Pharmacology, Biochemistry and Behavior 95 (2010) 390–400

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Acute novel stressors modify ethanol intake of psychosocially stressed rats

Larissa A. Pohorecky *

Center of Alcohol Studies, Rutgers University, 607 Allison Road, Piscataway, NJ 08854-1100, USA

ARTICLE INFO

Article history:
Received 1 October 2009
Received in revised form 22 February 2010
Accepted 28 February 2010
Available online 6 March 2010

Keywords:
Rank status
Housing aggression
Resident–intruder test
Elevated plus maze
Open field arena

ABSTRACT

Psychosocial stressors are known to alter ingestion of ethanol in humans and experimental animals. We evaluated the effect of novel acute stressors on ethanol ingestion of male triad-housed rats. Based on behavioral and body weight assessments triad members were designated as dominant, subdominant or subordinate rats, housed in triads designated as aggressive or non-aggressive triads. The triad-housed, and a group of single-housed rats, were sequentially subjected to three stressors (novel open field arena, elevated plus maze, and modified resident–intruder test) at 1–2 week intervals. Ethanol intake was measured for 21-h before and after each stressor. Prior to stressor exposure, ethanol intake of the triad-housed rats was higher than that of single-housed rats. In triads overall intake of ethanol was lower in dominant compared to non-dominant rats. The modified resident–intruder test decreased ethanol intake in non-dominant rats in aggressive triads, but increased its intake in non-aggressive triads. Since in non-dominant rats this stressor also increased ethanol preference but not total fluid intake, its effect on ethanol intake was specific. In non-dominant rats ethanol intake and preference declined after the elevated plus maze stressor, without an effect on total fluid intake, but water intake was increased only in the subdominant rats. Compared to triad-housed rats, single-housed rats were more resilient to the novel stressors. It can be concluded that novel acute stressors have specific effects on ethanol intake that are dependent on the subject's psychosocial stress level.

1. Introduction

It is widely recognized that many variables can significantly impact the consumption of ethanol. Genetic influences, including differences in taste sensitivity and in emotionality–anxiety dimensions are some of the subject-related factors that can regulate the consumption of ethanol. In turn, these individual differences in ethanol consumption can be modified by environmental factors. Stress is one of the major environmental factors that can alter the consumption of ethanol, as documented by several reviews (Björkqvist, 2001; Rospenda et al., 2000; José et al., 2000; Richman et al., 1996; Pohorecky, 1991, 1990). In particular, psychosocial stress has been shown to have a strong influence on the ingestion of ethanol both in humans and in experimental animals (Booth and Hasking, 2009; Miczek et al., 1994a,b; Blanchard et al., 1993; Pohorecky, 1990).

In experimental animals, a variety of non-social stressors, such as exercise, restraint, and footshock have been reported to increase the consumption of ethanol (Racz et al., 2008; Siegmund et al., 2005; Vengeliene et al., 2003; Werme et al., 2002; Lynch et al., 1999). Other investigators, on the other hand, found a negative effect of stress on ethanol consumption. For example, rodents subjected to chronic unpredictable restraint stress, repeated footshock stress or forced swim stress decreased the consumption of ethanol (Boyce-Rustay et al., 2008; Fidler and LoLordo, 1996). Ten days of immobilization stress also attenuated ethanol intake during the first five post-stress days, though only in rats that were genetically predisposed to high ethanol intake (Chester et al., 2004). This last finding is supported by studies that indicate the importance of genetic background on the effect stress on ethanol consumption (Boyce-Rustay et al., 2008; Matthews et al., 2008; Yang et al., 2008).

The impact of psychosocial stress on ethanol intake has been investigated in various animal models. Overall studies that focused on social isolation (single-housing) stress have been inconsistent. While many studies reported that isolation-stressed rodents ingested more ethanol compared to group-housed rodents (Ehlers et al., 2007; Juárez and Vázquez-Cortés, 2003; Hall et al., 1998; Roske et al., 1994; Schenk et al., 1990; Wolffgramm, 1990), others have reported a decline (Adams and Oldham, 1996) or no effect on ethanol intake (Doremus et al., 2005; Thorsell et al., 2005). Some of these studies evaluated ethanol drinking using the resident–intruder test. These studies reported a decline in ethanol intake in defeated rats (Funk et al., 2005; van Erp and Miczek, 2001). For mice, on the other hand, an acute defeat did not have significant effect on ethanol intake (Croft et al., 2005; Keeney and Hogg, 1999). Nevertheless, a slow increase in ethanol ingestion was noted when the resident–intruder test was repeated once per week for 5 weeks (Croft et al., 2005). Other studies of psychosocial stress evaluated ethanol intake in group-housed or pair-housed rodents. These studies reported that ethanol intake was

* Tel.: +1 732 445 3593; fax: +1 732 445 3500.
E-mail address: larissa@rci.rutgers.edu.

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.02.017

enhanced in group-housed *non-dominant* rats compared to the intake of the dominant rats (Pohorecky, 2008; Blanchard et al., 1993; Wolffgramm and Heyne, 1991; Weisinger et al., 1989; Blanchard et al, 1987; Ellison, 1987).

The triad-housing model developed in our laboratory (Blakley and Pohorecky, 2006; Steensland et al., 2005; Pohorecky et al., 2004a; 2004b; 1999) is particularly amenable for assessing the behavioral and neurobiologic consequences of psychosocial stress. The model allows the rapid development of a stable and robust social hierarchy of co-housed male rats. Employing this model we have reported that a novel stressor can alter the intake of ethanol (Pohorecky et al., 2008). In this study exposure to an elevated plus maze stressor had a negative impact on ethanol ingestion in triad-housed rats, but had no effect on ethanol intake in single-housed rats. Since stressors are known to have specific neurobiological and behavioral effects (Kvetnansky et al., 2009; McEwen, 2000), some of the discrepant findings on ethanol intake may, at least in part, be due to the type of stressor employed, as previously suggested by the discrepant evidence on the relationship of stress and alcohol intake in humans (Pohorecky, 1991). Therefore, the issue of stressor specificity and its impact on ethanol intake requires further scrutiny. The aim of the present study was twofold, (1) to confirm our previous report that a subject's psychosocial stress experience alters the effect of a novel acute stressor on ethanol intake, and (2) to test the hypothesis that in psychosocially stressed animals ethanol intake will be differentially affected by distinct novel stressors.

