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Voluntary alcohol intake in two rat lines selectively bred for learned helpless and non-helpless behavior

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Abstract *Rationale:* A high comorbidity between depression and alcoholism has been reported in several studies, but the mechanisms underlying this relationship remain unknown. *Objectives:* We tested whether learned helplessness in rats as a model for depression is associated with enhanced alcohol intake and relapse behavior. *Methods:* Congenital learned helplessness (cLH) and congenital non-learned helplessness (cNLH) rats were selectively bred for differences in an escape paradigm. Sucrose preference was tested at the first hour of the dark phase. In order to study an association with alcohol drinking behavior, rats underwent a free-choice procedure with access to water, and 5% and 20% alcohol solutions for 6 weeks. After acquisition of alcohol drinking behavior, the alcohol deprivation effect (ADE) was assessed. Sensitivity to the sedative-hypnotic effect of alcohol was measured by loss of the righting reflex. *Results:* cLH rats showed significantly lower preference for sucrose solutions during the second half hour of the dark phase than cNLH rats. Alcohol intake of male cLH rats was not significantly different from that of male cNLH rats. In contrast, cLH female rats consumed higher amounts of alcohol than female cNLH rats. The ADE was more pronounced in female animals, although the magnitude of the ADE was similar in both cNLH and cLH female rats. The time to regain the righting reflex was significantly higher in both male and female cLH rats than

in cNLH rats. *Conclusions:* In summary, these data suggest that an inborn depressive-like behavior in female rats is associated with enhanced alcohol intake.

Keywords Learned helplessness · Anhedonia · Voluntary alcohol drinking · Alcohol deprivation effect · Loss of righting reflex

Introduction

Comorbidity of alcoholism and depressive disorders has been extensively documented in both epidemiological and clinical investigations (Schuckit et al. 1969; Kessler et al. 1994; Merikangas et al. 1994, 1996; Swendsen et al. 1998; Angst et al. 2002). The association may be based on common neurobiological factors mediating depression and alcohol dependence (for review Markou et al. 1998). However, depression can be effectively treated with antidepressants whereas the use of these drugs is very limited in the treatment of alcohol dependence. No consensus has been reached regarding the specific mechanisms underlying the association of both disorders and it remains unclear whether one of the disorders causes or predisposes to the other. Although several studies have investigated the role of genetic components (Allen 1976; Cloninger 1987; Winokur and Coryell 1992; Merikangas et al. 1994; Schuckit and Smith 1996; Preisig et al. 2001), it is still not clear whether there are common or shared genes leading to the association of these psychiatric disorders (for review Markou et al. 1998).

The relationship between high alcohol intake and a depressed-like state has been studied in alcohol-preferring rat lines. However, results are not consistent—some of the studies indicate a positive correlation between high alcohol intake and a depressed-like state, whereas others do not (Kiianmaa et al. 1991; Overstreet et al. 1992; Godfrey et al. 1997; Ciccocioppo et al. 1999). In the present study, we used another approach: we examined alcohol drinking behavior in two lines of rats—congenital learned helplessness (cLH) and congenital non-learned

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helplessness (cNLH)—which have been developed on the basis of their behavior in learned helplessness testing.

Inescapable stress can lead to a deficit in escape or avoidance behavior termed “learned helplessness” (Overmier and Seligman 1967) or “motor activation deficit” (Weiss and Glazer 1975). Learned helplessness is a well-established pharmacologically specific model for major depression with a good construct and face validity (for review Willner 1984; Willner and Mitchell 2002). Based on the assumption that the predisposition for “depression-like” behavior may be at least in part genetically determined, several rat lines have been introduced as genetic models of depression: SwLo rats and SwHi rats were selectively bred for their differential susceptibilities to stress-induced changes in swim-test activity (Weiss et al. 1998). Flinders sensitive and resistant lines were selected for their differential hypothalamic responses to an acetylcholinesterase agent (Overstreet 2002). We started a selective breeding program in order to develop a genetic model of learned helplessness. On a Sprague–Dawley background, we performed mating after phenotypic selection of escape performance after inescapable stress. After more than 20 generations, this has resulted in two lines: (1) cLH demonstrating helpless behavior congenitally without previous exposure to inescapable shock and (2) cNLH being resistant to the effects of inescapable stress and used as a control strain. The escape deficit of cLH rats is not confounded by deficits in general locomotor activity or learning and memory (Vollmayr et al. 2004). Also, there is no difference in pain sensitivity during the hot-plate test between the two strains (unpublished observations). Furthermore, cLH rats exhibit a decreased sensitivity to sucrose under operant conditions which may indicate anhedonia or anergia and, thus, underlines the utility of the cLH strain as a genetic model of depression (Vollmayr et al. 2004).

