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Influence of age at drinking onset on the alcohol deprivation effect and stress-induced drinking in female rats

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Abstract

We have recently observed increased stress responsiveness with regard to alcohol consumption in male rats that consumed alcohol since their adolescent period. Thus, early age at drinking onset can induce enhanced stress-induced alcohol drinking in male rats. However, it is not known whether female rats respond in a similar way. Therefore, we compared the drinking behavior of two female Wistar rat groups — one that acquired alcohol consumption during adolescence (adolescent group) and the other that acquired their drinking during adulthood (adult group) in a model of long-term voluntary alcohol drinking with repeated deprivation and stress phases. Furthermore, we studied the influence of age at drinking onset on the efficacy of acamprosate treatment. Thirty-nine female Wistar rats aged 31 days (adolescents) and 71 days (adults) were given *ad libitum* access to water, as well as to 5% and 20% ethanol solutions during an observation period of 29 weeks. A deprivation phase of 14 days was introduced following 8 weeks of access to alcohol in order to measure the alcohol deprivation effect (ADE). After 15 and 25 weeks of alcohol access, all animals were subjected for 3 consecutive days of forced swim and electric foot-shock stress, respectively. After 29 weeks of access to alcohol all animals underwent a second deprivation phase and the subsequent ADE was measured either under acamprosate (200 mg/kg) or vehicle treatment. Drinking before the first deprivation phase was not different between animal groups. However, the expression of the first ADE was more pronounced in adult female rats and alcohol intake stayed increased for the remainder of the experiment in the adult group. Both repeated swim stress and foot-shock stress produced a more pronounced increase in ethanol consumption in the adolescent group compared to the adult group. Acamprosate reduced relapse-like drinking in the adult female rat group. However, it had no effect on the ADE in the adolescent group. In conclusion, female rats that initiate alcohol consumption during adolescence might be more susceptible to stress-induced alcohol consumption. Adolescent alcohol drinking might also result in a reduced response to acamprosate.

Keywords: Long-term alcohol self-administration; Alcohol deprivation effect (ADE); Swim stress; Foot-shock stress; Rats; Adolescence; Acamprosate

1. Introduction

Alcohol drinking during adolescence is a recurrent and serious problem in our society. Thus, the young adolescent brain shows higher sensitivity to alcohol-induced brain damage and cognitive impairment than the adult brain in man as well as in rodents (Crews et al., 2000; Slawecki et al., 2004; White and Swartzwelder, 2004). Furthermore, the onset of alcohol use during adolescence may have potentially long-lasting consequences that could lead to a greater risk for developing alcohol dependence in adulthood (Grant and Dawson, 1997). We have recently reported that alcohol consumption in male rats during adolescence has an influence on future alcohol intake. In particular, the early exposure to alcohol may lead to a higher susceptibility to stress-induced alcohol consumption in male rats (Siegmund et al., 2005). Although the early onset of alcohol drinking as well as alcohol dependence has been reported to be more common in men, several studies have shown a more rapid development of alcohol dependence and alcohol-induced brain damage in women (Fernandez-Sola et al., 1997; Mann et al., 2005). Thus, the present study was designed to investigate the consequences of exposure to alcohol at an early age in female rats. In particular, we aimed to study the influence of age at

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drinking onset on relapse behavior and on stress-induced alcohol drinking in a rat model of long-term voluntary alcohol consumption (Spanagel and Holter, 1999; Vengeliene et al., 2003; Siegmund et al., 2005).