2. Methods

2.1. Subjects and housing

The subjects were male adult Long Evans rats (Harlan, Indianapolis, Indiana) weighing approximately 300 g at the start of the experiment. Purina chow and water were available *ad libitum* throughout the study. The animal room was kept at 21 ± 1 °C, with controlled humidity and a reverse light/dark cycle (12 h each, lights off at 12:30 PM). To adapt to our animal room conditions, rats were initially individually housed in hanging wire-mesh stainless steel cages for 18 days. During the last 5 days prior to a study, all rats were handled daily to minimize stress from human contact. Subjects were randomly assigned to triad or individual housing based on body weight, namely, the body weight of the rats assigned to a triad differed by less than 5%. Rats were weighed prior to group housing, 24-h after group housing, and daily when the intake of ethanol was being assessed. The housing cages were made of Plexiglas and had a wire mesh floor. The triad cages (housing three rats) were 26 cm wide × 82 cm long × 30 cm high, and the single-housed cages were 25 × 25 × 30 cm, both were made of Plexiglas. To control for potential major differences in locomotor activity due to differences in cage size between triad and single-housing cages, the dimension of the cages were adjusted to approximately a similar floor footprint. The floor space per rat was 625 cm^2 and 710 cm^2 for the single and triad cages, respectively. The triad cages had two removable Plexiglas cage dividers that partitioned the triad cage into three compartments equivalent in size to the single-housing cages, the dividers were utilized only initially to separate the members of a triad for identification, based on video camera records, and for simultaneously starting the initial social interaction when the dividers were removed. Each triad cage had one drinking station located in the middle of one of the long walls of the triad cage. It consisted of a small square wall opening (7 × 7 cm) that allowed access of only a single rat's head and neck to the drinking station. At its far end there were two stainless steel spouts coming from drinking tubes attached to the outside of the drinking station. Our animal facility has been certified by AAALAC, and the experimental protocols were approved by the Rutgers University review Committee for the use of Animal Subjects; all the principles of laboratory animal care were strictly adhered to.

2.2. Agonistic behavior rating

Agonistic behaviors were assessed at the time triads were formed. On day one of the study, subjects were placed into a novel triad cage with the cage dividers in place. This kept the three rats separated from each other for a 5-minute adaptation period and for recording individual subject identification. The cage dividers were then simultaneously removed, and social interactions were recorded for the next 30 min. Agonistic behaviors were scored using an expanded and modified version (Steensland et al., 2005; Pohorecky et al., 1999, 2004b) of the method originally described by Peterson and Pohorecky (1989). Twenty-three different behaviors were scored and subsequently grouped for analysis into four major categories: self-centered, affiliative, defensive, and aggressive behaviors. The self-centered category consisted of behaviors associated with body care, and with horizontal and vertical activities. This category included autogrooming, genital grooming, and rearing behaviors. The affiliative category consisted of social behaviors directed at cage-mates. This category included approaching a cage-mate, sniffing the body or the genitals, allogrooming, playfully pouncing on the cage-mate, and stretch-attend postures directed towards a cage-mate. Defensive behaviors consisted of behaviors displayed by an animal in response to offensive displays by a cage-mate. The defensive category included immobility, defensive upright, defensive back chick, escape and attempts to jump out of the cage, and vocalizations. Offensive behaviors consisted of aggressive displays that communicated dominance and territorial protection, and ranged from intimidation of cage-mates to overt attack. The offensive category included piloerection, aggressive push-under, pounce on, nip other, cage mark, offensive body block/pacing, offensive kicking with back legs, whole body lateral threat, positioning on top of recumbent cage-mate, roll–tumble interaction. Assignment of rank status within a triad was based on the combined use of the behavioral scores, 22 kHz ultrasonic and audible vocalizations, and the body weight changes 24 h after triad formation (Pohorecky, 2006, 2004a,b). The ultrasonic calls were detected with a Mini Bat Detector (QMC Instruments Inc., London, UK). At intervals during the study triad agonistic interactions were verified to assess the stability of rank assignments. Triads were designated as being either high or low aggressive based on the mean offensive score for each triad. Triads with offensive behavioral score above this mean were labeled high aggressive, those with a score below this mean were designated low aggressive. On the last day of the study the number of scars and/or fresh wounds (very rare) on the tails of the triad-housed rats was also recorded. Our previous observations indicated that the tail was one of the most frequent sites of wounding during agonistic interactions. The overall mean score per rat was 1.1 ± 0.3, 1.9 ± 0.6, 3.4 ± 2.3 for dominant, subdominant and subordinate rats, substantiating the behavioral rank assessments.

2.3. Ingestion of ethanol

The monitoring of ethanol consumption was carried out as previously described (Pohorecky, 2008). The intake of fluids from hermetically sealed drinking tubes was determined from the pressure changes produced as the fluid was consumed. The determination of pressure changes, and consequent volume changes, were carried out according to the method described by Higgins et al. (1992). The drinkometers were custom made (Mr. Robert Simpson, Institute of Biomedical Engineering, University of Toronto, Ont., Canada), and the interfacing with the appropriate software was provided by consultation with Dr. Howard Kaplan (Alcohol Addictions Institute, Toronto, Ont., Canada). To identify drinking by individual triad members, each rat was implanted subcutaneously with a microchip (BioMedic Data