First, we studied whether animals with congenital helplessness to stress will exhibit decreased preference for sucrose solutions over water. Voluntary alcohol drinking behavior and the alcohol deprivation effect (ADE), which is used as a model of relapse-like drinking behavior (Spanagel and Holter 1999; Spanagel 2000; Vengeliene et al. 2003), were examined. Finally, the sensitivity to the sedative-hypnotic dose of alcohol was also measured in all animals.

While alcohol dependence is more common in men, epidemiological data demonstrate clearly that unipolar depression is approximately twice as common in women than in men (Weissman and Olfson 1995; Piccinelli and Wilkinson 2000; for review Kuehner 2003), and comorbidity of alcohol dependence and depression also is more common in women than in men (Hesselbrock et al. 1985; Davidson and Ritson 1993; Dunne et al. 1993; Dixit and Crum 2000). Therefore, in the present study both, male and female cLH and cNLH rat lines, were tested.

Materials and methods

Animals

Rats from the 47th, 48th and 52nd generations of helplessness colonies (cLH and cNLH) were used. Breeding and testing of the helplessness colonies has been described previously (Vollmayr et al. 2001, 2004; Vollmayr and Henn 2001). Inescapable shock consisted of a 0.8-mA foot shock of random length and intervals of between 3 s and 10 s with a total shock time of 20 min. Rats were tested in 15 trials of an escape paradigm where a 60-s, 0.8-mA foot-shock could be eliminated with a bar press. Intertrial time was 24 s. A trial not stopped at 20 s or beyond was considered a failure. Animals failing to respond in more than 9 of 15 trials were selected as helpless; animals with less than five failures were selected as not helpless. Helpless animals and non-helpless animals, respectively, were mated for the subsequent generations with occasional backcrosses to the paternal Sprague–Dawley outbred strain (Janvier, Le Genest St Isle, France). Brother-sister matings were avoided. Two selective lines resulted: the congenital helpless line, demonstrating helpless behavior without prior inescapable shock, and the congenital non-helpless line, resistant to the development of learned helplessness. The behavioral phenotype of both strains was confirmed by testing the animals at the age of 8 weeks. cLH rats were tested without inescapable shock exposure, cNLH rats received inescapable shock 24 h prior to testing. For all subsequent experiments, the observer was blind to the experimental group.

Four groups of adult animals were studied: male cNLH rats ($n=16$) weighing an average of 526 ± 12 g, male cLH rats ($n=16$) weighing an average of 537 ± 8 g, female cNLH rats ($n=14$) weighing an average of 320 ± 7 g, and female cLH rats ($n=14$) weighing an average of 323 ± 8 g at the start of the experiment. All animals were housed individually in standard rat cages under a 12-h/12-h artificial light/dark cycle (lights on at 0600 hours). Room temperature was kept constant (temperature: $22\pm 1^\circ\text{C}$, humidity: $55\pm 5\%$). Standard laboratory rat food and water were provided ad libitum throughout the experimental period. Body weights were measured weekly. The experiments were approved by the Regierungspräsidium Karlsruhe and carried out according the German Law on the Protection of Animals.