In the present report relapse behavior was studied by using the alcohol deprivation model. Thus, alcohol deprivation for several days/weeks leads to a temporary increase in voluntary alcohol intake over baseline drinking conditions. This robust phenomenon is called the alcohol deprivation effect (ADE). The ADE shows different aspects of relapse-like drinking behavior (Sanchis-Segura and Spanagel, 2006) and has become a standard model to study pharmacological agents in preventing relapse-like drinking behavior (Spanagel and Zieglgansberger, 1997; Heyser et al., 1998; Le and Shaham, 2002; Spanagel, 2005). Acamprosate which is in clinical use for several years has been proven to reduce/suppress the ADE in male rats (Spanagel et al., 1996; Heyser et al., 1998). However, the effect of acamprosate treatment in female rats has so far not been studied and moreover, it is not known whether adolescent alcohol drinking has an influence on treatment response to acamprosate. This question is of particular importance as many patients do not respond to acamprosate treatment and the underlying factor for non-responding with adolescent alcohol consumption being one of them have not been revealed so far. Thus, a further aim of the present study was to examine the influence of age at drinking onset in female rats on the response of acamprosate treatment during an ADE.

Finally, we studied the impact of different stressors on alcohol drinking in female rats that started alcohol drinking during adolescence versus animals that started alcohol drinking during adulthood. The effects of stressors on voluntary alcohol intake have been studied intensively (e.g. Pohorecky, 1990; Lynch et al., 1999; van Erp and Miczek, 2001; Vengeliene et al., 2003; Funk et al., 2004; Brunell and Spear, 2005) showing that a variety of factors can influence drinking behavior of an individual in response to stress, including the intensity and type of a stressor, the duration and timing of stress, housing conditions, food restriction and finally sex (Pohorecky, 1990; Schroff et al., 2004; Chester et al., 2006). In the present study we have used two different stressors — swim stress and inescapable electric foot-shock — as our previous studies have shown that both stressors can enhance alcohol consumption in different rat lines (Vengeliene et al., 2003; Siegmund et al., 2005).

2. Materials and methods

2.1. Animals

Two groups of female Wistar rats (73rd generation; CIMH, Mannheim, Germany) were used: adolescent female Wistar rats ($n=19$) started drinking with an age of 31 days and had an average body weight of 90.6±0.8 g and adult female Wistar rats ($n=20$) aged 71 days at the drinking onset and body weight of 191.2±1.8 g.

The animals were housed under constant conditions (temperature: 23±1 °C; humidity: 55±2%) under a 12 h artificial light–dark cycle (lights on at 7:00 am) in standard rat cages. Rat food and tap water were provided *ad libitum* throughout the experiment.

All experiments were approved by the "Committee on Animal Care and Use" of the relevant governmental body (Regierungspräsidium Karlsruhe) and carried out following the "German Law on the Protection of animals".

2.2. Long-term alcohol consumption

After 1 week of habituation to the animal room, rats were given free access to water, and 5% and 20% (v/v) ethanol solutions in three bottles per cage. Alcohol solutions were prepared from 96% ethanol diluted with tap water. To avoid specific location preferences, the positions of the bottles were changed weekly.

The ethanol intake (g of pure alcohol per kg of body weight) and preference (percentage of ethanol solutions consumed in total volume of fluid intake) was calculated as the daily average across 1 week.

2.3. Alcohol deprivation phase

A stable baseline of ethanol consumption was established before all rats underwent a deprivation phase of 2 weeks after 8 weeks of alcohol access leaving animals with food and water *ad libitum* (Table 1). After the deprivation period, alcohol solutions were returned and alcohol consumption was measured daily for the following 4 days.

2.4. Stress procedures

After 15 weeks of free alcohol access animals were exposed to swim stress (Table 1). All animals were placed in a cylindrical plastic tank (50 cm high, 35 cm diameter) filled with tap water (19 °C). To ensure that the animal could not reach the bottom, the water level was 35–40 cm high. Swim stress was performed for 3 consecutive days (10 min duration each day) at the same time (2:00 pm). The animals were observed by a video camera at the first and the third stress day and latency time until animals started to float was measured. After swim stress, rats were returned into their home cages.

