REPEATED EXPOSURE TO ALCOHOLIC BEER DOES NOT INDUCE LONG-LASTING CHANGES IN ALCOHOL SELF-ADMINISTRATION AND INTAKE IN SARDINIAN ALCOHOL-PREFERRING AND SARDINIAN NON-PREFERRING RATS

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Abstract — Aims: Rats avidly consume non-alcoholic beer, and addition of alcohol to non-alcoholic beer may function as a medium to induce intake of large amounts of alcohol in rats. The present study investigated whether Sardinian alcohol-preferring (sP) and Sardinian non-preferring (sNP) rats, initially exposed to non-alcoholic beer, and subsequently to non-alcoholic beer containing increasing concentrations of alcohol, would develop unusually high alcohol self-administration and drinking behaviours: (i) when alcohol was added to non-alcoholic beer, and (2) once beer was withdrawn and a plain alcohol solution was made available.

Methods: In Experiment 1, rats were exposed to operant, 30-min/day self-administration sessions of non-alcoholic beer with increasing concentrations of alcohol [0, 2.5, 5, 7.5, and 10% (v/v)] for a total of 45 days. After a brief ‘beer-fading’ phase, the rats were exposed to self-administration sessions of a plain 10% (v/v) alcohol solution. In Experiment 2, the rats were exposed to non-alcoholic beer with increasing concentrations of alcohol [0, 2.5, 5, 7.5, and 10% (v/v)] and water under the 2-bottle choice regimen, with unlimited access (24 h/day) for a total of 35 days. After a brief ‘beer-fading’ phase, the rats were exposed to the choice between a plain 10% (v/v) alcohol solution and water.

Results: In Experiment 1, sP and sNP rats did not differ in self-administration (Experiment 1) and intake (Experiment 2) of non-alcoholic beer. In Experiment 1, as alcohol content increased, the amount of self-administered alcohol increased progressively in sP rats (up to 1–1.2 g/kg) and remained stable in sNP rats (approximately 0.65 g/kg). When the plain 10% alcohol solution was available, the amount of self-administered alcohol in sP rats initially dropped, and tended to increase—up to approximately 0.6 g/kg—on continuing exposure. In sNP rats, their lever-pressing behaviour was rapidly extinguished after beer withdrawal. In Experiment 2, as alcohol content was increased, daily alcohol intake increased progressively in sP rats (up to 8–9 g/kg) and averaged approximately 2.4 g/kg in sNP rats. When the plain alcohol solution was available, daily alcohol intake in sP rats was initially low, reaching control values on continuing exposure; conversely, daily alcohol intake was completely suppressed in sNP rats.

Conclusions: These results suggest that exposure to alcoholic beer resulted in unusually high intakes of alcohol in both sP and sNP rats as long as non-alcoholic beer was added to alcohol; however, these high levels of alcohol self-administration and intake were not maintained once non-alcoholic beer was withdrawn.
(Vacca et al., 2002); *vice versa*, rats of the sNP line exposed to the ‘sucrose fading’ procedure displayed negligible—if any—levels of oral alcohol self-administration (Vacca et al., 2002), as they avoid alcohol under the 2-bottle regimen.

Specifically, the present study assessed whether the repeated exposure to a highly accepted, non-alcoholic beer containing increasing concentrations of alcohol would result in an increase in alcohol consumption, over the levels usually recorded in sP and sNP rats tested under standard conditions, when (i) the non-alcoholic beer was added to alcohol, and (ii) beer was withdrawn and only the plain alcohol solution was available. To this aim, we measured alcohol self-administration behaviour in sP and sNP rats exposed to daily 30-min sessions of an operant procedure of oral self-administration (Experiment 1), and voluntary alcohol intake in sP and sNP rats continuously (24 h/day) exposed to the home cage 2-bottle choice paradigm (Experiment 2). In other words, the present study was designed to address the following two experimental questions: (i) Does an environmental manipulation such as the repeated exposure to alcoholic beer lead to the development of unusually, and possibly long-lasting, high levels of alcohol self-administration and intake in sP rats? (ii) Does the above manipulation overcome the genetically based aversion to alcohol in sNP rats?

The 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’.

**MATERIALS AND METHODS**

**Experiment 1: Materials and methods**

**Animals.** Adult male sP (*n* = 11) and sNP (*n* = 10) rats, from the 61st generation of both lines, and 75 days old at the start of the experiment, were used. The rats were housed four per cage in an animal facility with inverted 12:12 h light/dark cycle (lights on at 8:00 p.m.), constant temperature of 22 ± 2°C and relative humidity of approximately 60%. Standard rat chow was always available in the home cage. Water was also always available in the home cage, except as noted (see below). Rats were alcohol-naive at the start of the experiment.

**Experimental procedure.** Self-administration sessions were conducted in modular chambers (Med Associates, Georgia, VT, USA) located in sound-attenuated cubicles, with fans for ventilation and background white noise. The front panel of each chamber was equipped with (i) one retractable response lever, (ii) one liquid dipper (0.1-ml cup), positioned close to the lever, and (iii) one white stimulus light mounted above the lever. Achievement of the response requirement (see below) resulted in the dipper presentation and flashing of the stimulus light. Each chamber was also equipped with a white ‘house’ light, centred at the top of the back wall. Self-administration sessions lasted 30 min and were conducted 6 days per week (Monday–Saturday) during the dark phase of the light/dark cycle.

The rats were trained to lever-press for a non-alcoholic beer (see below). To assure initial lever-pressing behaviour, the rats were deprived of water in their home cage solely for the 24 h preceding the first exposure to the operant chamber. The rats were initially shaped under a fixed ratio (FR) schedule of 1 (FR1) (i.e. the number of lever responses required for dipper presentation). Over 4–8 sessions, the FR schedule was progressively increased to FR4. Once reached, FR4 was maintained from then onwards.

The rats were initially exposed to nine consecutive daily sessions with non-alcoholic beer (Phase 1). They were then exposed to four phases, each comprising nine consecutive daily sessions, with an alcoholic beer containing four increasing concentrations of alcohol [2.5, 5, 7.5, and 10% (v/v)] in each phase (Phases 2–5). Subsequently, they underwent three daily sessions of ‘beer-fading’, when beer was diluted at 75, 50, and 25% (v/v) in water (the alcohol concentration was maintained at 10%, v/v) (Phase 6). Finally, the rats were exposed to daily sessions (nine in sNP rats and 27 in sP rats) with a plain 10% (v/v) alcohol solution.