Sucrose preference test

After 2 weeks of habituation to the animal room, rats from all four groups were tested for sucrose preference using the protocol originally established by D'Aquila et al. (1997). At the start of sucrose preference testing, all animals were first trained to drink 0.5% sucrose solution, by exposing them to the bottle with sucrose in the place of water for 24 h. Test procedures were performed on three consecutive days (for 60 min of total duration each day) preceded by

23 h food and water deprivation. Each animal was presented with two bottles simultaneously, one containing sucrose solution and the other water, as well as food ad libitum. The position of the two bottles (right/left) was varied randomly from trial to trial and counterbalanced across the animals. During the test, the bottles were weighed at two time points, after 30 min and after 60 min. The tests were carried out at the start of the dark cycle presuming that symptoms of anhedonia could be more pronounced at this time (D'Aquila et al. 1997). Sucrose preference (sucrose solution consumed over the total fluid intake) for each day and average preference for all 3 days were calculated.

Alcohol self-administration procedure

To observe alcohol-drinking behavior, rats were given access to tap water and to 5% and 20% ethanol solutions (v/v) ad libitum. Alcohol drinking solutions were made from 96% ethanol diluted with tap water to the different concentrations. Spillage and evaporation were minimized by the use of special bottle caps (TSE, Bad Homburg, Germany). With this procedure, the alcohol concentration in a given solution stayed constant for at least 1 week (Hölter et al. 1998). The positions of bottles were changed twice weekly to avoid location preferences. The 5% ethanol intake, 20% ethanol intake and total ethanol intake (g/kg body weight per day) values were calculated as the daily average across the seven measuring days.

Alcohol deprivation procedure

All rats underwent one deprivation period of 2 weeks after 6 weeks of continuous alcohol availability. To measure the ADE, alcohol consumption was measured daily until a stable baseline was established. Following the 14-day

deprivation phase, alcohol intake was measured daily for 4 days.

Loss of righting reflex

To examine the sensitivity to the sedative-hypnotic effect of alcohol, two separate groups of alcohol-naïve male rats—cNLH ($n=8$) and cLH ($n=8$)—were injected (i.p.) with ethanol (20% v/v) at the dose of 3.0 g/kg body weight. Two groups of alcohol-naïve female rats—cNLH ($n=8$) and cLH ($n=8$)—were administered with a dose of 3.8 g/kg of ethanol, since a preliminary study demonstrated a lower sensitivity to the sedative effects of ethanol in female rats. When animals became ataxic, they were placed on their backs, and the time until the righting response was recorded. Animals were judged to have regained their righting response when they could right themselves three times within 30 s. The observer was blind to the experimental design.

In addition, 25–30 μ l of tail vein blood was taken for blood alcohol level determination at 20, 40, 80 and 120 min after the ethanol injection. The blood alcohol concentration was evaluated via the nicotinamide dinucleotide phosphate enzyme spectrophotometric method (Rolf Greiner BioChemica GmbH, Germany).

Statistics

Data obtained from the sucrose preference testing was analyzed by means of three-way analysis of variance (ANOVA) (line, gender and time; i.e., first and second half an hour). Data obtained from alcohol self-administration experiments were analyzed by means of two-way ANOVA with repeated measures (between subjects—line and within subjects—time; i.e., weeks/days). Whenever significant differences were found, post-hoc Student Newman Keul's tests were performed. The difference in duration of

Table 1 The 5% and 20% ethanol intake values (g/kg) over the whole time course of the alcohol drinking experiment in male cLH ($n=16$) and cNLH ($n=16$) rats, and in female cLH ($n=14$) and cNLH ($n=14$) rats

