus, during the 1-h drinking session, rats focused their drinking behavior almost exclusively on alcohol solutions, limiting water intake to negligible amounts. Conversely, sizable

amounts of food were consumed over the drinking session; no effect of treatment with GS39783 was noted on this variable, suggesting that (a) the reducing effect of GS39783 was selective for alcohol intake, and (b) GS39783 was tested at doses devoid of sedative effects (that would have hampered the regular rates of drinking and feeding).

Data from Experiment 2 extend to binge-like drinking, the capacity of non-sedative doses of GS39783 to reduce alcohol drinking under the standard, 2-bottle choice procedure with unlimited access (Orrù et al., 2005), and oral alcohol self-administration under multiple operant procedures (Maccioni et al., 2007, 2008, 2010, 2012, 2015) in sP rats. These data are also consistent with those recently reported by Linsenbardt and Boehm (2014) on the ability of acutely administered GS39783 to selectively suppress binge-like drinking in C57BL/6J mice. Data from the study by Linsenbardt and Boehm (2014), together with those of the present study, suggest that the GABA_B receptor is part of the neural substrate underlying binge-like drinking in rodents. As binge drinking is currently an important medical and social problem (see Bava & Tapert, 2010; Patrick & Schulenberg, 2013; White & Hingson, 2013), these data might have therapeutic potential once GABA_B PAMs reach clinical testing.

Acknowledgments

The authors are grateful to Mrs. Carla Acciaro for animal breeding and care, Mr. Alessandro Capra for technical assistance, and Ms. Anne Farmer for language editing of the manuscript. Supported by NIAAA-funded “Integrative Neuroscience Initiative on Alcoholism” (INIA-Stress) Consortium and European Foundation for Alcohol Research (ERAB; grant # EA 13 20).

References

- Agabio R, Carai MA, Lobina C, Pani M, Reali R, Vacca G, et al. Alcohol stimulates motor activity in selectively bred Sardinian alcohol-preferring (sP), but not in Sardinian alcohol-nonpreferring (sNP), rats. *Alcohol*. 2001; 23:123–126. [PubMed: 11331110]
- Bava S, Tapert SF. Adolescent brain development and the risk for alcohol and other drug problems. *Neuropsychology Review*. 2010; 20:398–413. [PubMed: 20953990]
- Cagiano R, Cassano T, Coluccia A, Gaetani S, Giustino A, Steardo L, et al. Genetic factors involved in the effects of developmental low-level alcohol induced behavioral alterations in rats. *Neuropsychopharmacology*. 2002; 26:191–203. [PubMed: 11790515]
- Colombo G, Agabio R, Lobina C, Reali R, Zocchi A, Fadda F, et al. Sardinian alcohol-preferring rats: a genetic animal model of anxiety. *Physiology & Behavior*. 1995; 57:1181–1185. [PubMed: 7652041]
- Colombo G, Maccioni P, Acciaro C, Lobina C, Loi B, Zaru A, et al. Binge drinking in alcohol-preferring sP rats at the end of the nocturnal period. *Alcohol*. 2014; 48:301–311. [PubMed: 24680256]
- Cryan JF, Kelly PH, Chaperon F, Gentsch C, Mombereau C, Lingenhoehl K, et al. Behavioral characterization of the novel GABA_B receptor-positive modulator GS39783 (N,N'-dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine): anxiolytic-like activity without side effects associated with baclofen or benzodiazepines. *The Journal of Pharmacology and Experimental Therapeutics*. 2004; 310:952–963. [PubMed: 15113848]
- File SE, Kenny PJ, Ouagazzal AM. Bimodal modulation by nicotine of anxiety in the social interaction test: role of the dorsal hippocampus. *Behavioral Neuroscience*. 1998; 112:1423–1429. [PubMed: 9926824]
- File SE, Lippa AS, Beer B, Lippa MT. Animal tests of anxiety. *Current Protocols in Neuroscience*. 2004; Chapter 8(Unit 8.3)