Non-alcoholic ‘Tourtel’ beer (G.P.B., Rome, Italy) was used. The beer was thoroughly decarbonated by pouring it in a 5–1 beaker kept under continuous agitation with a magnetic stirrer for at least 2 h before the start of the self-administration session. This vigorous agitation was also needed for evaporation of the low alcohol content (<0.5%) in the ‘Tourtel’ beer. Alcoholic beer was prepared adding the corresponding amount of alcohol to decarbonated non-alcoholic beer, which was then presented to the rats at room temperature.

**Variables and statistical analysis.** The measured variables were: (i) number of responses on the lever in all self-administration sessions, (ii) amount of self-administered non-alcoholic beer (expressed in ml/kg) in self-administration sessions when non-alcoholic beer was presented, (iii) amount of self-administered alcohol (expressed in g/kg pure alcohol) in self-administration sessions when alcoholic beer or plain alcohol solution was presented.

Data on the number of lever responses and the amount of self-administered non-alcoholic beer collected in Phase 1 were analysed by separate 2-way (rat line; day) ANOVAs with repeated measures on the factor ‘day’. Data on the number of lever responses and the amount of self-administered alcohol collected in Phases 2–5 were analysed by separate 3-way (rat line; alcohol concentration; day) ANOVAs with repeated measures on the factor ‘day’. Data on the number of lever responses and the amount of self-administered alcohol collected in Phase 6 were analysed by separate 2-way (rat line; day) ANOVAs with repeated measures on the factor ‘day’. Data on the number of lever responses and the amount of self-administered alcohol collected in the first nine sessions of Phase 7 were analysed by separate 2-way (rat line; day) ANOVAs with repeated measures on the factor ‘day’; further, data on the number of lever responses and the amount of self-administered alcohol in sP rats only—over the total 27 sessions of Phase 7—were analysed by separate 1-way ANOVAs.

**EXPERIMENT 1: RESULTS**

**Phase 1 (non-alcoholic beer)**

When exposed to the non-alcoholic beer, rats of the sP and sNP lines displayed robust self-administration behaviour,
to daily 30-min sessions of self-administration, under a FR4, of: (i) non-alcoholic beer (Phase 1), (ii) alcoholic beer with increasing concentrations of alcohol [2.5, 5, 7.5, and 10% (v/v)] (Phases 2–5), (iii) decreasing concentrations of beer [75, 50, and 25% (v/v) in water, with the alcohol concentration maintained at 10% (v/v)] (Phase 6), and (iv) 'plain' 10% (v/v) alcohol solution (Phase 7). Each point is the mean ± SEM of n = 10–11 rats. The letter 'A' reads 'alcohol'.

Phases 2–5 (alcoholic beer with increasing concentrations of alcohol)

Addition of increasing concentrations of alcohol resulted in a progressive reduction in the number of lever responses in both rat lines, with sP rats performing, however, a significantly higher number of responses, in comparison to sNP rats, at each alcohol concentration (Fig. 1) [F_{line}(1, 19) = 42.11, P < 0.0001; F_{concentration}(3, 76) = 36.99, P < 0.0001; F_{day}(8, 608) = 8.15, P < 0.0001; F_{lineXday}(8, 608) = 2.66, P < 0.01; F_{concentrationXday}(24, 608) = 3.95, P < 0.0001; F_{lineXconcentrationXday}(24, 608) = 0.85, P > 0.05].

In sP rats, the amount of self-administered alcohol increased progressively as alcohol concentration was augmented; specifically, it averaged approximately 0.65, 0.95, 1.05, and 1.20 g/kg when beer contained 2.5, 5, 7.5, and 10% alcohol, respectively (Fig. 2). Conversely, in sNP rats the amount of self-administered alcohol remained rather stable over the four phases, averaging 0.55, 0.60, 0.60, and 0.65 g/kg, respectively.
0.70 g/kg when beer contained 2.5, 5, 7.5, and 10% alcohol, respectively (Fig. 2) [$F_{\text{line}}(1, 76) = 32.41, P < 0.0001$; $F_{\text{concentration}}(3, 76) = 6.62, P < 0.0005$; $F_{\text{day}}(8, 608) = 6.01, P < 0.01$; $F_{\text{lineXday}}(8, 608) = 2.55, P < 0.05$; $F_{\text{concentrationXday}}(24, 608) = 2.97, P < 0.0001$; $F_{\text{lineXconcentrationXday}}(24, 608) = 0.99, P > 0.05$].

**Phase 6 (‘beer-fading’)**

Beer was withdrawn over three consecutive sessions, during which it was progressively diluted in water [75, 50, and 25% (v/v)], while the alcohol concentration was maintained at 10% (v/v). This phase was included in the experimental design in order to allow the rats to become accustomed to the new taste of the solution and to recognize the plain alcohol solution (presented in the subsequent Phase 7) as deriving from the beer beverage.

As beer was progressively faded out, the number of lever responses and the amount of self-administered alcohol decreased in sP rats, while both variables remained relatively stable in sNP rats (Figs 1 and 2) [number of responses: $F_{\text{line}}(1, 19) = 3.19, P > 0.05$; $F_{\text{day}}(2, 38) = 5.25, P < 0.01$; $F_{\text{lineXday}}(2, 38) = 1.92, P > 0.05$; amount of self-administered alcohol: $F_{\text{line}}(1, 19) = 1.38, P > 0.05$; $F_{\text{day}}(2, 38) = 5.02, P < 0.05$; $F_{\text{lineXday}}(2, 38) = 1.71, P > 0.05$].

**Phase 7 (plain alcohol solution)**

Comparison between sP and sNP rats was performed during the first nine sessions. Two-way ANOVA for the number of lever responses yielded the following results: $F_{\text{line}}(1, 19) = 48.40, P < 0.0001$; $F_{\text{day}}(8,152) = 1.89, P > 0.05$; $F_{\text{lineXday}}(8,152) = 4.31, P < 0.0005$. Two-way ANOVA for the amount of self-administered alcohol yielded the following results: $F_{\text{line}}(1,19) = 61.87, P < 0.0001$; $F_{\text{day}}(8,152) = 1.81, P > 0.05$; $F_{\text{lineXday}}(8,152) = 4.65, P < 0.0001$.