Systems, Maywood, NJ). After swabbing the skin at the scapular region of lightly restrained un-anesthetized rats with disinfectant, the injection needle (provided by BioMedic Data Systems) containing the microchip was quickly inserted under the skin flap that was lifted between two fingers; no prior skin incision was required. After the chip was ejected from the needle by a small plunger the needle was retrieved and the area was again swabbed with disinfectant before the rat was returned to its cage. It should be noted that because the rats had been extensively handled, this procedure could be performed by a single person. A sensor (BioMedic Data Systems Reader) located above the drinking station monitored signals from the microchip. Signals from both the pressure transducers and the microchips were interfaced and further processed by a computer located in a room adjacent to the animal room. The single-housed rats were also implanted with microchips, and their cage had the same drinkometer set-up. The consumption of water and of the 6% (v/v, in tap water) solution of ethanol was monitored daily for 21 h. This allowed 3 h for weighing the rats and for servicing the drinkometer system (i.e., refilling and calibrating the drinking tubes). To avoid the development of a side preference, the location of the water and ethanol drinking tubes was alternated daily. A Metlar GT4000 electronic balance equipped via an RS-232 interface was used to record the drinking tube weight and body weight measurements. Ethanol intake was calculated by the formula: g/kg ethanol = ml fluid × .794 g/ml × .06 × 1/kg body weight. Ethanol preference ratio was defined as ml 6% v/v ethanol/(ml 6% ethanol + ml water). The ethanol intake change ratio was calculated as: ethanol g/kg post-stress/(ethanol g/kg pre-stress + ethanol g/kg post-stress). The 6% ethanol solution was diluted v/v from a 95% solution of ethanol.

2.4. Open field arena stressor

The subject was placed in a modified open field arena (100 cm × 100 cm; the floor was elevated 10 cm above the ground level, divided into 16 quadrants; in every other quadrant there were 8 equidistantly distributed centrally located 4-cm diameter holes) for a 10-min period (Pohorecky et al., 1999). Between tests the apparatus was cleaned thoroughly with water and dried.

2.5. Elevated plus maze stressor

The walls of the elevated plus maze apparatus were made of clear Plexiglas. Two opposite open arms (50 × 10 cm) had no walls but had a 0.5 cm high ledge (Fernandes and File, 1996), and the other two closed arms (50 × 10 cm) had 50 cm high walls. The open and closed arms were connected by a central square (10 × 10 cm); the four arms were elevated on a pedestal to a height 50 cm from the floor. Rats were gently placed in the central arena, for a 5-min period. Between tests the apparatus was cleaned thoroughly with water and dried.

2.6. Modified resident–intruder stressor

The modified resident–intruder stressor consisted of introducing into a triad cage an intruder Long Evans male rat, of approximately similar body weight as the triad-housed rats, for a period of 10 minutes. Generally only the dominant rat engaged in agonistic displays towards the intruder. We were prepared to terminate the test if the agonistic interactions became too intense (i.e., the resident inflicting severe biting attacks on the intruder).

2.7. Experimental design

To minimize circadian differences in stressor sensitivity, the novel stressor tests were conducted during the rat's active phase (between 1:30 and 5:00 PM) in an adjoining testing room illuminated with a red bulb (40 W). The study involved 42 triad-housed and 11 were single-housed (total of 53 rats). The experimental schedule is outlined in Table 1. After an 18-day period of acclimatization, rats were housed either as triads or singly. During the initial 6 days of differential housing only water was available from both drinking bottles. Beginning on the 28th day of the study, and for its duration, a 6% (v/v) solution of ethanol was available from one of the drinking tubes and water was available from the other drinking tube. The ingestion of ethanol was measured for the 21-h prior to and for the 21-h after exposure to each stressor. Starting on test day 38, and continuing at 1–2 week intervals, the subjects were subjected to the three sequential stressors (novel open field apparatus, elevated plus maze and the modified resident–intruder test). Because of the number of subjects involved, the study was carried out in two sequential cohorts consisting of 7 triads and of 5–6 single-housed rats each.

2.8. Statistical data analysis

The data are presented as the means and standard errors of the means. The data were analyzed using StatView version 5. A simple ANOVA was employed, with the between subjects factors being the rat's housing (triad and single) and rank status (dominant, subdominant, subordinate). Data analysis is based on 14 rats/rank in triads (7 rats/rank in aggressive triads and 7 rats/rank in non-aggressive triads) and 11 single-housed rats. The repeated measure ANOVA was employed to determine pre-stress to post-stress differences in ethanol ingestion. The between subjects factors for the repeated measure ANOVA were rank status (dominant, subdominant, subordinate) and triad aggression (aggressive, non-aggressive). When appropriate, statistical significance between groups was assessed using the post-hoc Bonferroni's test, with significance levels set at $P = /< .0167$.

3. Results

3.1. Rank status assessment, triad aggression, and body weight changes

Table 2 lists the behaviors assessed during the social interactions that occurred during the initial 30 min of triad housing. Based on these behavioral assessments, the rats that displayed significantly more frequent offensive behaviors against cage-mates were designated as the dominant rats ($F2,36 = 5.52$, $P = .0132$). The dominant rats directed their offensive behavior particularly towards the subdominant rats ($P = .0038$). The subordinate rat rapidly learned to display such subordinate behaviors as freezing and vocalization. As soon as the subordinate rats signaled submission to the dominant rat, they were subsequently largely ignored. Consequently, the subordinate rats generally remained immobile in one corner of the cage during agonistic interactions of its other two cage-mates. The subdominant rat, on the other hand, did not display subordinate behavior to the dominant rat and consequently was involved in repeated aggressive interactions with the dominant rat. Sometimes,

Table 1
Chronology of experimental procedures.

Time line	Performed procedures or tests	Available drinking fluid
Days 1–18	Pre-triad single-housing Animal room acclimatization	Water
Days 19–20	Microchip implantation	Water
Days 22–23	Pre-triad body weight and triad formation	Water
Days 23–24	24-h body weight determination	Water
Days 28–86	Daily choice of ethanol and water	6% v/v ethanol and water
Days 38–39	Novel open field arena	6% v/v ethanol and water
Days 45–46	Elevated plus-maze apparatus	6% v/v ethanol and water
Days 57–58	Modified resident–intruder test	6% v/v ethanol and water
Days 85–86	Final body weight	6% v/v ethanol and water

Table 2
Behavioral assessment and body weights at triad formation.