The foot-shock procedure was performed after 25 weeks of free alcohol access (Table 1). This stress procedure was performed for 3 subsequent days (4 min duration each day) at the same time

Table 1
The experimental design of the study

Time	Procedure	Free access to
Week 1–8	Free-choice drinking	Food, tap water, 5% and 20% ethanol
	2 weeks deprivation	Food and tap water
Week 9	ADE	Food, tap water, 5% and 20% ethanol
Week 10–15	Free-choice drinking	Food, tap water, 5% and 20% ethanol
Week 16	Swim stress	Food, tap water, 5% and 20% ethanol
Week 17–25	Free-choice drinking	Food, tap water, 5% and 20% ethanol
Week 26	Foot-shock	Food, tap water, 5% and 20% ethanol
Week 27–29	Free-choice drinking	Food, tap water, 5% and 20% ethanol
	2 weeks deprivation	Food and tap water
Week 30	ADE+ acamprosate treatment	Food, tap water, 5% and 20% ethanol

(2:00 pm). Animals were placed on steel rods (6 mm in diameter; 19.5 mm rod distance) in a fear conditioning box (TSE, Bad Homburg, Germany) with dimensions of 64×60×64 cm. Unpredictable foot-shocks of 0.8 mA intensity were delivered to the grid floor and the current was pulsating with a phase-duration of 20 ms. Total shock duration was 120 s in a 240 s session (shock and inter-shock times ranged from 2–10 s).

To see the effect of stress on animals, a stable baseline regarding alcohol consumption was established before stress procedures were applied. Alcohol consumption was measured daily before (pre-stress phase), during, and 2 days following each stress procedure (post-stress phase).

2.5. Acamprosate treatment

After 29 weeks of free access to alcohol all animals underwent a second deprivation phase for 2 weeks (Table 1). At the last day of the deprivation phase animals were divided into two groups, with approximately the same mean of basal alcohol intake. Each animal was administered a total of five intraperitoneal injections (starting at 7:00 pm in a 12 h interval) of acamprosate (200 mg/kg) or saline at a volume of 1 ml/kg. After the second injection the alcohol solutions were given back to the animals (at 9:00 am) and the occurrence of an ADE was determined. Thus, intake of different alcohol solutions, total alcohol intake (ml/kg/day), water intake and total fluid intake (ml/kg/day) were measured daily for one more week. Animals' body weight were measured 24 h before the first injection and 12 h after the last injection.

The locomotor activity was monitored by use of an infrared sensor (Mouse-E-Motion, Henstedt–Ulzburg, Germany). The sensor was placed on a holder above the cage (30 cm distance to bottom). Monitoring was done during rats' active phase (7:00 pm–7:00 am), starting 3 days before acamprosate treatment and was continued for 2 more days after the last treatment day.

2.6. Statistics

Data from all experiments (basal alcohol intake, ADE, swim stress, foot-shock and acamprosate treatment) were analysed using a one-/two-/three-way analysis of variance (ANOVA) with repeated measures. When necessary, to prevent a possible effect of baseline differences between two animal groups the data were transformed to percentages over these baseline values. Post-hoc Student Newman Keul's tests were performed, when significant differences were found. For the analysis of the alcohol drinking behavior over the whole time course of the experiment Bonferroni correction was applied. The software Statistica 6.1 was used for all statistical analysis.

3. Results

3.1. Alcohol drinking behavior in female rats

At the beginning of the acquisition of voluntary alcohol consumption both groups started with a relatively high ethanol intake. Alcohol consumption was not different during the first 3 days between groups (adolescent group: 12.2±0.5 g/kg/day, adult group: 13.8±0.8 g/kg/day) (Fig. 1). However, ethanol preference was significantly higher in the adult group during the first 3 days due to the higher preference of the 5% ethanol solution [factor age: $F(1,37)=19.8$, $p<0.001$]. (Fig. 1). Alcohol intake rapidly declined over the next few days and weeks in both groups. Surprisingly, following the alcohol deprivation phase adult rats progressively enhanced their intake (Fig. 2). Statistical analysis showed a significant difference in ethanol intake of the adult and adolescent rat groups over the whole time course of the experiment [age×week interaction effect on alcohol intake during the experiment: $F(28,1036)=8.4$, $p<0.001$]. Following the alcohol deprivation phase, we could observe a significantly higher alcohol intake in the adult Wistar rat group with respect to the adolescent rat group (Figs. 2A and 4A). Similar to the ethanol intake we could also show a significant difference in ethanol preference during the whole time course of the experiment [factor age: [$F(1,37)=19.9$, $p<0.001$] and significant age×week interaction effect [$F(28,1036)=2.6$, $p<0.001$] (Fig. 2B).

