Neuropharmacology 59 (2010) 388–394

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Reduced alcohol drinking in adult rats exposed to sucrose during adolescence

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ARTICLE INFO

Article history:
Received 23 February 2010
Received in revised form
24 May 2010
Accepted 25 May 2010

Keywords:
Natural reward
Pharmacological reward
Alcoholism
Consummatory behavior
Two-bottle free-choice paradigm
Operant intravenous self-administration

ABSTRACT

Intake of sweet-alcoholic drinks during adolescence is believed to favor alcohol abuse and dependence in adulthood. This study examined the influence of early exposure to ethanol with or without sucrose on the consumption of sweet or alcoholic solutions in adulthood. Adolescent rats (from post-natal day 30–46) were given continuous free access to tap water and either 5% sucrose, 5% ethanol or mixed 5% sucrose–5% ethanol. The control group was given access to water only. Upon reaching adulthood (post-natal day 60), rats were tested for saccharin (sweet), quinine (bitter) and ethanol consumption using a two-bottle free-choice paradigm. The results indicated that pre-exposure to ethanol did not alter the intake of sweet or ethanol solutions in adulthood. However, rats exposed to sucrose during adolescence showed a decreased consumption of both sweet and ethanol solutions. Because alcohol has a sweet taste component, an additional group of rats, pre-exposed to either 5% sucrose or water during adolescence, was tested for intravenous ethanol self-administration (preventing oral sensory stimulation) and in a new model of simultaneous access to oral saccharin and intravenous ethanol that results in higher total ethanol intake. Relative to controls, sucrose-exposed rats showed reduced operant self-administration of saccharin, yet no differences were found for intravenous ethanol self-administration. Altogether, these findings indicate that sucrose exposure during adolescence persistently affected the perception of sweet taste reward and thereby alcohol's acceptance in adulthood.

1. Introduction

Adolescence is a time of dramatic changes in brain organization and function that prepares the individual for adulthood. During this period, the individual is also more vulnerable to external insult. Exposure to drugs of abuse, including alcohol, during adolescence may elicit enduring changes in brain development and behavioral abnormalities in adulthood (Spear, 2000a, 2000b; Andersen, 2003; Adriani and Laviola, 2004; Crews et al., 2007; Vendruscolo et al., 2008). These effects may explain epidemiological observations showing a positive relationship between alcohol abuse during adolescence and future alcohol dependence (Bates and Labouvie, 1997).

Sweet-alcoholic beverages, known as *Alcopops* or *Premixed drinks*, have become increasingly popular over the last 20 years. These drinks, often served in bottles with attractive packaging, contain between 4 and 7% ethanol and may contribute to excessive ethanol consumption among adolescents (McKeganey, 1998; Metzer and Kraus, 2007; Copeland et al., 2007). The sweet flavor of these drinks is believed to be particularly important in increasing ethanol intake, but, the impact of this type of drink on alcohol abuse among adolescents has been controversial (Metzner and Kraus, 2008). Studies using animal models of alcohol intake may be useful in clarifying this issue.

A close relationship between alcohol drinking and the intake of sweet drinks has been demonstrated (Overstreet et al., 1997; Kampov-Polevoy et al., 1999; Terenina-Rigaldie et al., 2003). This could be explained by the sweet taste component of ethanol (Scinska et al., 2000; Blednov et al., 2008) or, alternatively, the rewarding effects of sweet and alcohol solutions may share overlapping brain mechanisms (Kampov-Polevoy et al., 2001). Interestingly, sweet flavors produce a sensation of intense reward (Berridge, 2003) that under certain conditions surpasses reward associated with drugs (Mormède et al., 2004; Lenoir et al., 2007). However, few studies have investigated the effects of free access to a sweet solution during adolescence on the intake of sweet or ethanol solutions in adulthood.

In rodents, the impact of ethanol exposure during adolescence on behavior during adulthood is not clear. Some reports have

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doi:10.1016/j.neuropharm.2010.05.015

indicated a subsequent increase in ethanol intake in adulthood (Ho et al., 1989; Siciliano and Smith, 2001; Yoshimoto et al., 2002; Rodd-Henricks et al., 2002; Blizard et al., 2004; Moore et al., 2010; Pascual et al., 2009), whereas others detected no change (Slawecki and Betancourt, 2002; Siegmund et al., 2005; Füllgrabe et al., 2007; Vetter et al., 2007; Hargreaves et al., 2009; Gurkovskaya et al., 2009; Moore et al., 2010). To our knowledge, only one study has investigated the effects of exposure to a sweetened alcoholic solution during adolescence on ethanol intake in adulthood. Vetter et al. (2007) have reported that adolescent rats having access to saccharin sweetened ethanol solution did not show changes in their ethanol intake in adulthood. However, the saccharin solution was unexpectedly not preferred compared to water by adolescent rats in this latter study leaving some kind of uncertainties on the exact nature of the effects.