Rank	Offensive behavior	Defensive behavior	Pre-triad body weight (g)	24-h percent change in body weight (%)	Final percent change in body weight (%)
Dominant	28.43 ± 8.61	9.86 ± 4.92	363.10 ± 4.52	−1.17 ± 0.54	39.37 ± 2.08
Subdominant	16.00 ± 5.36[a]	19.14 ± 8.69	363.42 ± 4.37	−5.64 ± 0.80[a]	20.33 ± 2.56[a]
Subordinate	0.86 ± 0.46	9.43 ± 1.84	362.77 ± 3.36	−6.80 ± 0.69[a]	21.30 ± 2.57[a]

Behaviors rats displayed during the initial 30-min of triad housing. The number of offensive and defensive behaviors was scored as described in the Methods section. Data are presented as the means ± SEM for groups of 42 triad-housed rats ($n = 14$/group). (a) Indicates statistically significant difference from the dominant rats as determined by the Bonferoni test.

the subdominant rat engaged in displaced aggression against the subordinate rat. Although the subdominant rats most frequently displayed defensive behaviors, these group differences did not attain statistical significance. Based on the severity of the agonistic interactions, the triads were subdivided into aggressive and non-aggressive triads. Differences in colony aggression are important to the interpretation of the behavioral data from group-housed rats, particularly since our preliminary observations indicated that the aggression level of triad-housed rats can vary significantly.

The subject's body weights 24-h prior to and after triad formation are also shown in Table 2. While at triad formation there were no group differences in body weights, within 24-h of triad-housing body weights differed significantly. There was a mean effect of rank status on body weight loss 24-h after triad formation ($F2,39 = 18.461$, $P<.0001$), the loss in body weight of the dominant rats was less than that of their cage-mates ($P<.0001$ for both). Triad aggression level did not have a significant effect of on the 24-h loss in body weight. At the end of the study, there was also an effect of rank status on body weight gain ($F2,39 = 19.707$, $P<.0001$). Again the dominant rats had gained more body weight than its two cage-mates ($P<.0001$ for both).

3.2. Acute stressors and ethanol ingestion

The intake of ethanol of triad-housed rats was evaluated before and after the subjects were exposed to three distinct stressors that are believed to differ in "stress severity". The exposure to each one of these brief stressors (5–10 min) was carried out at approximately 1–2 weekly intervals.

The initial mild novel stressor to which the rats were exposed to was an open field arena. A repeated measures ANOVA indicated that both rank status and aggression had significant main effects on ethanol intake ($F2,36 = 10.068$, $P = .0003$ and $F1,36 = 14.819$, respectively) (Fig. 1A). Overall, the subordinate rats ingested more ethanol compared to dominant rats and subdominant rats ($P = .0002$ and $P = .0015$, respectively). Furthermore, rats in aggressive triads ingested more ethanol compared to their counterparts in non-aggressive triads ($P = .0005$). Although ethanol intake tended to be higher during the pre-stress test compared to the post-stress test ($P = .0853$) this effect did not reach statistical significance. Additional statistical analysis indicated that there were significant interactions of rank status and aggression, and of rank status, aggression and test period on the intake ethanol ($F2,36 = 3.289$, $P = .0487$ and $F2,36 = 4.303$, $P = .0211$, respectively). These significant interaction effects indicate that both the level of triad aggression and the test period modified the existing rank status differences in ethanol ingestion. Specifically, compared to their non-aggressive counterparts, in aggressive triads more ethanol was ingested by the dominant rats prior to the stressor and by the subdominant rats after the stressor ($P = .0281$ and $P<.0001$, respectively). A similar trend in ethanol intake by the dominant rats was evident at the post-stress period ($P = .0528$), but this effect just missed statistical significance. The subordinate rats in non-aggressive triads ingested more ethanol compared to the dominant rats both before and after the novel open field stressor ($P = .0096$ and $P = .0002$, respectively), and also compared the subdominant rats after the stressor ($P<.0001$). While overall the novel open field stressor had little effect on the intake of ethanol, the intake of ethanol of the subdominant rats in non-aggressive triads was decreased as a result of this stressor ($P = .0422$). The absence of a statistically significant effect of rank status on the pre-/post-open field ratio in ethanol intake reiterated the overall small effect of this stressor, while the effect of triad aggression just missed statistical significance ($P = .0562$) (Fig. 1B).

Exposure to the elevated plus maze apparatus was the next stressor to be evaluated. Again a repeated measures ANOVA analysis indicated that there were significant effects of rank status, aggression, as well as of test period on the ingestion of ethanol ($F2,36 = 14.003$, $P<.0001$, $F1,36 = 40.626$, $P<.0001$ and $F1,36 = 16.620$, $P = .0002$, respectively) (Fig. 2A). Overall, dominant rats ingested less ethanol compared to subdominant and subordinate rats ($P<.0001$, for both). Furthermore, irrespective of rank status, rats in aggressive triads ingested more ethanol compared to their counterparts in non-aggressive triads ($P<.0001$), and ethanol intake was higher during the pre-stress test compared to the post-stress test ($P = .0002$). Additionally, the interactions of rank status and aggression, of rank status