The intake of the 5% and 20% alcohol solutions remained stable in the adult rat group during the whole time-course of the experiment (Fig. 3). We could not observe a shift of lower (5%) to higher (20%) alcohol concentration after 2 weeks of deprivation

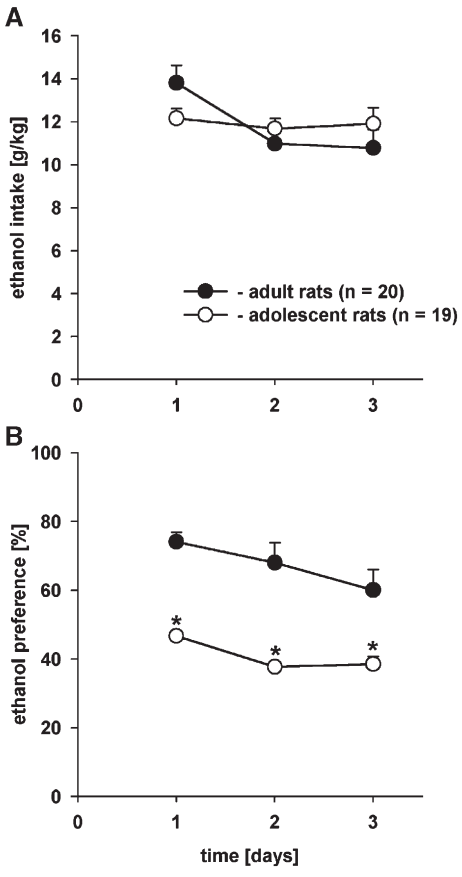

Fig. 1. Total ethanol intake (g/kg/day) (A) and ethanol preference (B) in adolescent ($n=19$) and adult ($n=20$) female rats during the first 3 days of acquisition period. Results are presented as means±SEM. *$p<0.01$ with respect to the adult group.