- Leggio B, Masi F, Grappi S, Nanni G, Gambarana C, Colombo G, et al. Sardinian alcohol-preferring and non-preferring rats show different reactivity to aversive stimuli and a similar response to a natural reward. *Brain Research*. 2003; 973:275–284. [PubMed: 12738071]
- Linsensardt DN, Boehm SL 2nd. Alterations in the rate of binge ethanol consumption: implications for preclinical studies in mice. *Addiction Biology*. 2014; 19:812–825. [PubMed: 23742054]
- Lobina C, Gessa GL, Colombo G. Anxiolytic effect of voluntarily consumed alcohol in Sardinian alcohol-preferring rats exposed to the social interaction test. *Journal of Alcoholism and Drug Dependence*. 2013; 1:132.
- Maccioni P, Fantini N, Froestl W, Carai MA, Gessa GL, Colombo G. Specific reduction of alcohol's motivational properties by the positive allosteric modulator of the GABAB receptor, GS39783--comparison with the effect of the GABAB receptor direct agonist, baclofen. *Alcoholism: Clinical and Experimental Research*. 2008; 32:1558–1564.
- Maccioni P, Flore P, Carai MA, Mugnaini C, Pasquini S, Corelli F, et al. Reduction by the positive allosteric modulator of the GABA(B) receptor, GS39783, of alcohol self-administration in Sardinian alcohol-preferring rats exposed to the "sipper" procedure. *Frontiers in Psychiatry*. 2010; 1:20. [PubMed: 21423431]
- Maccioni P, Pes D, Orrù A, Froestl W, Gessa GL, Carai MA, et al. Reducing effect of the positive allosteric modulator of the GABA(B) receptor, GS39,783, on alcohol self-administration in alcohol-preferring rats. *Psychopharmacology*. 2007; 193:171–178. [PubMed: 17393141]
- Maccioni P, Vargiolu D, Thomas AW, Malherbe P, Mugnaini C, Corelli F, et al. Inhibition of alcohol self-administration by positive allosteric modulators of the GABAB receptor in rats: lack of tolerance and potentiation of baclofen. *Psychopharmacology*. 2015; 232:1831–1841. [PubMed: 25420609]
- Maccioni P, Zaru A, Loi B, Lobina C, Carai MA, Gessa GL, et al. Comparison of the effect of the GABAB receptor agonist, baclofen, and the positive allosteric modulator of the GABAB receptor, GS39783, on alcohol self-administration in 3 different lines of alcohol-preferring rats. *Alcoholism: Clinical and Experimental Research*. 2012; 36:1748–1766.
- Mombereau C, Kaupmann K, Froestl W, Sansig G, van der Putten H, Cryan JF. Genetic and pharmacological evidence of a role for GABA(B) receptors in the modulation of anxiety- and antidepressant-like behavior. *Neuropsychopharmacology*. 2004; 29:1050–1062. [PubMed: 15039762]
- NIAAA National Advisory Council. NIAAA Council approves definition of binge drinking. *NIAAA Newsletter*. 2004; 3:3.
- Orrù A, Lai P, Lobina C, Maccioni P, Piras P, Scanu L, et al. Reducing effect of the positive allosteric modulators of the GABA(B) receptor, CGP7930 and GS39783, on alcohol intake in alcohol-preferring rats. *European Journal of Pharmacology*. 2005; 525:105–111. [PubMed: 16289452]
- Paterson NE, Hanania T. The modified Geller-Seifter test in rats was insensitive to GABAB receptor positive modulation or blockade, or 5-HT1A receptor activation. *Behavioural Brain Research*. 2010; 208:258–264. [PubMed: 20006648]
- Patrick ME, Schulenberg JE. Prevalence and predictors of adolescent alcohol use and binge drinking in the United States. *Alcohol Research*. 2013; 35:193–200. [PubMed: 24881328]
- Richter RM, Zorrilla EP, Basso AM, Koob GF, Weiss F. Altered amygdalar CRF release and increased anxiety-like behavior in Sardinian alcohol-preferring rats: a microdialysis and behavioral study. *Alcoholism: Clinical and Experimental Research*. 2000; 24:1765–1772.
- Roman E, Colombo G. Lower risk taking and exploratory behavior in alcohol-preferring sP rats than in alcohol non-preferring sNP rats in the multivariate concentric square field (MCSF) test. *Behavioural Brain Research*. 2009; 205:249–258. [PubMed: 19703494]
- Roman E, Stewart RB, Bertholomey ML, Jensen ML, Colombo G, Hyytiä P, et al. Behavioral profiling of multiple pairs of rats selectively bred for high and low alcohol intake using the MCSF test. *Addiction Biology*. 2012; 17:33–46. [PubMed: 21521426]
- Sweeney FF, O'Leary OF, Cryan JF. GABAB receptor ligands do not modify conditioned fear responses in BALB/c mice. *Behavioural Brain Research*. 2013; 256:151–156. [PubMed: 23933467]