The objective of this study was to investigate the effect of unlimited access to ethanol and/or sucrose during adolescence on the oral consumption of sweet and alcoholic solution in adulthood. We hypothesized that pre-exposure to ethanol or a sweet solution would induce changes in consummatory behavior later in life and that this effect would be more pronounced following exposure to a combined sweetened alcoholic solution. Additional rats were tested for intravenous ethanol self-administration, thus preventing interference by any chemosensory factors (taste and smell) of ethanol (Bachmanov et al., 2003). This effect was tested in two different situations: 1) in a regular ethanol operant self-administration paradigm (Gass and Olive, 2007) and 2) with concurrent access to oral saccharin and intravenous ethanol self-administration that elicited higher levels of ethanol intake.

2. Methods

2.1. Subjects

Fifty-one male Wistar rats weighing 50–75 g (post-natal day [P] 26) were purchased from Charles River Laboratories (IFFA/CREDO, France). Animals were individually housed in plastic cages and maintained under a 12 h light/dark cycle (lights on at 7:30) at 21 ± 2 °C. Food and water were provided *ad libitum*. This study was conducted in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC).

2.2. Exposure to different solutions during adolescence

As similar behavioral and neurobiological changes have been observed in rodents aged from P30-46 and in humans during adolescence (Spear, 2000a; Andersen, 2003; Crews et al., 2007), testing rats during this period is widely accepted as a valid model of adolescence and was therefore implemented in the present study. After four days of acclimatization to the animal facilities, adolescent rats (P30) were split into four experimental groups ($n = 8$ per group) matched by body weight. Across 16 days (P30-46) each group was given continuous free choice in their home cages between water and an additional bottle (treatment solution) containing either water, 5% sucrose (w/v; SUC), 5% ethanol (v/v, prepared from 95% ethyl alcohol; EtOH) or a mixture of 5% sucrose and 5% ethanol (SUC + EtOH; a mixture that approximates the amount of sugar and ethanol in commercially available *Premixed drinks*). After this period, rats had access to water only. Animals were then kept undisturbed in their home cages until reaching adulthood (>P60) at which time behavioral testing started.

2.3. Voluntary consumption of saccharin, quinine and ethanol in adulthood

A standardized testing procedure for saccharine, quinine and ethanol consumption was used (Vendruscolo et al., 2006, 2008), with some modifications. Saccharin and quinine were used to test the intake of sweet and bitter substances, respectively, since both of them are components of the ethanol taste (Scinska et al., 2000; Blednov et al., 2008; Bachmanov et al., 2003).

Across a 24-h period, rats were given a two-bottle free choice between saccharin solution (0.13% w/v; Sigma) and water in their home cages. For quinine consumption, the test followed the same protocol as for saccharin, with a 0.0001% quinine (w/v; Sigma) solution being used. The data were expressed in ml of saccharin (or quinine) and water consumed over 24 h.

For ethanol, rats were given free choice between water and ethanol (10% v/v) across 18 days. The bottles were weighed (refilled if necessary) every 2 days within the same 2-h interval. The data were expressed as average intake (g/kg/day) and preference (%) over the 18-day period of ethanol consumption. Interspersed between saccharin, quinine and ethanol conditions, water was given as the sole source of drinking fluid for two days. The body weight of the rats was recorded at least once a week.

2.4. Intravenous ethanol self-administration in adulthood

Because a significant effect of sucrose on ethanol intake was observed (see Results) and because the consumption of ethanol is motivated by both its taste and its pharmacological effect (Blednov et al., 2007), additional groups of adolescent rats were exposed to sucrose ($n = 10$) or water ($n = 9$) as described above and tested for intravenous operant ethanol self-administration in adulthood. This procedure prevents gustatory stimulation thus assessing specifically the pharmacological component of ethanol. These rats were individually housed in plastic cages and maintained under a reversed 12 h light/dark cycle (lights off at 8:00) at 21 ± 2 °C. Behavioral tests were performed between 13:00 and 17:00, 5–7 days a week.

Rats were anesthetized with an intraperitoneal injection of a mixture of Ketamine (100 mg/kg; Imalgène 1000®) and Xylazine (10 mg/kg; Rompum®) and surgically implanted with chronic intravenous Silastic catheters (Dow Corning, USA) into the right jugular vein. The catheter was secured to the vein with suture thread and was passed subcutaneously to exit dorsally on the animal's back. After surgery, catheters were flushed daily with 0.2 ml of a sterile antibiotic solution containing heparinized saline (280 IU/ml; Sanofi-Synthelabo, France) and ampicillin (Panpharma, France). Rats were allowed to recover for seven days before behavioral testing.