Time	Male rats				Female rats			
	5% Ethanol		20% Ethanol		5% Ethanol		20% Ethanol	
	cLH	CNLH	cLH	CNLH	CLH	cNLH	cLH	cNLH
Acquisition								
Days 1–4	0.35±0.1	0.51±0.2	0.45±0.1	0.47±0.1	1.13±0.2	0.79±0.1	0.96±0.1 ^a	0.50±0.1
Maintenance								
Week 1	0.32±0.1	0.50±0.2	0.41±0.0	0.42±0.1	0.91±0.2	0.65±0.1	0.85±0.1	0.46±0.1
Week 2	0.32±0.1	0.35±0.0	0.50±0.1	0.49±0.1	0.65±0.1	0.40±0.1	0.69±0.1	0.58±0.1
Week 3	0.39±0.1	0.46±0.1	0.47±0.1	0.46±0.1	1.04±0.2 ^a	0.45±0.1	0.71±0.1	0.55±0.1
Week 4	0.41±0.1	0.67±0.1	0.55±0.1	0.55±0.1	1.33±0.2 ^a	0.71±0.2	0.89±0.2	0.58±0.1
Week 5	0.32±0.1	0.52±0.2	0.60±0.1	0.53±0.1	1.32±0.3 ^a	0.67±0.2	0.80±0.1	0.53±0.1
Week 6	0.36±0.1	0.55±0.1	0.53±0.1	0.55±0.1	1.11±0.2 ^a	0.53±0.1	0.99±0.1	0.62±0.1
ADE								
Day 1	0.63±0.1	1.02±0.2 ^b	1.00±0.2 ^b	0.82±0.1 ^b	1.38±0.3	1.01±0.2	1.67±0.3 ^b	1.19±0.3 ^b
Day 2	0.43±0.1	0.61±0.2	0.54±0.1	0.52±0.1	1.09±0.3	0.78±0.3	1.05±0.2	0.76±0.2
Day 3	0.22±0.1	0.38±0.2	0.53±0.1	0.42±0.1	1.29±0.4	0.60±0.2	0.64±0.1	0.45±0.1
Day 4	0.20±0.1	0.35±0.1	0.59±0.1	0.67±0.1	1.43±0.4	1.01±0.3	0.87±0.2	0.53±0.1

Weekly ethanol intake was calculated as the average of 7 days measurements. Data are presented as means±SEM

^aSignificant differences compared with the cNLH group

^bSignificant differences to baseline drinking, $P<0.05$

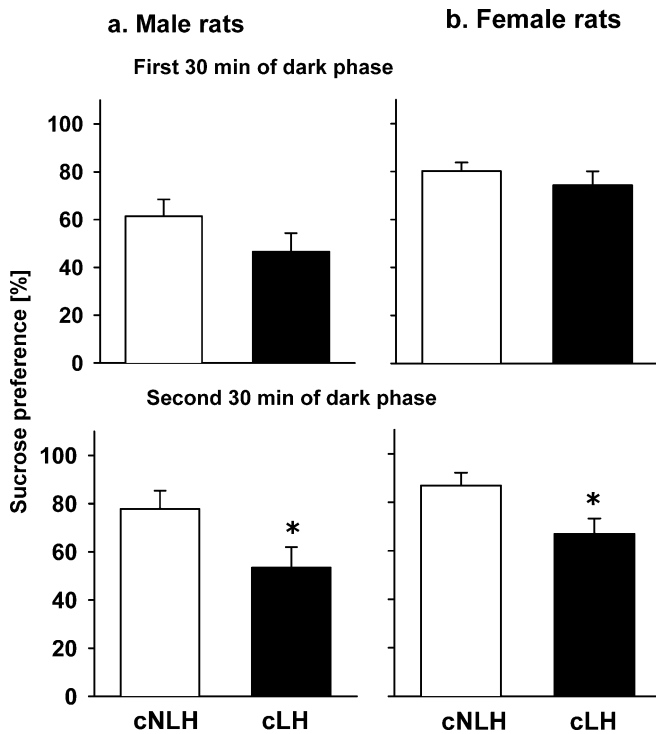


Fig. 1 Sucrose solution preference in male cLH ($n=16$) and cNLH ($n=16$) rats (a) and in female cLH ($n=14$) and cNLH ($n=14$) rats (b) during the first and second 30 min of the dark phase (average of 3 days). Data are presented as means \pm SEM. Asterisks Significant differences between the two lines, $P<0.05$

ethanol-induced sleep time between cLH and cNLH lines was evaluated using independent two-tailed t -tests for each gender separately. The chosen level of significance was $P<0.05$.