In sNP rats, beer removal and availability of the plain alcohol solution resulted in a virtually complete suppression of both, the number of lever responses and amount of self-administered alcohol from the first sessions and throughout the entire phase (Figs 1 and 2).

In sP rats, both, the number of lever responses and amount of self-administered alcohol were reduced by approximately 80% in comparison to values recorded in the last sessions of Phase 5, over the first three sessions of Phase 7, and tended to increase on continuing exposure (Figs 1 and 2) [results of 1-way ANOVA for number of responses: $F(25, 285) = 3.93, P < 0.0001$; results of 1-way ANOVA for amount of self-administered alcohol: $F(25, 285) = 3.80, P < 0.0001$]. Specifically, self-administered alcohol averaged approximately 0.3 g/kg over the first three sessions and then progressively increased, reaching an average value of 0.6 g/kg [i.e. the amount of alcohol usually consumed by sP rats in 30-min sessions of plain alcohol solution under a FR4 schedule (Vacca et al., 2002; Maccioni et al., 2005)] after 16 sessions (Fig. 2). From then onwards, alcohol self-administration remained stable at these levels (Figs 1 and 2).

**EXPERIMENT 1: DISCUSSION**

Availability of non-alcoholic beer (Phase 1) resulted in a sustained self-administration behaviour in both sP and sNP rats, without any significant line difference. Rats of both lines pressed the lever on an average 600–700 times over the 30-min session, gaining access to approximately 150–175 reinforcers, and consuming approximately 40 ml/kg/session of nonalcoholic beer. The lack of any line difference suggests that the reinforcing properties of non-alcoholic beer were virtually identical in sP and sNP rats.

As alcohol was added (at increasing concentrations from 2.5 to 10%) to non-alcoholic beer (Phases 2–5), the self-administration behaviour of sP and sNP rats was influenced—apparently in opposite directions—by two different factors: the gustatory attributes of non-alcoholic beer and the pharmacological effects of alcohol. In both rat lines, the gustatory attributes of beer apparently promoted addictive-like behaviour, which was conversely likely stopped by the occurrence of aversive and—at least in sP rats—in intoxicating (see below) effects of alcohol. In sP rats, the amount of self-administered alcohol averaged 1–1.2 g/kg/session during Phases 3–5 (5–10% alcohol), levels virtually double those (approximately 0.6 g/kg) usually achieved by sP rats when exposed to daily 30-min sessions of operant self-administration of a plain alcohol solution without addition of any tastant (Vacca et al., 2002; Maccioni et al., 2005). These levels of alcohol self-administration also match the highest alcohol consumption observed in sP rats under particular ‘promoting’ experimental conditions, including short-term alcohol access, re-exposure to alcohol after a period of alcohol abstinence (the so-called alcohol deprivation effect), and acute pretreatment with drugs—such as opioid and cannabinoid receptor agonists—known to stimulate alcohol intake in rodents (see Colombo et al., 2005, 2006). Notably, in the present study, signs of intoxication, including mild motor incoordination, ataxia, and muscle-relaxation, were occasionally observed in sP rats upon removal from the operant chamber immediately after the end of the self-administration session; these signs were frequent in those rats displaying the highest self-administration behaviour, with amounts of self-administered alcohol higher than 1.7 g/kg. Although based on the experimenter’s evaluation and not scored objectively, these observations confirm that alcohol self-administration during Phases 3–5 likely occurred at the highest levels that sP rats could perform.

In sNP rats, the amount of self-administered alcohol during Phases 2–5 (2.5–10% alcohol) was relatively high and stable, averaging approximately 0.65 g/kg/session, i.e. more than 20-fold higher than that recorded in sNP rats tested in daily 30-min sessions of operant self-administration of a plain alcohol solution (Vacca et al., 2002). This comparison clearly indicates the strong contribution of the gustatory attributes of beer to the alcohol self-administration behaviour in sNP rats. The different ‘roof’ limit of alcohol self-administration between sP (1–1.2 g/kg) and sNP (approximately 0.65 g/kg) rats is likely due to their inherent differences in sensitivity to the aversive effects of alcohol, limiting alcohol ingestion at different levels in the two rat lines.

The large amounts of alcohol self-administered by both sP and sNP rats during Phases 2–5 led to hypothesize that some...
degree of neuro-adaptation to alcohol could develop, resulting in a sustained self-administration of alcohol even when the beer taste was removed and only the plain 10% alcohol solution was available. According to this hypothesis, both sP and sNP rats should maintain, during Phase 7 (plain alcohol solution), levels of alcohol self-administration comparable to those recorded in Phases 2–5.

In order to test this hypothesis, beer was rapidly withdrawn over the three sessions of Phase 6, when it was progressively diluted with water. This procedure was preferred over an abrupt removal as it allowed the rat to become accustomed to the new taste of the solution and to recognize the plain alcohol solution as deriving from the alcoholic beer.

In sP rats, availability of the plain alcohol solution (Phase 7) resulted in an immediate, marked reduction in the number of lever responses and amount of self-administered alcohol. The average amount of self-administered alcohol dropped from approximately 1.2 g/kg/session (Phase 5) to approximately 0.3 g/kg/session over the first three sessions of Phase 7. Subsequently, the average amount of self-administered alcohol tended to increase, reaching the value of approximately 0.6 g/kg/session after 16 sessions, and remaining rather stable from then onwards. This initial drop and subsequent increase to the ‘usual’ level of alcohol self-administration (Vacca et al., 2002; Maccioni et al., 2005) may be interpreted as the consequence of a devaluation process of the reinforcing properties of the plain alcohol solution, when compared to those of alcoholic beer, and the need of several self-administration sessions before sP rats were able to evaluate the reinforcing properties of the plain alcohol solution. Pertaining to sNP rats, exposure to the plain alcohol solution resulted in an immediate and virtually complete stop of lever responding, with a subsequent suppression of alcohol self-administration that persisted over the entire Phase 7.