Self-administration experiments were conducted in standard operant chambers (30 × 40 × 36 cm; Imétronic, France) located in a dimly lit room with a background white noise (75 dB). The chambers were individually enclosed in wooden cubicles fitted with a ventilation fan that also screened extraneous noise. Each operant chamber had two opaque panels on the right and left walls and two clear Plexiglas walls on the back and front walls. The floor consisted of 6-mm diameter steel bars spaced 15 mm apart. Two retractable levers (2 × 4 × 1 cm) were mounted 7 cm above the grid floor on the right operant panel. A white light diode was mounted 8.5 cm above each lever. A bulb (2 W) fixed on the top of the back wall provided a diffuse white light to the chamber. Spring-covered Tygon tubing connected the rats' catheter through a fluid swivel to a syringe containing ethanol solution that was placed outside the chamber. Ethanol delivery was controlled by the activation of a syringe pump. A computer controlled the delivery of fluids, presentation of visual stimuli and recorded behavioral data.

Rats were initially trained to press one of the two levers for food pellets (45 mg each) during daily 1-h sessions on a fixed-ratio 1 schedule (every active lever press was reinforced). Reinforced responses were followed by a 4 s time-out period, during which the cue-light (above the active lever) was turned on and active lever presses did not result in additional reinforcement. Lever presses on the other lever were registered as a measure of non-specific behavior, but had no programmed consequences. The position of the active and inactive levers was counterbalanced among rats. After acquisition of food self-administration (3 days), the reinforcer was changed to intravenous ethanol (1% v/v in sterile saline, delivered in a volume of 50 μl over 2 s, equivalent to approximately 1 mg/kg/infusion) and tested for 21 sessions. This dose of ethanol (i.e., 1%) is typically used for intravenous self-administration (e.g., Gass and Olive, 2007). During this experiment, rats received their daily food ration (20 g of food per rat) immediately after the self-administration test. The data were expressed as the average number of infusions over the 21-day period and as percentage preference for the active lever relative to the inactive lever.

2.5. Concurrent access to oral saccharin and intravenous ethanol

In order to reach higher levels of alcohol, rats were tested in a new paradigm of concurrent access to oral saccharin and intravenous ethanol. Saccharin is a strong reinforcer and rats typically show high responding levels for saccharin (Vendruscolo et al., 2010). Hence, after establishing saccharin self-administration, increasing ethanol injections were concomitantly delivered with saccharin, thus propagating high levels of ethanol exposure. This experiment was carried out in similar operant chambers as for ethanol self-administration except that a drinking reservoir (volume capacity 0.4 ml), positioned 4 cm above the grid floor in the center of the two levers, was present for delivery of saccharin. The drinking cup was connected to a syringe containing saccharin and connected to a pump placed outside the chamber. The same rats tested for intravenous ethanol self-administration and with patent catheters were used in this experiment ($n = 6$ for the sucrose group and $n = 5$ for the water group). Rats were initially allowed to press the active lever to obtain 100 μl of saccharin (0.13%) plus an intravenous injection of saline (25 μl) in 1-h daily sessions. Once stable levels of responding were observed (7 sessions), saline was substituted by increasing doses of ethanol: 1% (5 sessions), 3% (5 sessions), 6% (3 sessions), 12% (1 session) and 24% (4 sessions). Baseline was calculated as the average responding for the last 3 sessions of the saccharin plus saline condition. The results of concurrent access to saccharin and ethanol were presented as percentage change from the baseline to adjust data for baseline differences between animals.

2.6. Blood-alcohol determination

For the determination of blood-alcohol levels (BAL), we collected blood from the intravenous catheters at the end of an operant session and BAL was measured by the spectrophotometric alcohol dehydrogenase method (Sigma, Saint Louis, MO, USA).

2.7. Statistical analysis

All data are presented as the mean and standard error of the mean (SEM). For consumption of different solutions during adolescence and for consumption of quinine and saccharin in adulthood, statistical analyses were performed using three-way ANOVA for repeated measures, with SUC (exposed or not exposed) and EtOH (exposed or not exposed) as between-subject variables, and Session (PD32-46) or Bottles (water vs. treatment solution) as a within-subject factor. For ethanol intake (in g/kg/day) during adolescence, only rats having access to ethanol (EtOH and EtOH + SUC) were included in the analysis. For body weight, food intake during adolescence, and for ethanol intake and ethanol preference in adulthood, statistical analyses were conducted using a two-way ANOVA with SUC (exposed or not exposed) and EtOH (exposed or not exposed) as between-subject variables. For intravenous ethanol self-administration and preference for the ethanol lever in adulthood, experimental groups (EtOH vs. SUC + EtOH; water vs. SUC) were compared using the Student's *t*-test. For concurrent access to oral saccharin and intravenous ethanol self-administration in adult rats, statistical analyses were carried out using a two-way ANOVA for repeated measures, with SUC (exposed or not exposed) as a between-subject factor and session as a within-subject factor. Whenever a significant interaction was detected by ANOVA, the Fisher LSD test was used for post-hoc comparisons. Pearson correlation was used to investigate the relationship among adolescent and adult data, and ethanol intake and BAL. The accepted level of significance for all tests was $p < 0.05$. The analyses were performed using the Statistica® software package (StatSoft Inc., USA).