Results

Sucrose preference test

Three-way ANOVA data analysis revealed a significant overall difference in sucrose preference between the two rat lines (line: $F_{1,56}=7.0$, $P<0.01$). Sucrose preference was also dependent on time (time: $F_{1,56}=7.9$, $P<0.01$) but not on gender. There were no difference found between cLH and cNLH animal lines in the sucrose preference measured during the first half hour of testing, but during the second half an hour of testing cLH animal group showed a significantly lower preference for sucrose solution compared to cNLH animals (line: $F_{1,28}=6.4$, $P<0.05$; Fig. 1).

Alcohol drinking behavior

During the first 4 days of measurement, which resemble the acquisition period of alcohol drinking, male cLH rats consumed 0.8 ± 0.1 g/kg while male cNLH rats consumed 1.0 ± 0.2 g/kg per day. During the acquisition period, female rats had a significantly different alcohol intake

(line: $F_{1,26}=9.6$, $P<0.01$); female cLH rats consumed 2.1 ± 0.2 g/kg and female cNLH rats 1.3 ± 0.1 g/kg of alcohol per day. The intake of both 5% ethanol and 20% ethanol solutions was not different between the two lines of male rats, while the female cLH rat line had a higher 20% ethanol intake during the acquisition period (line: $F_{1,26}=6.8$, $P<0.05$; Table 1).

Over the whole time course of the experiment (6 weeks=maintenance phase), total alcohol intake of male cLH rats was not significantly different from that of male cNLH rats (Fig. 2a). Intake for both 5% ethanol and 20% ethanol solutions was not significantly different between the two lines of male rats (Table 1). In contrast, during the maintenance phase, total alcohol intake by female cLH rats remained significantly higher than in female cNLH rats (line: $F_{1,26}=12.9$, $P<0.001$; Fig. 2b). In particular, a significantly higher intake of 5% ethanol was found in cLH female rats (line: $F_{1,26}=7.0$, $P<0.05$; Table 1).

Alcohol deprivation effect

Alcohol deprivation led to an increase in alcohol consumption, indicating the occurrence of an ADE (Fig. 3). Two-way ANOVA data analysis indicated a significant effect of day on alcohol intake in male rats (day:

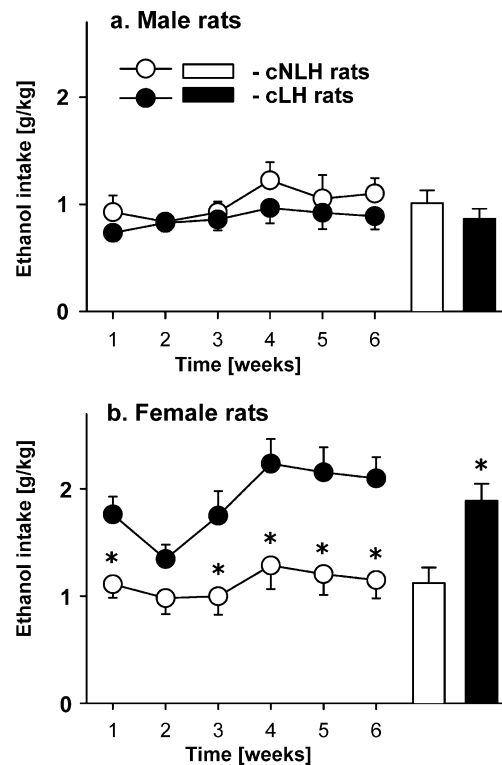


Fig. 2 Total ethanol intake (g/kg body weight per day) over the time course of 6 weeks in male cLH ($n=16$) and cNLH ($n=16$) rats (a) and in female cLH ($n=14$) and cNLH ($n=14$) rats (b). White and black circles Ethanol intake by cLH and cNLH rats, respectively (average of 7 days). Bars Average alcohol consumption in each group. Data are presented as means \pm SEM. Asterisks Significant differences between the two lines, $P<0.05$