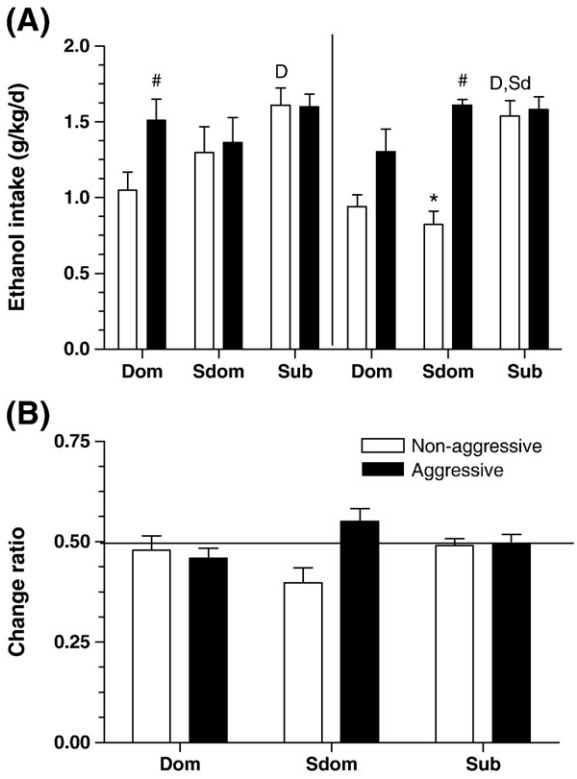

Fig. 1. Ethanol intake of triad-housed rats prior to and after exposure to an acute novel open field stressor. After 18 days of triad housing rats were exposed to a novel open field. (A) Intake (g/kg) of a 6% (v/v) solution of ethanol using a 2-bottle choice paradigm was assessed for a period of 21 h prior to and 21-h after each stressor. (B) Ratio of pre-stress to post-stress change in ethanol intake. Triads were categorized as being either aggressive ($n = 7$) or non-aggressive ($n = 7$) on the basis of behavioral assessments. Data are presented as the mean ± SEM. Statistically significant group differences due to rank status (D = dominant, Sd = subdominant), aggression (#), and pre- and post-stress test periods (*), were determined by the Bonferoni test with significance set at $P = .0167$.

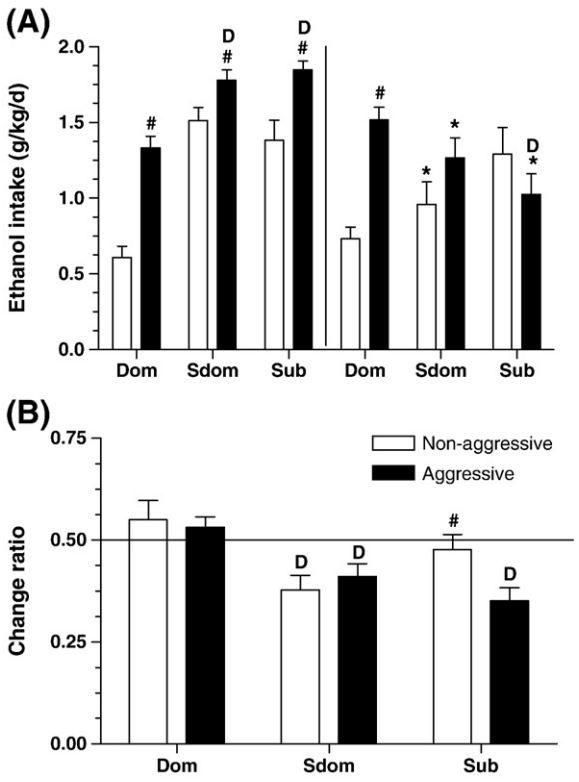

Fig. 2. Ethanol intake of triad-housed rats prior to and after exposure to an elevated plus-maze stressor. One week after the open field stressor rats were exposed to an elevated plus-maze. (A) Intake (g/kg) of a 6% (v/v) solution of ethanol using a 2-bottle choice paradigm was assessed for 21 h prior to and for 21-h after each stressor. (B) Ratio of pre-stress to post-stress change in ethanol intake. Triads were categorized as being either aggressive ($n=7$) or non-aggressive ($n=7$) on the basis of behavioral assessments. Data are presented as the mean ± SEM. Statistically significant group differences due to rank status (D = dominant), aggression (#) and pre- and post-stress test periods (*) were determined by the Bonferoni test with significance set at $P=.0167$.

and test period, and of rank, aggression and test period were also significant ($F2,36=10.611$, $P=.0002$, $F2,36=10.362$, $P=.0003$ and $F1,36=3.606$, $P=.0374$, respectively). These significant interactions indicate that both the level of triad aggression and the test period modified the differences in ethanol ingestion associated with rank status. Prior to the stressor the subdominant and subordinate rats in aggressive triads ingested more ethanol compared to the corresponding dominant rats ($P=.0002$ and $P<.0001$, respectively). Subdominant and subordinate rats in aggressive triad also ingested more ethanol compared to their counterparts in non-aggressive triads during the pre-stress test ($P=.0293$ and $P=.0083$). Both prior to and after the stressor, the dominant rats in aggressive triads ingested more ethanol compared to their non-aggressive counterparts ($P<.0001$ for both). Furthermore, the elevated plus maze stressor depressed ethanol intake in subdominant rats irrespective of triad aggression level ($P=.0174$ and $P=.0258$ for non-aggressive and aggressive triads, respectively), but for subordinate rats only for those in aggressive triads ($P=.0031$). Interestingly, these changes indicate that for subordinate rats triad aggression had a significant differential impact on ethanol intake engendered by the elevated plus maze stressor. Consequently, compared to the corresponding dominant rats, the subordinate rats in aggressive triads ingested less ethanol after the stressor in contrast to the pre-stressor test, while those in non-aggressive triads ingested more ethanol ($P=.0094$ and $P=.0123$, respectively). Rank status had a significant effect on ethanol intake as assessed by the pre-/post-stress ratio ($F2,36=10.265$, $P=.0003$), but there was no overall effect of aggression, and the interaction of rank and aggression missed significance ($P=.0831$) (Fig. 2B). For the subdominant rats in both the aggressive and non-aggressive triads the pre-/post-stress ratio in ethanol intake was significantly lower compared to the dominant rats ($P<.0001$ and $P=.0072$, respectively). Similarly, in aggressive triads the pre-/post-stress ratio for ethanol intake was lower in subordinate rats compared to the corresponding dominant rats ($P=.0004$). Additionally, for the subordinate rats in aggressive triads, the pre-/post-stress ratio for ethanol intake was smaller than that of the subordinate rats in non-aggressive triads ($P=.0237$).