3. Results

3.1. Consummatory behavior for sucrose, ethanol and mix sucrose–ethanol during adolescence

The consumption showed by adolescent rats increased across days (Session: $F_{(7, 196)} = 27.1$, $p < 0.0001$) and the total fluid intake strongly varied across the solutions offered to the adolescent rats (SUC × EtOH × Session: $F_{(7, 196)} = 5.5$, $p < 0.0001$, Fig. 1A). Rats having access to sucrose showed an increased fluid intake during the whole period of exposure compared to all other groups ($p < 0.0001$). The preference for the treatment solution also varied across days (Session: $F_{(7, 196)} = 4.2$, $p < 0.001$) and rats having access to sucrose showed higher preference for the treatment solution in relation to water (SUC × EtOH: $F_{(1, 28)} = 15.0$, $p < 0.001$) compared to all the other groups ($p < 0.0001$). Moreover, rats having ethanol (without sucrose) showed lower preference compared to rats having water ($p < 0.05$, Fig. 1B). Ethanol intake varied across sessions (Session: $F_{(7, 98)} = 3.1$, $p < 0.01$) and although sucrose was highly palatable to adolescent rats, the presence of alcohol markedly inhibited the consumption of this solution. The average daily ethanol intake (g/kg/day) was only modestly higher for the SUC + EtOH group compared to the EtOH group (SUC: $F_{(1, 14)} = 3.9$, $p = 0.067$, Fig. 1C).

All groups gained weight at a similar rate across the period of adolescent exposure to the different solutions (data not shown). Mean body weight for all groups: P32, 103 ± 1 g; P46, 205 ± 3 g. However, the ANOVA revealed a significant difference between groups for the average daily food intake (SUC × EtOH interaction: $F_{(1, 28)} = 4.4$, $p < 0.05$). Rats having access to sucrose consumed less food (16.9 ± 0.4 g per day) than rats having access to any other solution (19.8 ± 0.3 g per day; $p < 0.01$).

3.2. Impact of exposure to sucrose, ethanol or a mix of ethanol–sucrose during adolescence on consumption of saccharin, quinine and ethanol in adulthood

The ANOVA indicated a significant SUC × Bottle interaction ($F_{(1, 28)} = 7.1$, $p < 0.05$) for saccharin intake. Rats previously exposed to sucrose drank less saccharin than rats not exposed to sucrose

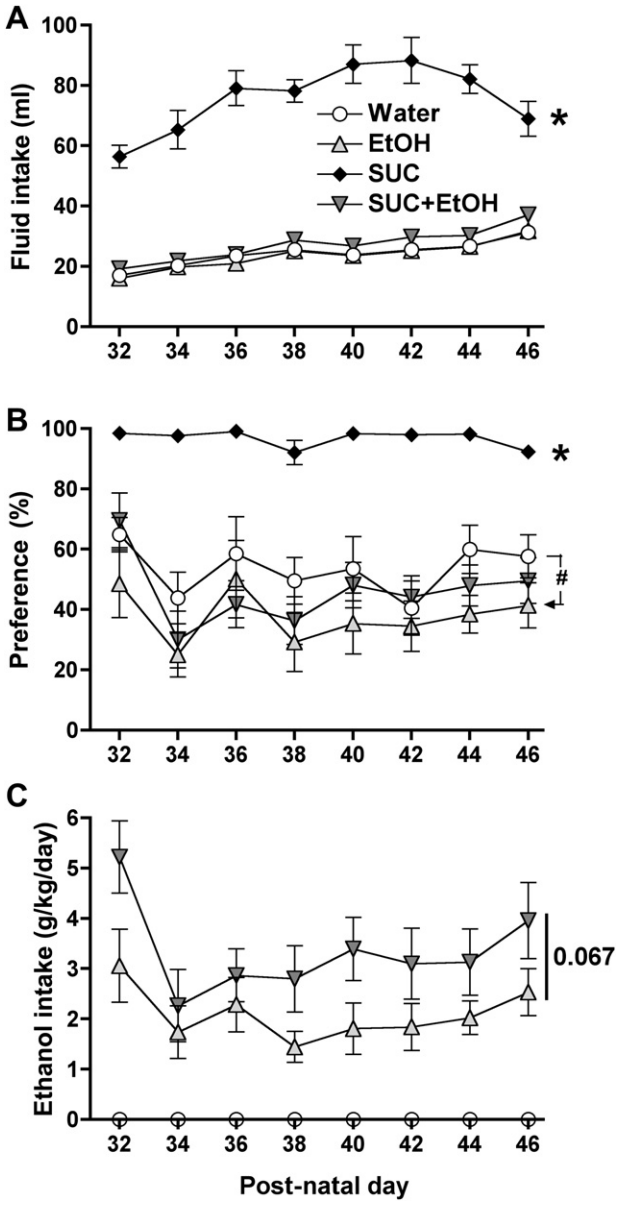

Fig. 1. Consumption of different solutions during adolescence. Adolescent (P30-46) rats were given continuous free-choice in their home cages between water and an additional bottle (treatment solution) containing either water, 5% sucrose (SUC), 5% ethanol (EtOH) or a mixture of 5% sucrose and 5% ethanol (SUC + EtOH). (A) Fluid intake (ml/day) over 16 days, (B) Percent preference between water and the treatment solution and (C) ethanol intake (g/kg/day). * indicates a significant difference compared to the consumption (or preference) of any other solution ($p < 0.0001$). # indicates a significant difference between Water and EtOH groups ($p < 0.05$). $n = 8$ per group.