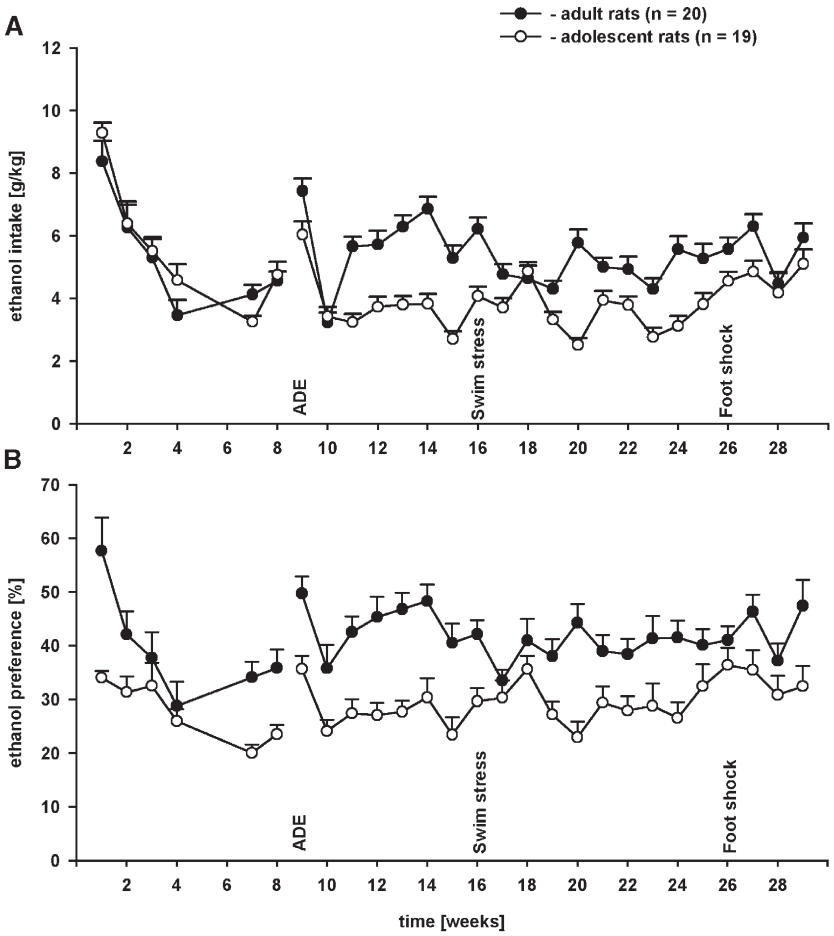

Fig. 2. Overview of a 29 weeks drinking period shown as ethanol intake (g/kg/day) (A) and preference (B) in adolescent ($n=19$) and adult ($n=20$) female rats with deprivation and stress phases. Results are presented as means±SEM.

as reported before in male rats (Spanagel et al., 1996; Rodd-Henricks et al., 2001; Vengeliene et al., 2003; Siegmund et al., 2005). In contrast, there was a significant drop of the 20% alcohol preference in the adolescent female rat group after the deprivation phase [factor week: [$F(4,148)=4.9$, $p<0.001$] (Fig. 3B) resulting in significant differences for the 5% and 20% ethanol preference between the two groups [factor age: [$F(1,37)=10.0$, $p<0.01$ and $F(1,37)=8.8$, $p<0.01$] (Fig. 3).

Both stress episodes did not have an influence on alcohol intake and preference in both groups over the remaining time course of the experiment (Figs. 2 and 3).

3.2. Alcohol deprivation effect (ADE)

After 2 weeks of alcohol deprivation we could observe a significant increase in alcohol intake in both groups indicating the occurrence of an ADE [factor deprivation: [$F(4,148)=148.2$, $p<0.001$]. A significant difference in alcohol intake was still seen on the fourth post-deprivation day (Fig. 4A). Corresponding to previous studies, animals increased their ethanol intake after the withdrawal phase for more than two folds that declined to the baseline level after the 4th or 5th post-deprivation day (Vengeliene et al., 2003). In respect to adolescent drinking a significant difference in ethanol intake between the two groups [factor age: [$F(1,37)=7.9$, $p<0.01$] could be detected in a way that adult animals showed a higher post-deprivational alcohol consumption.

3.3. Stress-induced alcohol drinking

Swim stress was performed for 10 min on each of three consecutive days. As shown by two-way ANOVA (factors: age and day), swim stress significantly increased the total alcohol intake in both groups [factor day: [$F(4,148)=64.8$, $p<0,001$] (Fig. 4B). However, during the first two consecutive days of swim stress, adolescent female rats increased alcohol intake more than 2-fold compared to baseline drinking [age×day interaction effect: $F(4,148)=50.5$, $p<0.001$] (Fig. 4B) showing a different reactivity to stress between the two animal groups. There was no significant difference in latency to float on day one or day three between both groups [day one: 118.4±6.2 s (adolescent) and 119.9±7.0 s (adult); day three: 134.6±19.7 s (adolescent) and 125.3±7.6 s (adult)].

Foot-shock procedure was also done on three consecutive days. During this time the alcohol intake was significantly increased in both groups [factor day: [$F(4,148)=76.0$, $p<0.001$] (Fig. 4C). A two-way ANOVA revealed a significant age×day interaction effect [$F(4,148)=26.5$, $p<0.001$]. Similarly to the