$F_{4,120}=24.5$, $P<0.0001$; Fig. 3a). Further analysis demonstrated a significant effect of alcohol deprivation on 5% ethanol intake and 20% ethanol intake in both cLH and cNLH male rats (day: $F_{4,120}=10.8$, $P<0.0001$ and $F_{4,120}=11.3$, $P<0.0001$, respectively; Table 1). Alcohol deprivation significantly increased alcohol intake also in female cLH and cNLH rats (day: $F_{4,104}=7.7$, $P<0.0001$; Fig. 3b). Moreover, alcohol intake during the first four post-deprivation days was found to be significantly higher in female cLH animals than in female cNLH rats (line: $F_{1,26}=5.8$, $P<0.05$). However, the magnitude of the ADE was similar in both cLH and cNLH female rats. Thus, only during the first day following deprivation could a significant increase in alcohol intake relative to baseline drinking be observed. In respect to the intake of different ethanol solutions, alcohol deprivation led to a significant increase of the 20% ethanol solution in both lines (day: $F_{4,104}=18.7$, $P<0.0001$; Table 1).

Loss of righting reflex

The sleep time after the administration of an acute dose of alcohol (3 g/kg for male rats and 3.8 g/kg for female rats, i. p.) was significantly shorter in cNLH rats than in cLH rats for both male and female animals ($F_{1,14}=6.5$, $P<0.05$ and

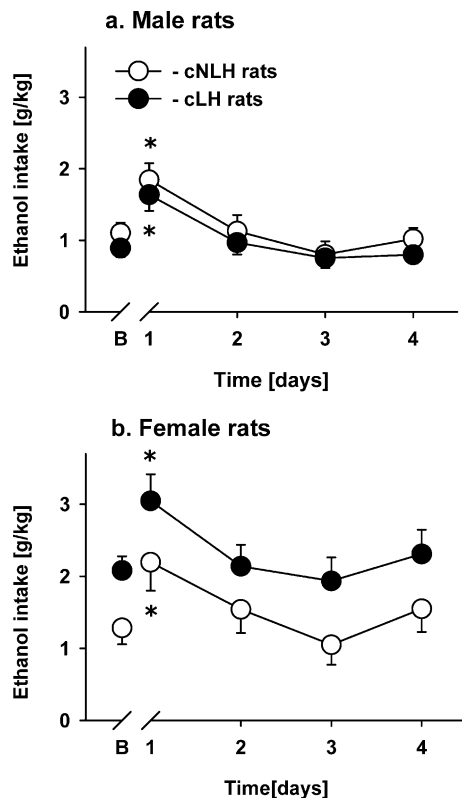


Fig. 3 Ethanol intake (g/kg per day) in male cLH ($n=16$) and cNLH ($n=16$) rats (a) and in female cLH ($n=14$) and cNLH ($n=14$) rats (b) before and after an alcohol deprivation period of 2 weeks. B Baseline drinking prior to deprivation as the average of 4 days measurements. Data are presented as means \pm SEM. Asterisks Significant differences to baseline drinking, $P<0.05$

$F_{1,14}=4.7$, $P<0.05$, respectively; Fig. 4). Thus, male cNLH rats needed 252 ± 17 min and male cLH 372 ± 46 min to recover their righting reflex, while female cNLH rats needed 286 ± 11 min and female cLH 339 ± 25 min (Fig. 4). Blood alcohol concentrations were maximal after 20 min (cLH: 269 ± 15 mg/dl and cNLH: 280 ± 13 mg/dl) but did not differ significantly at any time point (data not shown).

Discussion

In the present study, the relationship between inborn depressed-like behavior and alcohol drinking behavior was studied in male and female cLH and cNLH rat lines. Thus, acquisition and maintenance of alcohol drinking behavior, the effect of alcohol deprivation, and sensitivity for alcohol was examined in all animals. Before studying these acute and chronic alcohol effects, a sucrose preference test revealed the same degree of anhedonia in both male and female cLH rats. We found that alcohol intake by male cLH and cNLH rat lines was not significantly different. On the contrary, female cLH rats consumed higher amounts of alcohol than female cNLH rats. Following an alcohol deprivation phase of 2 weeks, a significant transient increase in voluntary alcohol intake and preference ensued in both male and female rats; however, the magnitude of the ADE was similar in both cNLH and cLH animals. Sensitivity to the sedative-hypnotic effect of alcohol was significantly higher in both male and female cLH rats than that in cNLH rats.