($p < 0.001$), yet they did not differ in water intake (Fig. 2A). For quinine intake, a significant interaction between SUC × EtOH × Bottle was observed ($F_{(1, 28)} = 5.1$, $p < 0.05$), with rats pre-exposed to ethanol (without sucrose) consuming more quinine than rats pre-exposed to sucrose or water ($p < 0.05$, Fig. 2B).

For ethanol drinking, the statistical analysis revealed an overall SUC effect for ethanol intake ($F_{(1, 28)} = 4.9$, $p < 0.05$, Fig. 3A) and preference ($F_{(1, 28)} = 6.1$, $p < 0.05$, Fig. 3B), indicating that rats pre-exposed to sucrose (with or without alcohol) showed less intake of and less preference for ethanol than rats not exposed to sucrose.

We sought to determine whether there was a relationship among sucrose consumption during adolescence and the consumption of saccharin and ethanol during adulthood. There was no significant

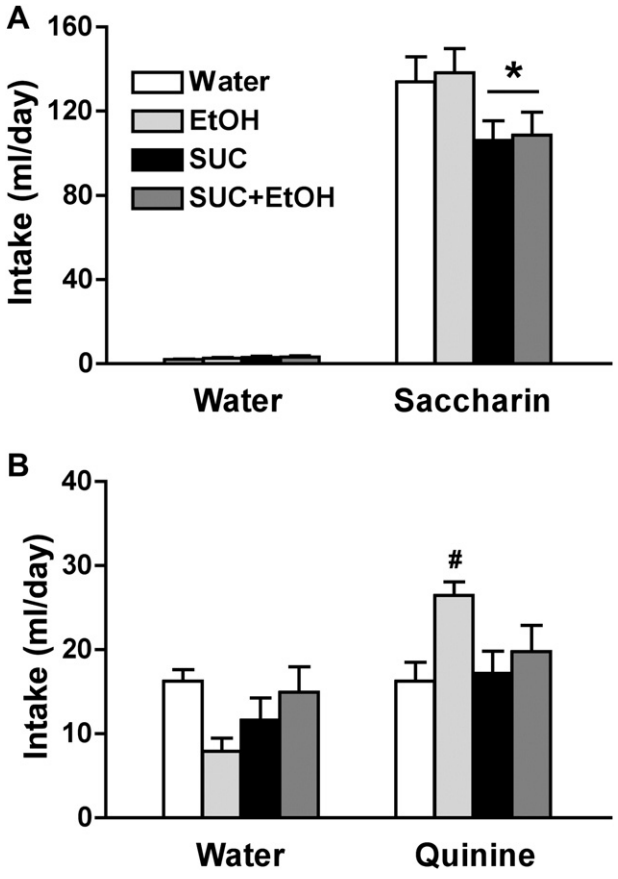

Fig. 2. (A) Intake (ml/day) of saccharin (0.13%, w/v) and (B) quinine (0.0001%, w/v) solutions in free choice with water for 24 h displayed by adult rats exposed to different solutions during adolescence. * indicates an overall sucrose effect ($p < 0.05$). # indicates a significant difference from water and EtOH groups ($p < 0.05$). $n = 8$ per group.

correlation among sucrose intake during adolescence and saccharin ($r = 0.29$, $p = 0.10$) or ethanol ($r = 0.18$, $p = 0.33$) intake in adulthood.

3.3. Impact of exposure to sucrose during adolescence on adult intravenous ethanol and/or oral saccharin self-administration

For intravenous ethanol self-administration the Student's *t*-test indicated that sucrose pre-exposed and control rats showed similar levels of intravenous ethanol self-administration (Fig. 4A) and preference (Fig. 4B) for the lever associated with ethanol delivery.

When animals were given a concurrent access to oral saccharin and intravenous saline (baseline), the Student's *t*-test revealed that rats pre-exposed to sucrose responded significantly less than control rats ($t_{10} = 2.5$, $p < 0.05$, Fig. 5A) replicating our previous findings (Vendruscolo et al., 2010). When animals were given increasing ethanol concentrations, the ANOVA revealed only an effect of concentration ($F_{(17, 170)} = 24.1$, $p < 0.0001$). Rats showed a significant reduction in responding at 24% ethanol concentration compared to all other concentrations ($p < 0.05$), regardless of the treatment during adolescence (Fig. 5B).