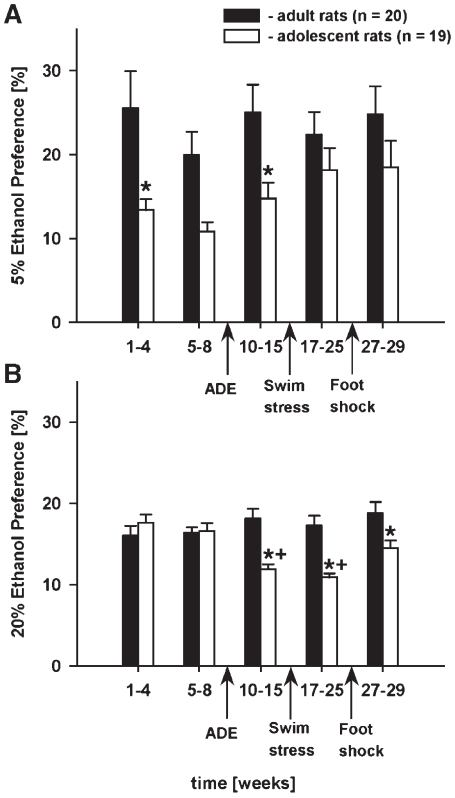

Fig. 3. 5% (A) and 20% (B) ethanol preference over the whole alcohol drinking experiment in adolescent ($n=19$) and adult ($n=20$) female rats. Weekly alcohol preference was calculated as average of 7 days measurements. Experimental weeks (9th, 16th and 26th) were excluded. Results are presented as means ± SEM. *$p<0.05$ with respect to adult group.+ $p<0.001$ with respect to pre-deprivation phase (5–8) of the corresponding group.

swim stress, the adolescent group had higher ethanol intake on the second stress day. On the fourth day both rat groups reached baseline drinking levels again.

3.4. Acamprosate treatment

Acamprosate treatment (200 mg/kg) started at the end of a second deprivation phase of 2 weeks. A three-way ANOVA (factors: age, treatment and deprivation) showed a significant increase in alcohol intake after a deprivation phase [factor deprivation: $F(3,105)=193.0$, $p<0.0001$]. Interestingly, the second ADE, contrary to the first, was more pronounced in the adolescent group [factor age: [$F(1,35)=18.7$, $p<0.001$] (Fig. 5). Consequently, the interaction of deprivation × age was also found significantly different [$F(3,105)=12.0$, $p<0.0001$]. Acamprosate treatment did not affect the expression of ADE in adolescent animals whereas a reduction of alcohol intake during treatment days could be observed in adult rats. Furthermore, both saline and acamprosate treated adolescent groups reached baseline drinking levels at the fifth day of regained access to alcohol, while it took only 3 days for the adult saline treated animals and only 2 days for the acamprosate treated adults (Fig. 5). Locomotor activity during treatment days was not affected significantly in any of the animal groups (age × treatment × days interaction effect, $p=0.96$) (data not shown).

4. Discussion

In the present study drinking behavior over 29 weeks in two female Wistar rat groups — one that initiated ethanol consumption during adolescence (adolescent group, aged 31 days) and the