A repeated measures ANOVA again indicated that rank status and test period had significant effects on the ingestion of ethanol when rats were subjected to the modified resident–intruder stressor ($F2,36=3.569$, $P=.0385$ and $F1,36=7.032$, $P=.0118$, respectively) (Fig. 3A). Overall, the subdominant rats ingested more ethanol than the subordinate rats ($P=.0114$). Furthermore, ethanol intake was higher during the post-stress compared to the pre-stress period ($P=.0118$). Additionally, the interactions of rank status and aggression, of aggression and test period, and of rank status, aggression and test period, were all significant ($F2,36=F2,36=3.732$, $P=.0337$, $F1,36=37.971$, $P<.0001$ and $F2,36=6.896$, $P=.0029$, respectively). As with the previous stressors, these significant interactions indicate that both the level of triad aggression and the test period significantly modified the effect of rank status on ethanol ingestion. In non-aggressive triads the dominant rats ingested more ethanol compared to their aggressive counterparts prior to the stressor ($P=.0323$), while the reverse was true for the non-dominant rats, though this difference was statistically significant only for the subordinate rats ($P<.0001$). Focusing on ethanol intake of rats before the modified

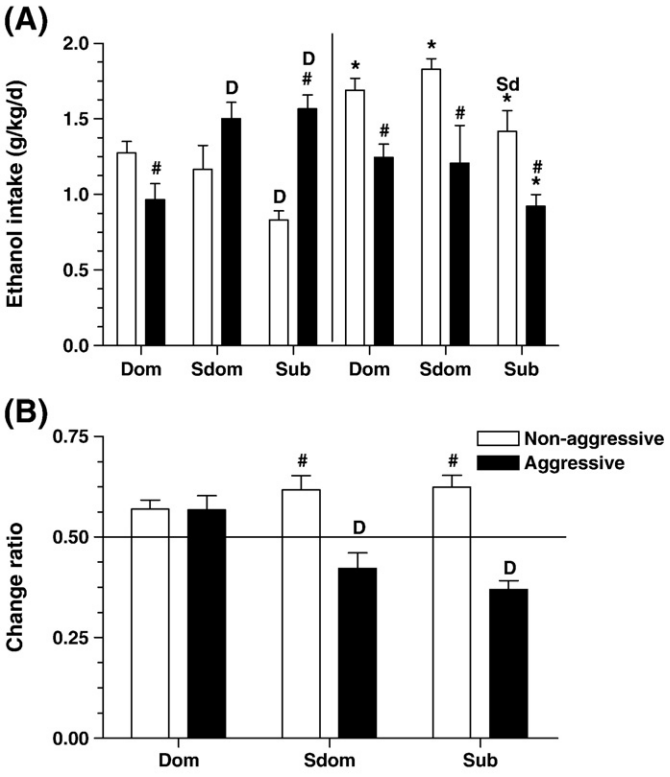

Fig. 3. Ethanol intake of triad-housed rats prior to and after exposure to a modified resident–intruder stressor. Two weeks after the elevated plus-maze stressor rats were subjected to a modified resident–intruder test. (A) Intake (g/kg) a 6% (v/v) solution of ethanol using a 2-bottle choice paradigm was assessed for 21 h prior to and 21-h after each stressor. (B) Ratio of pre-stress to post-stress change in ethanol intake. Triads were categorized as being either aggressive ($n=7$) or non-aggressive ($n=7$) on the basis of behavioral assessments. Data are presented as the mean ± SEM. Statistically significant group differences due to rank status (D = dominant, Sb = subordinate), aggression (#) and pre- and post-stress test periods (*) were determined by the Bonferoni test with significance set at $P=.0167$.

resident–intruder stressor, we find that the dominant rats in non-aggressive triads ingested more ethanol than did the subordinate rats ($P=.0087$), while the dominant rats in the aggressive triads ingested less ethanol than did the corresponding subdominant and subordinate rats ($P=.0016$ and $P=.0006$, respectively). The effect of the modified resident–intruder stressor on ethanol intake was generally dependent on triad aggression. In non-aggressive triads the stressor increased ethanol intake, while in aggressive triads ethanol intake was either unaffected (dominant rats) or depressed (subordinate rats). More specifically, all the rats in non-aggressive triads increased their ethanol intake as a result of the modified resident–intruder stressor ($P=.0169$, $P=.0170$, and $P=.0069$ for dominant, subdominant and subordinate rats, respectively). By contrast, only the subordinate rats in aggressive triads decreased their intake of ethanol ($P=.0016$); if anything, the corresponding dominant rats tended to show a non-statistically significant increase in their ethanol intake after the stressor ($P=.0847$). Lastly, after the modified resident–intruder stressor all the rats in non-aggressive triads ingested more ethanol compared to their counterparts in aggressive triads ($P=.0019$, $P=.0335$ and $P=.0078$ for the dominant, subdominant and subordinate rats, respectively). The effect of rank status on the pre-/post-stress ratio on ethanol intake after the modified resident–intruder stressor missed significance ($P=.0734$), but the effect of aggression was significant ($F1,36=35.345$, $P<.0001$) (Fig. 3B). Moreover, the interaction of rank status and aggression on the pre-/post-stress ratio in ethanol intake was significant ($F2,36=9.029$, $P=.0007$). The interaction effect was evident from the lower pre-/post-stress ratio in ethanol intake of both the subdominant and subordinate rats in aggressive triads compared to their counterparts in non-aggressive triads ($P=.0028$ and $P<.0001$, respectively), though it was unchanged in dominant rats. In aggressive triads the pre-/post-stress ratio in ethanol intake was higher for the dominant rats the compared to the corresponding ratio for the subdominant and subordinate rats ($P=.0053$ and $P=.0004$, respectively).

We also evaluated whether acute stressors modified the intake of ethanol of single-housed rats. To this end, we compared the intake of ethanol of the single-housed rats with that of triad-housed rats. For the open field stressor, housing had a significant effect on ethanol intake ($F1,51=8.298$, $P=.0058$) (Table 3). The test period also had a significant effect on ethanol intake ($F1,51=5.612$, $P=.0217$), but the interaction of housing and test period was not significant. Overall, the single-housed rats tended to ingest less ethanol than the triad-housed rats, however this effect was significant only at the post-stress test ($P=.0081$). For the elevated plus maze stressor the effect of housing on ethanol intake just missed statistical significance ($P=.0509$). The effect of test period was significant ($F1,51=4.091$, $P=.0484$), with higher ethanol intake before the stressor compared to the post-stress period. When individual ANOVAs were determined for these two experimental groups, ethanol intake was significantly lower in the triad-housed rats at the post-stress test ($P=.0026$), but there was no effect of the stressor on ethanol intake for the single-housed rats.