It is known that animals display impairments of reward behavior following exposure to stressors in chronic mild stress and learned helplessness models of depression. Decreased sensitivity of brain reward mechanisms (anhedonia) is one of the core diagnostic criteria of human depressive episodes according to ICD-10 or DSM-IV and can be modeled in animals by reduced consumption of a low concentrated sucrose solution (for review Willner 1991). Worsening of the depressive symptoms in the morning is recognized as having special clinical significance by ICD-10. Diurnal variations with symptoms worst

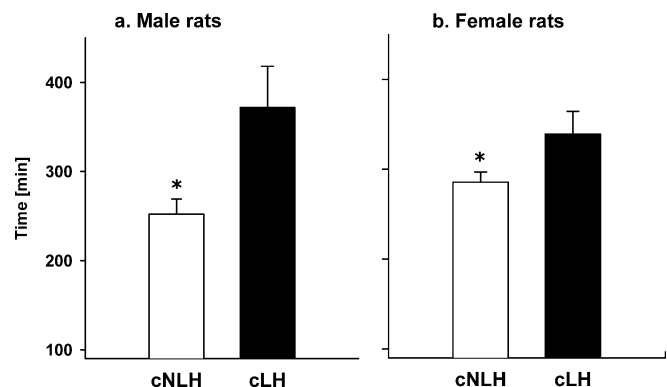


Fig. 4 Duration of loss of righting reflex (min) in male cLH ($n=8$) and cNLH ($n=8$) rats (a) and in female cLH ($n=8$) and cNLH ($n=8$) rats (b). Data are presented as means \pm SEM. Asterisks Significant differences between the two lines, $P<0.05$

at the start of active phase have been also demonstrated in animals (D'Aquila et al. 1997). Therefore, in our study, the preference for sucrose solution was examined at the first hour of the active (dark) phase. Although no difference in sucrose preference was found during the first half hour, both male and female cLH animals exhibited significantly lower preferences for sucrose solution than cNLH rats in the second half hour that indicates the presence of innate subsensitivity to natural rewards of both male and female cLH rats. In this respect, differences in gustatory sensitivity in cLH and cNLH rat lines should be considered as a possible confounding factor which could contribute to a reduced preference for sucrose. However, the finding of lower preference for a sucrose solution in cLH animals is limited to specific situations, such as limited access or higher response requirements in an operant task (Vollmayr et al. 2004). Furthermore differences in sucrose preference between these lines do not occur under baseline conditions, suggesting that differences in gustatory sensitivity are most likely not involved in reduced sucrose preference in LH animals. Decreased sensitivity to reward or anhedonia validates, at least in part, the congenitally learned helpless line as an animal model of predisposition to depression (Willner and Mitchell 2002).

Although the comorbidity of alcoholism and depressive disorder has been extensively documented in both clinical and epidemiological studies, the relationship between these disorders remains unclear. Thus, it is not known whether one of the disorders causes or predisposes to the other. A reason for this might be that in many cases only depressive mood disturbances or alcoholism are diagnosed but not both together, and in some individuals depressive symptoms may be directly related to chronic alcohol intoxication and are likely to improve markedly within days or weeks of abstinence (Davidson 1995; Schuckit et al. 1997). Results of studies in laboratory animal models are also contradictory: a positive relationship between high alcohol preference and a depressive-like behavior, measured by immobility time in a forced swim test model (Porsolt et al. 1978), has been seen in genetically selected alcohol-preferring AA rats (Kianmaa et al. 1991) and in Sardinian alcohol-preferring rats (Ciccocioppo et al. 1999). In contrast, alcohol non-preferring rats spend more time immobile than P rats, and between high alcohol drinking rats and low alcohol drinking rats there was no difference found (Godfrey et al. 1997). Correlation between high immobility score and high alcohol preference has been documented also in the Fawn-Hooded rat line, but low rates of alcohol intake and preference were found in Flinders sensitive line, a potential animal model of depression (Overstreet et al. 1992).