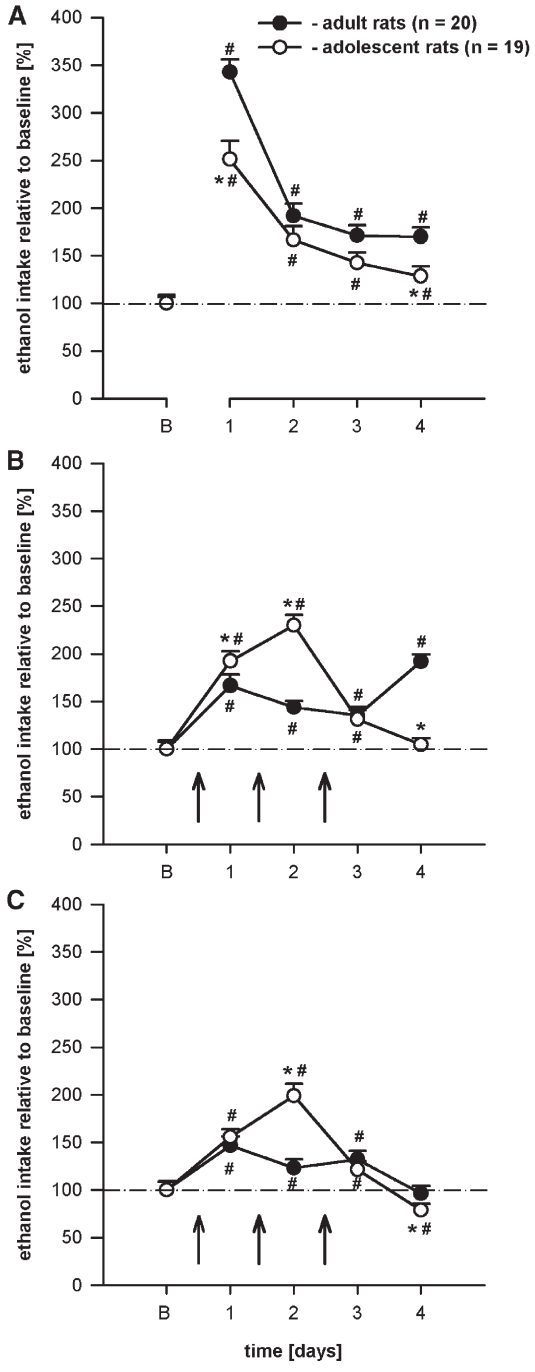

Fig. 4. Total ethanol intake relative to baseline in adolescent ($n=19$) and adult ($n=20$) female rats after ADE of 2 weeks (A) and in response to swim stress (B) and foot-shock stress (C) for 3 consecutive days. The average of 4 days measurements prior to experimental procedures was set as baseline "B". Arrows indicate stress procedures. Change of ethanol intake (g/kg/day) for each rat was calculated as percentage relative to baseline drinking. Results are presented as means ± SEM. *$p<0.05$ with respect to adult group. # $p<0.05$ with respect to baseline drinking.

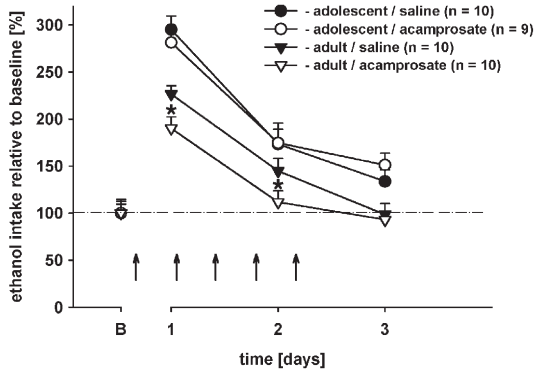

Fig. 5. The effect of acamprosate (200 mg/kg) and saline treatment on the expression of ADE in adult and adolescent female rat groups. The average of 4 days measurements prior to 2-weeks deprivation period was set as baseline "B". Arrows indicate acamprosate treatment. Change of ethanol intake (g/kg/day) for each rat was calculated as percentage relative to baseline drinking. Results are presented as means±SEM. *$p<0.05$ with respect to adult / saline group.

other group that initiated drinking during adulthood (adult group, aged 71 days) — was studied. The influence of age at drinking onset on later relapse-like drinking behavior and the effects of different stressors were studied as well. Finally, the influence of age at drinking onset on the treatment response to acamprosate was examined. Our data show that alcohol consumption during adolescence enhances the vulnerability to stress-induced alcohol drinking and diminishes treatment response to acamprosate in female rats.

Previous studies in male rats have shown that ethanol intake during adolescence can be 2–3 times higher than in adult rats (Lancaster et al., 1996; Brunell and Spear, 2005; Doremus et al., 2005). This enhanced alcohol intake could be driven by a higher novelty seeking, high-risk taking behavior and increased exploration in adolescents (Kampov-Polevoy et al., 2004; Stansfield et al., 2004). Our data show in female rats that during the first 3 days of access to ethanol — which resembles the beginning of the acquisition period of ethanol drinking — both groups started with a relatively high ethanol intake. Although ethanol intake did not differ between groups during this initiation phase, ethanol preference was significantly higher in the adult group due to a higher preference of the 5% ethanol solution. In agreement to our previous study using male rats (Siegmund et al., 2005) the later ethanol intake and preference was significantly lower in the adolescent group, although this difference became apparent only after a two-week alcohol deprivation phase.