Together, the results of Phase 7 suggest that exposure to elevated amounts of alcohol in Phases 2–5 did not apparently induce any degree of neuro-adaptive process of ‘psychological dependence’ capable of inducing sP and sNP rats to display abnormal alcohol self-administration behaviour after removal of the beer taste. A possible reason for this failure may be related to the shortness of the daily self-administration session (30 min), during which alcohol drinking may resemble ‘binge’ drinking (as the amounts of self-administered alcohol were exceptionally high, although drinking episodes were distant in time), lacking, however, of a continuous exposure which might be a necessary condition for the hypothesized development of longer-lasting changes in the rat’s drinking behaviour.

In order to explore this possibility, a second independent experiment was conducted. In this experiment (named Experiment 2), sP and sNP rats were exposed to a non-alcoholic beer with increasing concentrations of alcohol (the same concentrations used in Experiment 1) for a relatively long period of time (28 consecutive days) under the standard, home cage, 2-bottle choice regimen with unlimited (24 h/day) access.

### EXPERIMENT 2: MATERIALS AND METHODS

#### Animals

Adult male sP (n = 18) and sNP (n = 20) rats, from the 65th generation of both lines, were used. The rats were individually housed in an animal facility with inverted 12:12 h light/dark cycle (lights on at 8:00 p.m.), constant temperature of 22 ± 2 °C and relative humidity of approximately 60%.

Standard rat chow was always available. Rats were alcohol-naïve at the start of the experiment.

#### Experimental procedure

At the age of 75 days, rats of each line were divided into two groups of n = 9–10 based on their body weight. Both groups were exposed to the standard, home cage, 2-bottle choice regimen with unlimited access for 24 h/day. One group (‘beer’) was given the choice between a non-alcoholic beer added with alcohol and water. The other group (‘plain’) was given the choice between a plain alcohol solution and water.

The experiment started with an alcohol-free, 7-day Phase 1, during which rats of the ‘beer’ group were exposed to two bottles containing a non-alcoholic beer and water, respectively, and rats of the ‘plain’ group were exposed to two bottles both containing water. In the subsequent Phases 2–5, alcohol concentration was increased progressively (0, 2.5, 5, 7.5, and 10%, v/v) every 7 days in both rat groups (‘beer’ and ‘plain’). Then, the beer was diluted over three consecutive days, at 75, 50, and 25% (v/v) in order the alcohol concentration was maintained at 10%, v/v (Phase 6).

Finally, also, rats of the ‘beer’ group were exposed to the plain 10% (v/v) alcohol solution for seven consecutive days (Phase 7). Bottles were refilled every day with fresh solution and their left-right positions interchanged at random to avoid development of position preference. Daily intake of each fluid was monitored by weighing the bottles (0.1–g accuracy) immediately before the start of the dark phase. Possible fluid spillage was calculated by using 20 bottles filled with the different fluids and positioned in empty cages interspersed in the cage racks; mean spill volumes were subtracted before data analysis. Non-alcoholic beer and alcoholic beer were prepared as detailed above.

#### Variables and statistical analysis

The measured variables were: (i) daily intake of non-alcoholic beer (expressed in ml/kg) over Phase 1, (ii) daily intake of alcohol solution (in the ‘plain’ rat groups) and alcoholic beer (in the ‘beer’ rat groups) (expressed in ml/kg) over Phases 2–6, (iii) daily intake of plain alcohol solution (expressed in ml/kg) in Phase 7, (iv) daily alcohol intake (expressed in g/kg pure alcohol) over Phases 2–7 (v) daily water intake in each phase, (vi) daily total fluid intake in each phase.

Data on daily fluid (non-alcoholic beer or water) and water intake during Phase 1 were analysed by separate 3-way ANOVAs (rat line; type of fluid; day) with repeated measures on the factor ‘day’; in the ‘plain’ rat groups, that during Phase 1 were exposed to two water bottles, one of them was associated with that of non-alcoholic beer of the ‘beer’ rat
groups for statistical analysis. Data on fluid (alcoholic beer or plain alcohol solution), pure alcohol, and water intake over Phases 2–5 were analysed by separate 4-way ANOVAs (rat line; type of fluid; concentration; day) with repeated measures on the factor ‘day’. Data on fluid (alcoholic beer or plain alcohol solution), pure alcohol, and water intake in Phase 6 were analysed by separate 3-way ANOVAs (rat line; type of fluid; day) with repeated measures on the factor ‘day’. Data on intake of plain alcohol solution, pure alcohol, and water in Phase 7 were analysed by separate 3-way ANOVAs (rat line; type of fluid; day) with repeated measures on the factor ‘day’.

EXPERIMENT 2: RESULTS

Phase 1 (non-alcoholic beer)
Both ‘beer’ sP and sNP rats displayed high levels of daily intake of non-alcoholic beer that averaged approximately 300 ml/kg without any line difference (Fig. 3). Results of ANOVAs are the following: \( F_{\text{line}}(1, 34) = 0.04, P > 0.05; F_{\text{fluid}}(1, 34) = 308.45, P < 0.0001; F_{\text{day}}(6, 204) = 0.83, P > 0.05; F_{\text{line} \times \text{fluid}}(1, 34) = 0.05, P > 0.05; F_{\text{line} \times \text{day}}(6, 204) = 0.30, P > 0.05; F_{\text{fluid} \times \text{day}}(6, 204) = 0.29, P > 0.05; F_{\text{line} \times \text{fluid} \times \text{day}}(6, 204) = 0.29, P > 0.05.

![Phase 1 Intake](https://academic.oup.com/alcalc/article-abstract/42/6/513/118861/518A.35x863.png)
Daily total fluid intake was approximately five-fold higher in both ‘beer’ sP and sNP rat groups than in the corresponding ‘plain’ rat groups (offered only water) (data not shown). Daily water intake was limited to very few ml in both ‘beer’ sP and sNP rat groups (Fig. 5).

Phases 2–5 (alcoholic beer with increasing concentrations of alcohol)

Addition of increasing concentrations of alcohol to non-alcoholic beer resulted in a progressive reduction in the amount of alcoholic beer consumed daily by ‘beer’ sP rats, being approximately 25, 45, 60, and 65% lower in Phases 2 (2.5% alcohol), 3 (5%), 4 (7.5%), and 5 (10%), respectively, than non-alcoholic beer volumes recorded in Phase 1 (Fig. 3, top panel). Addition of increasing concentrations of alcohol to non-alcoholic beer resulted in a reduction in the amount of alcoholic beer consumed daily by ‘beer’ sNP rats; this reduction was already evident in Phase 2 and evolved to a virtually complete suppression in Phases 4 and 5 (Fig. 3, bottom panel).