BAL was determined on some animals at the end of an operant session. Means BAL were 51 mg/dl for 3%, 71 mg/dl for 6%, 158 mg/dl for 12% and 235 mg/dl for 24%. There was a significant positive correlation between the amount of ethanol self-administrated per session and the BAL ($r = 0.84$, $p < 0.0001$), as well as between active lever presses during a session and BAL (e.g., 3% dose, $r = 0.82$, $p < 0.05$).

4. Discussion

The present results show that free access to sucrose, but not ethanol, during adolescence reduced the subsequent consumption of both sweet and ethanol solutions in adulthood. This effect on ethanol intake is likely due to changes in the gustatory acceptance of ethanol given that intravenous ethanol self-administration was not modified in rats pre-exposed to sucrose.

Sucrose exposure during adolescence significantly reduced the consumption of both sweet and alcoholic solutions in adulthood. Our recent study investigating saccharin reinforcement indicated that rats pre-exposed to sucrose during adolescence perceive the sweet taste to a similar extent compared to control rats, yet in sucrose pre-exposed rats, saccharin had a reduced reinforcing efficacy (Vendruscolo et al., 2010). Moreover, sucrose exposure during adolescence, but not adulthood, leads to a considerable and sustained reduction in the motivation for saccharin (sweet) and maltodextrin (non-sweet) in an operant paradigm, suggesting a decreased "wanting" (Berridge et al., 2009) for various tasteful rewards (Vendruscolo et al., 2010). We did not test the hedonic response (liking) to saccharin taste in our rats. However, additional experiments (unpublished findings) indicate an anhedonia-like state in sucrose pre-exposed rats that might be translated in a decreased hedonic response to sweet taste. This issue would be very interesting to test in the future.

A possible explanation for the difference between sucrose and water pre-exposed adolescent rats is that an unusually frequent and intense firing of brain reward systems induced by the consumption of a sweet solution during adolescence alters neural developmental

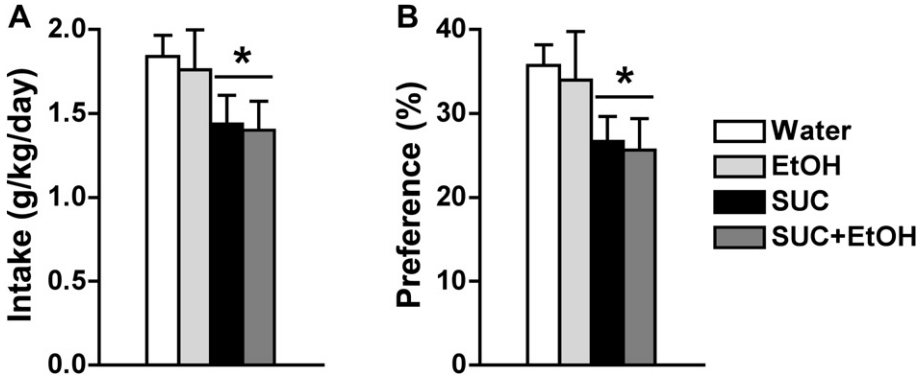

Fig. 3. (A) Intake (g/kg/day) of ethanol (10%, v/v) and (B) percent preference for ethanol versus water over 18 days displayed by adult rats exposed to different solutions during adolescence. * indicates an overall sucrose effect ($p < 0.05$). $n = 8$ per group.

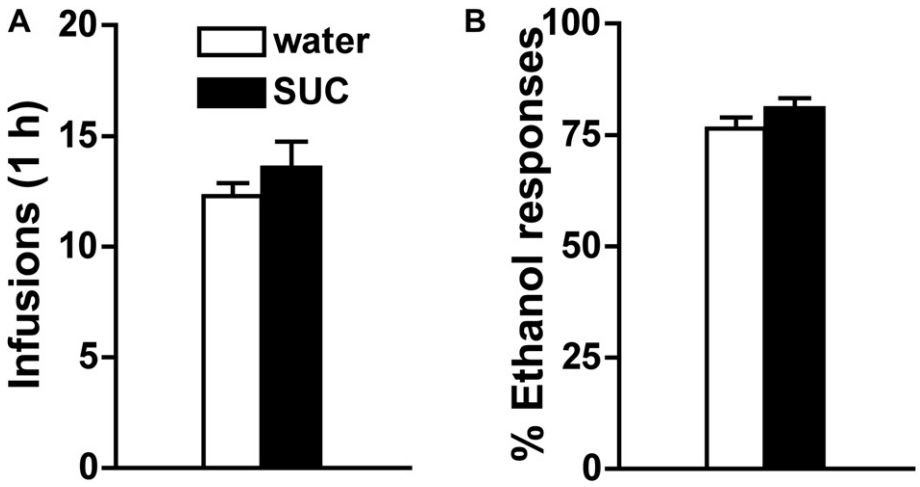

Fig. 4. (A) Responding for intravenous (i.v.) ethanol (1%, v/v, 50 μl) self-administration. The intake of ethanol per session was about 10–15 mg/kg. (B) Percent preference for the ethanol lever relative to the inactive lever, over twenty-one 1-h daily sessions, displayed by adult rats exposed to either sucrose or water during adolescence. $n = 9–10$ per group.