Table 3
Acute novel stressors and ethanol intake of single-housed and triad-housed rats.

Housing	Test period	Open field stress	Elevated plus maze stress
Single	Pre-stress	1.17 ± 0.07	1.09 ± 0.11
	Post-stress	0.92 ± 0.13	1.02 ± 0.07
Triads	Pre-stress	1.41 ± 0.06	1.41 ± 0.07
	Post-stress	1.30 ± 0.06*	1.13 ± 0.06**

Ethanol intake of single-housed and triad-housed rats was determined prior to and after exposure to two brief novel stressors. Ethanol intake (g/kg, 6% v/v solution) was determined for 21-h prior to and 21-h after each stressor using a 2-bottle choice paradigm. Data are presented as the means ± SEM for groups of 42 triad-housed and 11 single-housed rats. (*) Indicates statistically significant difference from the corresponding single-housed group, and (**) from the corresponding pre-stress group, as determined by the Bonferoni test.

3.3. Water intake

To determine whether the acute stressor-induced differences in ethanol intake were specific to ethanol, we also measured the daily consumption of water. A repeated measures ANOVA indicated that there was a significant effect of rank status on the intake of water as a result of the open field stressor ($F2,39=8.719$, $P=.0007$) (Fig. 4A, right). Overall, water intake was lower in the dominant rats compared to the subdominant and subordinate rats ($P=.0004$ and $P=.0024$, respectively). The interaction of rank status and test period was also

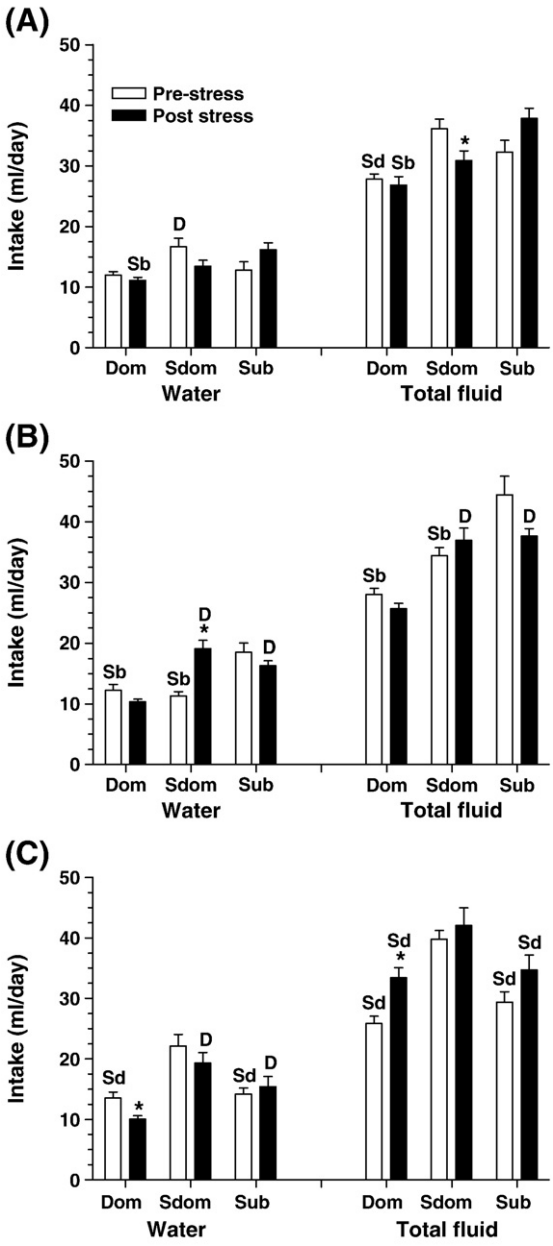

Fig. 4. Water and total fluid intakes of triad-housed rats prior to and after exposure to acute novel stressors. Rats were exposed to three distinct novel stressors: (A) open field apparatus, (B) one week later to an elevated plus maze apparatus, and (C) two weeks later to a modified resident–intruder test. Using a 2-bottle choice paradigm, water intake (in milliliters) was determined during 21 h prior to and 21-h after each stressor (left side of each panel). The right side of each panel shows the total fluid intake calculated as the sum of the intakes of ethanol and water (in milliliters) during the just stated time intervals. Data are presented as the mean ± SEM ($n=14$ subjects per rank). Statistically significant group differences due to rank status (D = dominant, Sd = subdominant, Sb = subordinate) and the pre- to post-stress test periods (*) were determined by the Bonferoni test with significance set at $P=.0167$.

significant on water intake ($F2,39 = 4.154$, $P = .0231$) indicating that the effect of rank status varied with the test period. Prior to this stressor the subdominant rats ingested more water than did the dominant rats ($P = .0061$). After this stressor the subordinate rats ingested more water compared to the dominant rats ($P = .0004$).

Similarly, rank status also had a significant effect on the water intake of rats as a result of the elevated plus maze stressor ($F2,39 = 15.682$, $P < .0001$) (Fig. 4B, right). Again, the water intake of the dominant rats was lower than that of the subdominant and subordinate rats ($P < .0001$ for both). The interaction of rank status and test period was also significant on water intake ($F2,39 = 17.549$, $P < .0001$). Reflecting this interaction effect, water intake was increased only in the subdominant rats in response to the elevated plus maze stressor ($P < .0001$). Prior to the stressor the dominant and subdominant rats ingested less water compared to the subordinate rats ($P = .0003$ and $P < .0001$, respectively). After the stressor water intake of the dominant rats was lower compared to their cage-mates ($P < .0001$ for both).