Addition of increasing concentrations of alcohol to the plain alcohol solution resulted in a relatively stable daily intake of the solution in ‘plain’ sP rats over Phases 2–5 (2.5–10% alcohol) (Fig. 3, top panel). Conversely, a progressive reduction was observed in ‘plain’ sNP rats over the four phases (Fig. 3, bottom panel).

When the two fluids (alcoholic beer and plain alcohol solution) were compared (Figs 3 and 4, bottom panels). Daily intake of alcoholic beer, plain alcohol solution, and pure alcohol were compared (Figs 3 and 4, bottom panels). Daily intake of alcoholic beer and pure alcohol was steadily lower than 1.0 g/kg over each phase, being even lower than that observed in the ‘beer’ sNP rat group (Fig. 4, top panel). These amounts were however 2–4 times lower than those recorded in the ‘beer’ sP rat group. In ‘plain’ sNP rats, daily intake of pure alcohol was steadily lower than 1.0 g/kg, being lower than that observed in the ‘beer’ sNP rat group (Fig. 4, bottom panel).

Results of ANOVAs are the following: $F_{\text{line}}(1, 136) = 505.01, P < 0.0001; F_{\text{fluid}}(1, 136) = 249.57, P < 0.0001; F_{\text{concentration}}(3, 136) = 43.01, P < 0.0001; F_{\text{day}}(6, 816) = 14.53, P < 0.0001; F_{\text{lineXfluid}}(1, 136) = 41.61, P < 0.0001; F_{\text{lineXconcentration}}(3, 136) = 0.67, P > 0.05; F_{\text{fluidXconcentration}}(3, 136) = 26.33, P < 0.0001; F_{\text{lineXday}}(6, 816) = 13.68, P < 0.0001; F_{\text{fluidXday}}(6, 816) = 6.29, P < 0.0001; F_{\text{concentrationXday}}(18, 816) = 5.95, P > 0.0001; F_{\text{lineXfluidXconcentration}}(3, 136) = 2.46, P > 0.05; F_{\text{lineXfluidXday}}(6, 816) = 12.17, P < 0.0001; F_{\text{lineXconcentrationXday}}(18, 816) = 6.19, P < 0.0001; F_{\text{fluidXconcentrationXday}}(18, 816) = 3.20, P < 0.0001; F_{\text{lineXfluidXconcentrationXday}}(18, 816) = 5.01, P < 0.0001.

Addition of increasing concentrations of alcohol to non-alcoholic beer resulted in a progressive increase in daily intake of pure alcohol in ‘beer’ sP rats, averaging approximately 4.7, 6.8, 7.5, and 8.4 g/kg in Phases 2, 3, 4, and 5, respectively (Fig. 4, top panel). Conversely, in ‘beer’ sNP rats, the addition of increasing concentrations of alcohol to non-alcoholic beer resulted in a relatively stable daily intake of pure alcohol over Phases 2–4 (averaging approximately 2.4 g/kg), followed by a marked reduction in Phase 5 (when daily alcohol intake averaged approximately 1.5 g/kg) (Fig. 4, bottom panel).

Addition of increasing concentrations of alcohol to the plain alcohol solution resulted in a progressive increase in daily intake of pure alcohol in ‘plain’ sP rats, averaging approximately 1.3, 2.9, 4.2, and 4.6 g/kg in Phases 2, 3, 4, and 5, respectively (Fig. 4, top panel). These amounts were however 2–4 times lower than those recorded in the ‘beer’ sP rat group. In ‘plain’ sNP rats, daily intake of pure alcohol was steadily lower than 1.0 g/kg, being lower than that observed in the ‘beer’ sNP rat group (Fig. 4, bottom panel).

Results of ANOVAs are the following: $F_{\text{line}}(1, 136) = 505.01, P < 0.0001; F_{\text{fluid}}(1, 136) = 249.57, P < 0.0001; F_{\text{concentration}}(3, 136) = 21.26, P < 0.0001; F_{\text{day}}(6, 816) = 10.19, P < 0.0001; F_{\text{lineXfluid}}(1, 136) = 50.39, P < 0.0001; F_{\text{lineXconcentration}}(3, 136) = 0.97, P < 0.05; F_{\text{lineXday}}(6, 816) = 13.42, P < 0.0001; F_{\text{fluidXday}}(6, 816) = 4.67, P < 0.0005; F_{\text{concentrationXday}}(18, 816) = 3.03, P < 0.0001; F_{\text{lineXfluidXconcentration}}(3, 136) = 1.12, P > 0.05; F_{\text{lineXfluidXday}}(6, 816) = 10.13, P < 0.0001; F_{\text{lineXconcentrationXday}}(18, 816) = 3.38, P < 0.0001; F_{\text{fluidXconcentrationXday}}(18, 816) = 2.25, P < 0.0005.

Daily water intake was steadily higher in sNP than sP rats, irrespective of the type of the alternate solution (non-alcoholic beer or water) (Fig. 5). Starting from Phase 3 (5% alcohol), daily water intake in sNP rats was steadily higher in ‘beer’ than ‘plain’ group (Fig. 5, top panel). Conversely, no difference in daily water intake was recorded in ‘beer’ and ‘plain’ sNP rat groups (Fig. 5, bottom panel).

Results of ANOVAs are the following: $F_{\text{line}}(1, 136) = 275.59, P < 0.0001; F_{\text{fluid}}(1, 136) = 7.69, P < 0.01; F_{\text{concentration}}(3, 136) = 21.84, P < 0.0001; F_{\text{day}}(6, 816) = 25.08, P < 0.0001; F_{\text{lineXfluid}}(1, 136) = 46.03, P < 0.0001; F_{\text{lineXconcentration}}(3, 136) = 2.51, P > 0.05; F_{\text{fluidXconcentration}}(3, 136) = 5.25, P < 0.0001; F_{\text{lineXday}}(6, 816) = 133.42, P < 0.0001; F_{\text{fluidXday}}(6, 816) = 5.82, P < 0.0001; F_{\text{concentrationXday}}(18, 816) = 13.18, P < 0.0001; F_{\text{lineXfluidXconcentration}}(3, 136) = 1.56, P > 0.05; F_{\text{lineXfluidXday}}(6, 816) = 3.00, P < 0.0001; F_{\text{lineXconcentrationXday}}(18, 816) = 6.68, P < 0.0001; F_{\text{fluidXconcentrationXday}}(18, 816) = 2.27, P < 0.0005; F_{\text{lineXfluidXconcentrationXday}}(18, 816) = 2.86, P < 0.0001.