processes (e.g., neuronal proliferation, migration, differentiation, etc) leading to enduring changes in reward-related behavior that emerge during adulthood. In contrast to the present results, Pian et al. (2009) have recently reported that limited/short access to sucrose during adolescence (1 h per day, 5 days/week, from P29-51) increased intake of sucrose and of a mixed solution of 10% sucrose and 2.5% ethanol in adulthood. A reason for this discrepancy could be that sporadic exposure to sucrose would result in increased sucrose acceptance later in life due to the memory of the rewarding effect of sucrose, whereas a continuous access as that of the present study may produce enduring changes in the brain reward system. Using a protocol of extended intermittent sucrose exposure (12-h access to a sucrose solution, followed by 12 h of deprivation daily), Avena et al. (2008) have reported that adult rats receiving daily intermittent access to sucrose demonstrate more severe behavioral and neurochemical alterations compared with continuous sucrose access (24 h). Moreover, intermittent, but not continuous, ethanol intake increased the subsequent intake of sucrose, whereas sucrose intake increased the subsequent intake of ethanol, suggesting that bingeing on either ethanol or sucrose fosters intake of the other (Avena et al., 2004). In a recent study we found that adolescent rats given 12-h access to sucrose showed a similar decrease in the intake of saccharin in adulthood (unpublished data). It would therefore be of interest to more closely compare the effects of intermittent versus continuous exposure to sucrose and/or ethanol during adolescence on the behavioral changes in adulthood.

Another major finding of the present study is that sucrose consumption during adolescence decrease ethanol intake in adulthood. Because ethanol intake is influenced both by its taste and its pharmacological effects, we sought to determine the impact of sucrose pre-exposure on intravenous ethanol self-administration (which prevents oral sensory stimulation). The results suggest that sucrose did not change the pharmacological effect of alcohol given that no differences were found between sucrose-exposed and control rats. It is noteworthy that rats of both groups showed a clear preference for the lever associated with ethanol, confirming that ethanol at very low doses may serve as a positive reinforcer when intravenously self-administered (Gass and Olive, 2007). However, the levels of responding and total ethanol intake were very low in this test and thus would have perhaps masked the emergence of any group differences. For this reason we tested these rats on a novel model of concurrent self-administration of oral saccharin

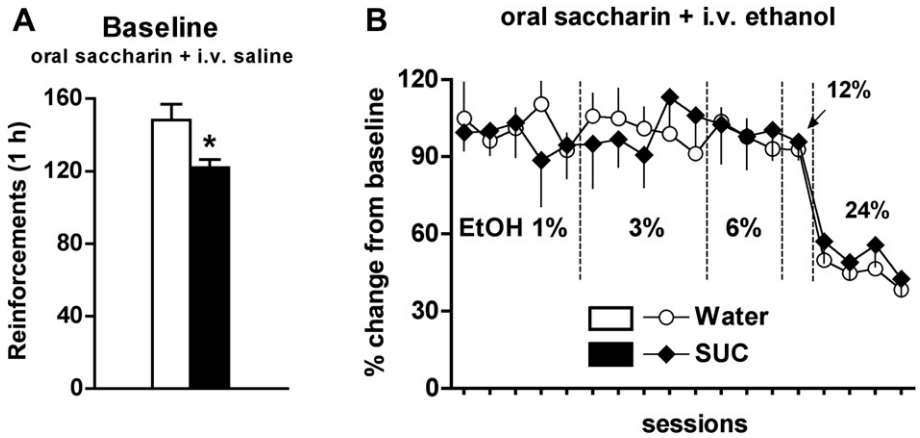

Fig. 5. (A) Number of reinforcements obtained in 1 h in a fixed-ratio 1 schedule (every active lever press was reinforced). Reinforcements consisted in a dose of oral saccharin (0.13%, w/v; 100 μl) plus an i.v. injection of saline (25 μl/injection) (Baseline). (B) Percent change in the number of reinforcements obtained in 1 h, relative to baseline, for oral saccharin (0.13%, w/v; 100 μl) plus increasing concentrations of i.v. ethanol (1–24%, v/v, 25 μl/injection) displayed by adult rats exposed to either sucrose or water during adolescence. Approximated ethanol intake per session: 70 mg/kg for 1%; 200 mg/kg for 3%; 400 mg/kg for 6%; 800 mg/kg for 12% and 24%. * indicates a significant difference from control rats ($p < 0.05$). $n = 5–6$ per group.