Following alcohol deprivation the adult group transiently increased their ethanol intake by approximately 250% compared to baseline drinking, while the adolescent group showed 150% increase over baseline consumption. These results demonstrate the occurrence of a typical ADE in female with a more pronounced effect in the adult group. This is not seen in male rats (Siegmund et al., 2005) and is contradictory to a previous report by Rodd-Henricks et al. (2002) who observed that periadolescent ethanol drinking by alcohol-preferring rats increases the potential for relapse during abstinence. We also analyzed the intake of 5% and 20% alcohol solutions in detail during the ADE. Alcohol deprivation usually leads to a preference shift towards the higher concentrated alcohol solution in male rats (Spanagel et al., 1996; Rodd-Henricks et al., 2001; Vengeliene et al., 2003; Colombo et al., 2006). However, this preference shift is not observed in female rats. Contrary, the adolescent female group even had a significant decrease in 20% ethanol preference after the deprivation period, which resulted in the lower total ethanol intake and preference. Interestingly, contrary to the first ADE, expression of the second ADE was found to be higher in adolescent animals as compared to that of adult animals. The reason for this could be the residual effect of two earlier stress procedures, given that adolescents are more sensitive to stress when compared to adults with respect to alcohol consumption (Brunell and Spear, 2005; Siegmund et al., 2005; current report). Additionally, the second ADE was accompanied with the vehicle/drug administration, which can also be considered as stressful.

To study the effects of different stressors we performed forced swim and foot-shock stress. Both groups increased alcohol intake as compared to baseline drinking levels after swim stress. However, the adolescent group achieved considerably higher intake levels (150% over baseline) compared to the adult group. The effect of foot-shock on alcohol intake was comparable to swim stress. Thus, female animals that started to drink alcohol during their adolescent age, similar to male animals (Siegmund et al., 2005), exhibit a higher susceptibility to stress-induced alcohol drinking.

Overall female rats in our study consumed greater amounts of alcohol than it was previously shown in our laboratory in male rats (Siegmund et al., 2005). This is in agreement with previous studies reporting that there is a gender difference in ethanol ingestion (e.g. Lancaster and Spiegel, 1992; Adams, 1995; Lancaster et al., 1996; Almeida et al., 1998) and that female rats consume significantly greater amounts of alcohol. At first view this is in stark contrast to what is observed in humans as epidemiological and clinical studies demonstrate that females do consume less alcohol than males. However, we have recently reported that if alcohol intake in humans would be also calculated on a g/kg basis instead of the number of drinks consumed, consumption in females would be pretty much the same or even more compared to males (Kiefer and Spanagel, 2006). Thus, opposing gender differences in humans and animals are mainly related to social barriers in different populations and to an artefact in calculating exact alcohol intake. The reasons for gender differences in alcohol consumption are still poorly understood. However, it is obvious that intrinsic sex differences in brain organization and the actions of circulating gonadal steroids could contribute to the enhanced voluntary alcohol intake in female animals (Almeida et al., 1998; Becker et al., 2001).

Acamprosate is a clinically used drug that acts on the glutamatergic system (De Witte et al., 2005; Spanagel et al., 2005). Acamprosate is also effective in reducing ethanol drinking and relapse behavior in rodents (Spanagel et al., 1996; Zornoza et al., 2003; Cowen et al., 2005; De Witte et al., 2005). Our present study shows that acamprosate was able to reduce relapse-like drinking behavior after long-term voluntary alcohol consumption

in adult female rats. However, acamprosate had no effect on relapse-like alcohol drinking in the adolescent group, showing that female rats that initiated their drinking at an early age do not respond to acamprosate treatment.

In summary, our data confirm that alcohol use during adolescence constitutes a risk factor for alcohol-related problems and dependence in later life. Thus, we could demonstrate in both male (Siegmund et al., 2005) and female (the present report) rats that initiation of alcohol drinking during adolescence may lead to increased alcohol intake following exposure to stress. Moreover, there is a reason to suggest that early onset of alcohol drinking could result into non-responsiveness to acamprosate treatment.

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