- Thiele TE, Navarro M. "Drinking in the dark" (DID) procedures: a model of binge-like ethanol drinking in non-dependent mice. *Alcohol*. 2014; 48:235–241. [PubMed: 24275142]
- Urwyler S, Pozza MF, Lingenhoebl K, Mosbacher J, Lampert C, Froestl W, et al. N,N'-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine (GS39783) and structurally related compounds: novel allosteric enhancers of gamma-aminobutyric acidB receptor function. *The Journal of Pharmacology and Experimental Therapeutics*. 2003; 307:322–330. [PubMed: 12954816]
- White A, Hingson R. The burden of alcohol use: excessive alcohol consumption and related consequences among college students. *Alcohol Research*. 2013; 35:201–218. [PubMed: 24881329]

Highlights

- Alcohol drinking in sP rats is highly sensitive to time schedule of alcohol access
- Unpredictability of time of alcohol availability exacerbates this sensitivity
- We found that alcohol expectation was associated to anxiety-related behaviors
- Thus, sP rats might cope with this negative affective state by alcohol drinking
- The anxiolytic compound, GS392783, effectively reduced excessive alcohol intake

A Experiment 1A – Social Interaction Test **B** Experiment 2 – Effect of treatment with GS39783 **Fig. 1.**

Schematic representation of the experimental design of Experiments 1A and 2.

Fig. 2.

Left panel: Alcohol intake in Sardinian alcohol-preferring (sP) rats exposed to 12 consecutive daily 1-h drinking sessions under the 4-bottle “alcohol (10%, 20%, and 30%, v/v) vs. water” choice regimen. Drinking sessions occurred during one of the 12 hours of the dark phase of the daily light/dark cycle; time of the drinking session was changed daily in a semi-random order and was unpredictable to rats. Each point is the mean $\pm$ SEM of $n = 34$ rats. Right panel: Time spent in social interaction (SI) behaviors by pairs of sP rats exposed to the SI test at the 1st or 12th hour of the dark phase of the day after completion of the above-mentioned 12-day drinking period. Alcohol was not available on the test day. Data were collected in two equal time intervals (0–5 and 6–10 min) of the 10-min session; the right portion of this panel depicts data from the total session (0–10 min). Each bar is the mean $\pm$ SEM of $n = 8$ –9 rat pairs. * $p < 0.05$ and ** $p < 0.005$ in comparison to the “1st-Hour” rat group of the corresponding time interval (Newman-Keuls test). § $p < 0.01$ in comparison to the “1st-Hour” rat group (t test).

Fig. 3.

Time spent in social interaction (SI) behaviors by pairs of alcohol-naïve sP rats exposed to the SI test at the 1st or 12th hour of the dark phase. Data were collected in two equal time intervals (0–5 and 6–10 min) of the 10-min session. The right portion of this panel depicts data from the total session (0–10 min). Each bar is the mean $\pm$ SEM of $n = 12$ rat pairs.

Fig. 4.

Left panel: Alcohol intake in Sardinian alcohol-preferring (sP) rats exposed to 12 consecutive daily 1-h drinking sessions under the 4-bottle “alcohol (10%, 20%, and 30%, v/v) vs. water” choice regimen. Drinking sessions occurred during one of the 12 hours of the dark phase of the daily light/dark cycle; time of the drinking session was changed daily in a semi-random order and was unpredictable to rats. Each point is the mean $\pm$ SEM of $n = 32$ rats. Right panel: Effect of acute, intragastric administration of the positive allosteric modulator of the GABA_B receptor, GS39783, on alcohol intake in sP rats exposed to an additional drinking session conducted at the 12th hour of the dark phase of the day after completion of the above-mentioned 12-day drinking period. Each bar is the mean $\pm$ SEM of $n = 8$ rats. * $p < 0.0005$ in comparison to 0 mg/kg GS39783-treated rat group (Newman-Keuls test).

Table 1

Effect of acute, intragastric administration of the positive allosteric modulator of the GABA_B receptor, GS39783, on water and food intake in Sardinian alcohol-preferring (sP) rats exposed to an additional drinking session conducted at the 12th hour of the dark phase of the day after completion of an initial 12-day drinking period made of daily 1-h drinking sessions, during the dark phase of the daily light/dark cycle, under the 4-bottle “alcohol (10%, 20%, and 30%, v/v) vs. water” choice regimen, and with unpredictable time schedule

	GS39783 (mg/kg)			
	0	25	50	100
Water intake (mL/kg)	0.25 ± 0.08	0.09 ± 0.03	0.14 ± 0.05	0.09 ± 0.06
Food intake (g/kg)	7.96 ± 1.55	4.96 ± 0.96	6.87 ± 2.27	5.41 ± 0.75

Each value is the mean ± SEM of $n = 8$ rats.