Phase 6 (‘beer-fading’)

Similarly to Experiment 1, beer was withdrawn over three consecutive days, during which it was progressively diluted in water [75, 50, and 25% (v/v)] while alcohol concentration was maintained at 10% (v/v). Reasons for including this phase in the experimental design are given above (see Experiment 1). In ‘beer’ sP rats, dilution of beer from 75 to 25% resulted in a reduction in daily intake of alcoholic beer (Fig. 3, top panel) and daily intake of pure alcohol (Fig. 4, top panel); values of both variables were, however, markedly higher than those observed in the ‘plain’ sP rat group (displaying a constant consumption of approximately 4.3 g/kg/day over the 3 days). No change in daily intake of alcoholic beer and pure alcohol was observed in sNP rats when (i) compared to data collected in Phase 5, and (ii) data from ‘beer’ and ‘plain’ rat groups were compared (Figs 3 and 4, bottom panels). Daily intake of alcoholic beer, plain alcohol solution, and pure alcohol were steadily higher in sP than sNP rats (Figs 3 and 4).

Results of ANOVAs for fluid intake and alcohol intake are the following: $F_{\text{line}}(1, 24) = 145.44,
Fig. 4. Intake of alcohol (expressed in g/kg) in Sardinian alcohol-preferring (sP) (top panel) and Sardinian alcohol non-preferring (sNP) (bottom panel) rats exposed to the home cage 2-bottle choice with water and unlimited access for 24 h/day. Rats of the ‘beer’ groups were exposed to: (i) non-alcoholic beer (Phase 1), (ii) alcoholic beer with increasing concentrations of alcohol [2.5%, 5%, 7.5%, and 10% (v/v)] (Phases 2–5), (iii) decreasing concentrations of beer [75%, 50%, and 25% (v/v) in water, with the alcohol concentration maintained at 10% (v/v)] (Phase 6), and (iv) plain 10% (v/v) alcohol solution (Phase 7). Rats of the ‘plain’ groups were exposed to: (i) water (Phase 1), (ii) plain alcohol solution with increasing concentrations of alcohol [2.5%, 5%, 7.5%, and 10% (v/v)] (Phases 2–5), (iii) plain 10% (v/v) alcohol solution (Phases 6 and 7). Each point is the mean ± SEM of n = 9–10 rats. The letter ‘A’ reads ‘alcohol’.

Phase 5 (10% A in beer)

Phase 7 (plain alcohol solution)

Beer withdrawal resulted in a marked reduction in daily intake of plain alcohol solution in the ‘beer’ sP rat group. Specifically, over the first 3 days of Phase 7, daily intake of the plain alcohol solution was approximately 70% lower than that recorded in the last days of Phase 5 (Fig. 3, top panel). Accordingly, intake of pure alcohol on the first 3 days

\[ F_{\text{line}}(1,34) = 57.55, \quad P < 0.0001; \quad F_{\text{fluid}}(1,34) = 6.10, \quad P < 0.05; \quad F_{\text{day}}(2,68) = 1.79, \quad P > 0.05; \quad F_{\text{line} \times \text{fluid}}(1,34) = 4.47, \quad P < 0.05; \quad F_{\text{line} \times \text{day}}(2,68) = 0.22, \quad P > 0.05; \quad F_{\text{fluid} \times \text{day}}(2,68) = 1.23, \quad P > 0.05. \]

In each rat group, the pattern of daily water intake was exceedingly similar to that observed in Phases 3–5. Specifically, daily water intake was steadily higher (i) in sNP than sP rats, irrespective of the type of alternate solution (non-alcoholic beer or water), and (ii) in ‘beer’ than ‘plain’ sP rat group (Fig. 5).

Results of ANOVAs are the following: \( F_{\text{line}}(1,34) = 57.55, \quad P < 0.0001; \quad F_{\text{fluid}}(1,34) = 6.10, \quad P < 0.05; \quad F_{\text{day}}(2,68) = 1.79, \quad P > 0.05; \quad F_{\text{line} \times \text{fluid}}(1,34) = 4.47, \quad P < 0.05; \quad F_{\text{line} \times \text{day}}(2,68) = 0.22, \quad P > 0.05; \quad F_{\text{fluid} \times \text{day}}(2,68) = 1.23, \quad P > 0.05. \)
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Fig. 5. Intake of water (expressed in ml/kg) in Sardinian alcohol-preferring (sP) (top panel) and Sardinian alcohol non-preferring (sNP) (bottom panel) rats exposed to the home cage 2-bottle choice, with water and unlimited access for 24 h/day. Rats of the ‘beer’ groups were exposed to: (i) non-alcoholic beer (Phase 1), (ii) alcoholic beer with increasing concentrations of alcohol [2.5, 5, 7.5, and 10% (v/v)] (Phases 2–5), (iii) decreasing concentrations of beer [75, 50, and 25% (v/v) in water, with the alcohol concentration maintained at 10% (v/v)] (Phase 6), and (iv) plain 10% (v/v) alcohol solution (Phase 7). Rats of the ‘plain’ groups were exposed to: (i) water (Phase 1), (ii) plain alcohol solution with increasing concentrations of alcohol [2.5, 5, 7.5, and 10% (v/v)] (Phases 2–5), (iii) plain 10% (v/v) alcohol solution (Phases 6 and 7). Each point is the mean ± SEM of n = 9–10 rats. The letter ‘A’ reads ‘alcohol’.

of Phase 7 averaged 2.6–3.2 g/kg/day (Fig. 4, top panel). However, alcohol consumption increased on the following days, averaging approximately 4.3 g/kg on days 6 and 7 (Fig. 4, top panel). In the ‘plain’ sP group, not subjected to change in the alcohol solution to which it was exposed, daily alcohol intake remained stable, replicating the same values recorded in Phases 5 and 6 (approximately 4.6 g/kg) (Fig. 4, top panel).