According to the self-medication hypothesis, depressive symptomatology may underlie the initial motivation to drink alcohol (for review Markou et al. 1998). This would predict higher intake of alcohol in the cLH rats than in cNLH rats. In our study, differences in the amount of alcohol consumed between female cLH and cNLH rats had already been seen at the first 4 days of alcohol exposure (acquisition period). Moreover, higher alcohol

intake by female cLH rats remained stable over the whole time course of the experiment. Following alcohol deprivation, a typical ADE could be observed but no line differences could be measured in the expression of the magnitude of the ADE. Thus, cLH and cNLH female rats drank more after the deprivation period, and the cLH continued to drink more than the cNLH. Both male rat lines increased their alcohol intake during post-deprivation days, but there were still no differences seen between male cLH and cNLH rats. However, it should be noted that the levels of alcohol intake in this study were low, which is not surprising since the cLH and cNLH lines derive from a Sprague-Dawley colony well known as outbred rats with a low intake of alcohol (Khanna et al. 1990; Pare et al. 1999; Henniger et al. 2002). Nevertheless, it is known that significant changes in a rat's behavior can be induced by ingestion of as little as 0.2–0.3 g/kg of ethanol (Wolffgramm and Heyne 1995). In summary, our drinking studies indicate that individuals who are congenitally helpless and have reduced sensitivity to the rewarding effects of sucrose are more likely to drink alcohol, although they are not different in alcohol relapse-like drinking behavior. However, this association was only found in female cLH animals. An explanation of this gender difference is lacking at the moment since it is not known how male and female cLH rats differ in neurobiological reward mechanisms. A suggestion is that both intrinsic sex differences in brain organization and the actions of circulating gonadal steroids can contribute to the enhanced voluntary alcohol intake in female cLH animals (Almeida et al. 1998; Becker et al. 2001).

The ability to consume relatively high amounts of alcohol from an early age with comparatively little effect is hypothesized to be one factor that might increase an individual's risk to alcoholism (Schuckit and Smith 1996; Schuckit 1998; Schuckit et al. 2000). Therefore, we have also measured the acute response to alcohol in cLH and cNLH animals. Sensitivity to the sedative-hypnotic effect of alcohol was measured as the time to recover the righting reflex after acute administration of a high intoxicating dose of alcohol. In general, female rats showed a lower sensitivity to the sedative effect of alcohol, an effect that in female rats is inversely correlated with the ability to consume higher amounts of alcohol. It has been shown in previous studies that female rats have higher alcohol consumption and higher metabolic rates than males, which corresponds to a lower response to alcohol in female rats (Almeida et al. 1998; Lancaster 1995; Thomasson 1995; Matsumoto and Fukui 2002). With respect to line differences, female and male cNLH rats were found to be less sensitive to alcohol than cLH animals. Although initial sensitivity to alcohol may be an important factor in determining alcohol intake, at least in some cases the critical factor appears to be the individual organism's innate propensity to find alcohol reinforcing. In agreement, the initial sensitivity to alcohol has also been reported to vary widely among rat lines selectively bred for opposite alcohol preference (Colombo et al. 2000; Bell et al. 2001; Li et al. 2001; for review George 1993).

In summary, we could show that male and female cLH rats show a reduced sucrose preference, which indicates reduced sensitivity to reward associated with learned helplessness. Reduced sensitivity to reward which is used as a measure for anhedonia might be a reason why cLH animals consume more alcohol in comparison with the cNLH line. However, this relationship is gender specific. Thus, only female animals consume more alcohol. At the moment, it is not clear which neurobiological mechanisms in the reward pathway drive these gender differences, however, there are some similarities to the human situation since, among alcohol-dependent patients, women are more likely than men to suffer additionally from primary or secondary depression (Hesselbrock et al. 1985; Davidson and Ritson 1993; Dunne et al. 1993; Dixit and Crum 2000).

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