and intravenous ethanol hypothesizing that in these conditions they would consume more alcohol intravenously. It was observed that rats pre-exposed to sucrose responded less than control rats when they were given simultaneous access to oral saccharin and intravenous saline (baseline). This difference in saccharin-motivated behavior confirms that sucrose exposure during adolescence decreases saccharin intake in adulthood (Vendruscolo et al., 2010). When saline was switched to increasing ethanol concentrations, the level of responding of both groups remained unaffected up to 12% ethanol. However, a similar dramatic reduction of responding was observed for both groups when the highest dose of ethanol was tested (24%). Taken together, these results suggest that sucrose exposure did not modify the pharmacological effects of alcohol. It is important to note here that much higher levels of ethanol intake (more than 50 times higher) were achieved with this model of simultaneous access to oral saccharin and intravenous ethanol than with typical intravenous ethanol self-administration procedures and with this protocol rats consumed amounts of alcohol sufficient to produce detectable blood-alcohol levels. Considering the rapid onset of effects achieved with intravenous drug infusion, an interesting issue to be investigated in future studies is to determine whether adjustment of this new paradigm (e.g., longer self-administration sessions) could result in even higher levels of alcohol intake and whether the development of physical alcohol dependence could be observed. One possibility to achieve this goal would be to provide contingent and intermittent delivery of food or saccharin solution (with or without food deprivation) to produce schedule-induced polydipsia or adjunctive behaviors (see, for example, Wayner, 2002; Grant et al., 2008).

Although some studies have reported that ethanol exposure during adolescence increases ethanol consumption (Ho et al., 1989; Siciliano and Smith, 2001; Yoshimoto et al., 2002; Rodd-Henricks et al., 2002; Blizard et al., 2004; Moore et al., 2010), the results of the present study are consistent with data showing no effect of ethanol pre-exposure on subsequent ethanol consumption (Slawecki and Betancourt, 2002; Siegmund et al., 2005; Füllgrabe et al., 2007; Vetter et al., 2007; Hargreaves et al., 2009; Gurkovskaya et al., 2009). The only effect observed here was an increased quinine preference by rats pre-exposed to ethanol (without sucrose), suggesting that this pre-treatment persistently affected the rewarding value of diluted bitter solutions.

The lack of ethanol effect on the subsequent ethanol consumption could not be simply attributed to the modest ethanol intake displayed by adolescent rats. The consumption of ethanol by adolescent rats was moderate and, contrary to our prediction, only slightly increased when sucrose was added to the ethanol solution (increased intake around 53%). This was a surprising result because the 5% sucrose solution was very rewarding to adolescent rats. However, it is consistent with a study by Truxell et al. (2007) reporting that the addition of saccharin to an ethanol solution did not profoundly modify ethanol consumption by adolescent rats. This indicates that either alcohol's taste or its pharmacological effects restrict adolescent ethanol ingestion, as has previously been shown in adults (Terenina-Rigaldie et al., 2004). However, the amount of ethanol consumed by adolescent rats in the present study should be sufficient to produce rewarding effects: ethanol stimulates dopamine release in the nucleus accumbens when orally self-administered at doses around 0.53 g/kg (Weiss et al., 1993), produces measurable brain ethanol levels at doses between 0.41 and 1.13 g/kg (Ferraro et al., 1991) and at doses up to 0.8 g/kg facilitates rewarding electrical brain stimulation (Moolten and Kornetsky, 1999). Moreover, procedures that produce higher blood ethanol levels (e.g., ethanol as the sole source of fluid or exposure to ethanol vapor) have also failed to induce an increase in ethanol consumption in adulthood (Tolliver and Samson, 1991; Slawecki and Betancourt, 2002). The pattern of exposure to ethanol may also be important. Although ethanol drinking under continuous/free access to ethanol generally produces lower intake and blood ethanol levels compared with intermittent/limited access (Wise, 1973), most studies that showed increased ethanol intake following adolescent ethanol exposure employed continuous access to ethanol (Ho et al., 1989; Siciliano and Smith, 2001; Rodd-Henricks et al., 2002; Blizard et al., 2004). Gender, strain and different procedures may also influence the effects of ethanol pre-exposure on subsequent ethanol intake (Rodd-Henricks et al., 2002; Moore et al., 2010) and further comparative studies are needed to identify these factors.

In conclusion, sucrose exposure during adolescence leads to persistent effects on the perception of sweet taste reward and thereby alcohol drinking in adulthood. This suggests that free access to sucrose during adolescence is able to "reprogram" the homeostatic function of the brain reward system. Given the widespread consumption of sweetened beverages in our modern societies, particularly by adolescents (Guthrie and Morton, 2000), these data may have implications for understanding the factors linked to the vulnerability to alcoholism.

Acknowledgments

The authors thank Anne Fayoux and Stéphane Lelgouach for animal care, Pierre Gonzalez for technical assistance, Caroline Vouillac for help with some experiments and Marie-Helene Bruyères for administrative help. LFV received financial support from Fondation Fyssen, France. ABG received financial support from Mission Interministérielle de la Lutte contre les Drogues et la Toxicomanie (MILDT # 0901), France. KJC received funding from the Association pour la Recherche sur les Nicotianées. MC was financially supported by CNRS, MD by University of Lille 1 and PM by INRA. Research was supported by CNRS, INRA, University Bordeaux 1&2, Conseil régional d'aquitaine, MILDT, IREB (Institut de Recherche sur les Boissons).

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