In ‘beer’ sNP rats, beer withdrawal resulted in a virtually complete suppression of daily alcohol intake [both in terms of intake of alcohol solution (Fig. 3, bottom panel) and pure alcohol (Fig. 4, bottom panel)] throughout the entire phase. Values recorded in ‘beer’ sNP rat group were steadily lower (by approximately 75%) than those recorded in ‘plain’ sNP rat group.

Results of ANOVAs are the following: $F_{\text{line}}(1, 34) = 86.76$, $P < 0.0001$; $F_{\text{fluid}}(1, 34) = 6.98$, $P < 0.05$; $F_{\text{day}}(6, 204) = 6.54$, $P < 0.0001$; $F_{\text{lineXday}}(1, 34) = 0.18$, $P > 0.05$; $F_{\text{fluidXday}}(6, 204) = 4.86$, $P < 0.0005$; $F_{\text{lineXfluidXday}}(6, 204) = 0.94$, $P > 0.05$; $F_{\text{lineXfluidXday}}(6, 204) = 2.07$, $P > 0.05$.

Previous exposure to beer apparently influenced water intake in Phase 7, as both ‘beer’ sP and sNP rat groups had higher daily intakes of water than the two corresponding ‘plain’ rat groups (Fig. 5). Nevertheless, daily water intake tended to be higher in sNP than sP rats (Fig. 5). Finally, in the ‘beer’ sP rat group, daily water intake was initially...
high (approximately 45 ml/kg) and decreased markedly on continuing exposure (Fig. 5), in a negative correlation with daily alcohol intake that, instead, tended to progressively increase (Fig. 3, top panel); as a result, daily total fluid intake was unaltered (data not shown).

Results of ANOVAs are the following: $F_{\text{line}}(1, 34) = 33.56$, $P < 0.0001$; $F_{\text{fluid}}(1, 34) = 20.99$, $P < 0.0001$; $F_{\text{day}}(6, 204) = 6.40$, $P < 0.0001$; $F_{\text{line} \times \text{fluid}}(1, 34) = 1.52$, $P > 0.05$; $F_{\text{line} \times \text{day}}(6, 204) = 6.44$, $P < 0.0001$; $F_{\text{fluid} \times \text{day}}(6, 204) = 3.85$, $P < 0.005$; $F_{\text{line} \times \text{fluid} \times \text{day}}(6, 204) = 3.30$, $P < 0.005$.

EXPERIMENT 2: DISCUSSION

Availability of non-alcoholic beer (Phase 1) resulted in a polydipsic-like intake in both sP and sNP rats, without any significant line difference. Indeed, rats from the ‘beer’ groups of both lines consumed approximately 300 ml/kg non-alcoholic beer daily; this intake was approximately five-fold higher than water intake observed in the two ‘plain’ rat groups (corresponding to the daily fluid requirement of a healthy rat of the age concerned).

Addition of alcohol to non-alcoholic beer (at increasing concentrations from 2.5 to 10%; Phases 2–5) resulted in patterns of drinking behaviour similar to those of alcohol self-administration behaviour observed in Experiment 1. Specifically, in sP rats, daily alcohol intake progressively rose to levels of 8–9 g/kg, up to approximately 50% higher than those (around 6 g/kg) usually observed in sP rats exposed to the standard 10% alcohol solution versus water choice (see Colombo et al., 2006). The levels of daily alcohol intake observed in ‘beer’ sP rats were even double those recorded in ‘plain’ sP rats. Quite unexpectedly, in the latter group, exposure to increasing concentrations of alcohol, rather than to the 10% alcohol solution from the very beginning [as usually done with sP rats (see Colombo et al., 2006)], resulted in relatively lower daily intakes of alcohol, also at the 10% concentration. These data, confirmed in a subsequent, independent experiment (this laboratory, unpublished results), suggest that the concentration at which alcohol is presented may have an impact on the acquisition of alcohol drinking behaviour in sP rats.

In ‘beer’ sNP rats, daily alcohol intake was markedly higher than that observed in ‘plain’ sNP rats exposed to the original 10% alcohol solution. However, daily alcohol intake in ‘beer’ sNP rats was relatively low (Phases 2–4, 2.5–7.5% alcohol), while it approached control levels (around 1 g/kg) at the 10% alcohol concentration.

These results of Phases 2–5 apparently complement those collected in Experiment 1, and suggest that alcohol intake, as well as alcohol self-administration of ‘beer’ sP and sNP rats was influenced by two factors: the gustatory attributes of non-alcoholic beer, and the pharmacological effects of alcohol. In both rat lines, the gustatory attributes of beer promoted alcohol drinking up to levels where the likely occurrence of the aversive effects of alcohol (set at markedly different levels in the two rat lines) presumably stopped drinking.

Again, the unusually large amounts of alcohol consumed by both ‘beer’ sP and sNP rats during Phases 2–5, associated with the relatively long (28 days) and continuous (24 h/day) exposure to alcohol led to hypothesize that some degree of neuro-adaptation to alcohol could develop, and daily alcohol consumption be set at high levels also when the beer taste had been removed and only plain 10% alcohol solution was available. However, as seen in Experiment 1, this was not the case.

In ‘beer’ sP rats, beer removal and availability of the plain alcohol solution (Phase 7) were associated with an immediate, marked reduction in daily alcohol intake, dropping to levels (around 3 g/kg) approximately 70% lower than those observed in Phase 5, and even lower than those recorded in the ‘plain’ sP rat group (averaging 4.6 g/kg). The average daily intake of alcohol in ‘beer’ sP rats tended to increase over the following days, reaching control values (represented by the values of the ‘plain’ sP rats) after 5 days. This pattern of alcohol drinking closely resembles that of alcohol self-administration observed in Phase 7 of Experiment 1, and is suggestive of an initial devaluation of the plain alcohol solution, when compared to alcoholic beer.

In ‘beer’ sNP rats, beer withdrawal and the availability of the plain alcohol solution resulted in an immediate and virtually complete suppression of alcohol drinking; daily alcohol intake in ‘beer’ sNP rats was even lower, on all 7 days of Phase 7, than that recorded in ‘plain’ sNP rats.

DISCUSSION

Phases 1 of both experiments (when rats were exposed to non-alcoholic beer) clearly confirm previous literature data on the high acceptability of non-alcoholic beer by rats (see the Introduction for references), as both sP and sNP rats consumed extremely large amounts of non-alcoholic beer (approximately 40 ml/kg/30-min and 300 ml/kg/day in Experiments 1 and 2, respectively). Notably, no difference in self-administration or intake of non-alcoholic beer was observed between sP and sNP rats. These data are in close agreement with previous observations demonstrating that sP and sNP rats—unlike the majority of other pairs of rat lines selectively bred for opposite alcohol preference and consumption—consumed comparable quantities of highly palatable fluids, including solutions of sucrose or saccharin, and chocolate-flavoured beverages (see Colombo et al., 2006).

The large intakes of non-alcoholic beer observed in the present study also confirm the hypothesis that non-alcoholic beer can be used as a medium to induce rats to consume large amounts of alcohol (Lancaster et al., 1987; Gallate et al., 2003). Indeed, both sP and sNP rats increased—to unusually high levels—the amount of self-administered alcohol (Experiment 1) or alcohol consumed under the 2-bottle choice regimen (Experiment 2) when alcohol was added to non-alcoholic beer. In sP rats (a) the average amount of self-administered alcohol over the daily 30-min session peaked up to 1.2 g/kg, approximately two-fold higher than that usually recorded in sP rats exposed to a plain alcohol solution (Vacca et al., 2002; Maccioni et al., 2005), and (b) the average daily intake of alcohol under the 2-bottle choice regimen peaked up to 9 g/kg, approximately 50% higher than that usually observed in sP rats exposed to a plain alcohol solution (see Colombo et al., 2006). In sNP rats (i) the average amount...
of self-administered alcohol over the daily 30-min session achieved the level of 0.6 g/kg, approximately 20-fold higher than that usually recorded in sNP rats exposed to a plain alcohol solution (Vacca et al., 2002), and (ii) the average daily intake of alcohol under the 2-bottle choice regimen peaked up to 2.5–3 g/kg, approximately five-fold higher than that usually observed in sNP rats exposed to a plain alcohol solution (see Colombo et al., 2006).

These data suggest that both the gustatory attributes of non-alcoholic beer and the pharmacological effects of alcohol contributed to alcohol self-administration and intake in both rat lines. Specifically, beer taste apparently promoted alcohol self-administration and intake to these unusually high levels; the promoting effect of beer taste on alcohol self-administration and intake was particularly evident in sNP rats, suggesting its capability in overcoming animals' gustatory aversion to the plain alcohol solution that otherwise limits alcohol self-administration and intake to the very low levels usually observed. Self-administration and intake of the alcoholic beer was then likely limited by perception of the aversive effects of alcohol. In other words, it is possible that a dual ‘set-point’ regulates alcohol drinking in sNP rats: the lower set-point reflects a gustatory aversion to the plain alcohol solution, as addition of beer permitted to increase alcohol self-administration (Experiment 1) and alcohol intake (Experiment 2); the higher set-point reflects the pharmacological aversive properties of alcohol.

In sP rats, alcohol self-administration and intake was likely promoted by the sum of the gustatory attributes of beer and the reinforcing effects of alcohol, resulting in an upward move of the ‘set-point’ that limits alcohol consumption in these rats. It is of interest to note that, in spite of the ‘promoting’ effect of beer taste, the inherently determined limits to alcohol self-administration and intake in sP and sNP rats were set at greatly distant levels.

 Exposure to alcohol when alcohol was mixed with non-alcoholic beer (Phases 2–5), both under the ‘binge’-like drinking of Experiment 1, and the continuous access of Experiment 2, did not lead to sustained levels of alcohol self-administration or intake after beer removal (Phase 7) in any rat line. In sP rats, alcohol self-administration and intake were even under the control levels over the first days of Phases 7; in sNP rats, availability of the plain alcohol solution resulted in an immediate and virtually complete suppression of alcohol self-administration and intake.

Together, the results of Phase 7 of both Experiments 1 and 2 suggest that exposure to unusually elevated amounts of alcohol (Phases 2–5) did not induce any neuro-adaptive process of ‘psychological dependence’ that would lead sP and sNP rats to abnormal alcohol self-administration and drinking behaviours. A possible limitation of the present study is the duration of exposure to alcohol, as it could have been not long enough to produce these changes; additional experiments may add to this point.

These data closely complement those of a previous study (Brunetti et al., 2003) demonstrating that the prolonged (108 daily 30-min sessions) exposure to a palatable ‘alcohol plus sucrose’ mixture did not result in any aberrant alcohol intake in sP and sNP rats when sucrose was faded out. In the previous study (Brunetti et al., 2003), both sP and sNP rats displayed high levels of alcohol intake for as long as a high concentration of sucrose was mixed to alcohol (up to 1.2 g/kg and 1.8 g/kg alcohol in sNP and sP rats, respectively). However, sucrose withdrawal was associated with an average intake of alcohol of approximately 0.2 g/kg/session (and of 1–1.5 g/kg when alcohol was available with unlimited access for 24 h/day) and of 0.6–1.2 g/kg/session (5.5–6.5 g/kg/day) in sNP and sP rats, respectively. These values were similar to those repeatedly recorded in rats of both lines when exposed to a plain alcohol solution with comparable temporal periods of access to alcohol (see Colombo et al., 2006). Notably, the above prolonged exposure to the ‘alcohol plus sucrose’ mixture induced a long-lasting intake of pharmacologically relevant amounts of alcohol in selectively bred, Indiana alcohol non-prefering (NP) rats (Gauvin et al., 1998), suggesting that this procedure was able to totally overcome the genetically determined aversion of these rats to alcohol.

In conclusion, the results of the present study suggest that an environmental manipulation represented by exposure to a highly palatable and reinforcing alcoholic beer promoted high levels of alcohol self-administration and intake in both sP and sNP rats only when alcohol was mixed with beer. Indeed, removal of non-alcoholic beer and exposure to a plain alcohol solution was associated with (i) marked reduction in alcohol self-administration and intake in sP rats, and (ii) suppression of alcohol self-administration and intake in sNP rats.

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