As with the previous two stressors, with the modified resident–intruder test rank status had a significant effect on the water intake ($F2,39 = 13.051$, $P < .0001$) (Fig. 4C, right). Overall, the subdominant rats ingested more water compared to the dominant and subordinate rats ($P < .0001$ and $P = .0020$, respectively). Test period and the interaction of rank status and test period also had significant effects on water intake ($F1,39 = 6.062$, $P = .0183$ and $F2,39 = 4.451$, $P = .0182$, respectively). This stressor lowered water intake in specifically the dominant rats ($P = .0018$). Water intake was higher in subdominant rats compared to the dominant and the subordinate rats both prior to the stressor ($P < .0001$ and $P = .0002$, respectively), as well as after the stressor ($P < .0001$ and $P = .0111$, respectively).

3.4. Total fluid intake

A repeated measures ANOVA indicated a significant effect of rank status on total fluid intake as a result of the open field stressor ($F2,39 = 22.653$, $P < .0001$) (Fig. 4A, left). Total fluid intake was lower in the dominant rats compared to the subdominant and subordinate rats ($P < .0001$ for both). The interaction of rank status and test period also had a significant effect on the total fluid intake ($F2,39 = 9.679$, $P = .0004$) indicating that the effect of rank status varied with the test period. Indeed, the subdominant rats decreased significantly their total fluid intake after this stressor ($P = .0032$). Prior to the open field stressor the subdominant rats ingested more total fluid than did their cage-mates ($P < .0001$ and $P = .0007$ for the dominant and subordinate rats, respectively). After the stressor the total fluid intake of the subordinate rats was higher compared to both the dominant and subordinate rats ($P < .0001$ and $P = .0032$, respectively).

Rank status similarly had a significant effect on the total fluid intake of rats subjected to the elevated plus maze stressor ($F2,39 = 37.213$, $P < .0001$) (Fig. 4B, left). Again the total fluid intake of the dominant rats was lower than that of the subdominant and subordinate rats ($P < .0001$ for both) and the intake of subordinates was higher than that of the subdominant rats ($P = .0025$). However neither the effect of test period nor the interaction of rank status was significant. Prior to the stressor the dominant and subdominant rats ingested less total fluid compared to the subordinate rats ($P < .0001$ and $P = .0011$, respectively). After the stressor total fluid intake of the dominant and subdominant rats was lower compared to the subordinate rats ($P < .0001$ for both cage-mates).

As with the previous two stressors, with the modified resident–intruder test rank status had a significant effect on the total fluid intake ($F2,39 = 14.961$, $P < .0001$) (Fig. 4C, left). Overall, the subdominant rats ingested more total fluid compared to the dominant and subordinate rats ($P < .0001$ and $P = .0001$, respectively). Test period had significant effect on the total fluid intake ($F1,39 = 19.017$, $P < .0001$). This stressor lowered the total fluid intake in specifically the dominant rats ($P = .0001$). Total fluid intake was lower in dominant rats compared to the subdominant and subordinate rats both prior to the stressor ($P < .0001$ and $P = .0011$, respectively), as well as after the stressor ($P < .0001$ for both).

3.5. Ethanol preference

In contrast to the observed differences in the intake of water and of total fluid, rank status had no effect on the percent ethanol preference after both the open field and the elevated plus maze stressors (Fig. 5A and B). However, with the elevated plus maze stressor there were significant effects of test period and for the interaction of test period and rank status ($F1,39 = 14.100$, $P = .0006$ and $F2,39 = 20.660$, $P < .0001$). Specifically, the elevated plus maze stressor decreased the percent ethanol preference only in the subdominant rats ($P < .0001$). Prior to this stressor the percent ethanol preference of

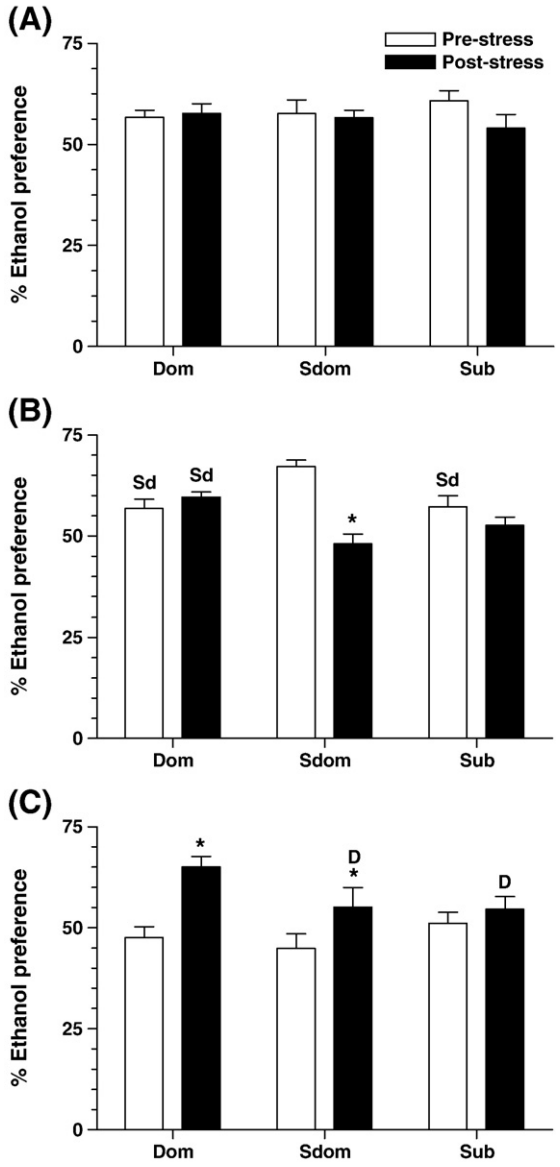

Fig. 5. Ethanol preference of triad-housed rats prior to and after exposure two acute novel stressors. Using a 2-bottle choice paradigm, ethanol and water intakes (in milliliters) were determined during 21 h prior to and 21-h after each stressor. Ethanol preference was calculated as described in the Methods section. Data are presented as the mean ± SEM ($n = 14$ subjects per rank). Statistically significant group differences due to rank status (D = dominant, Sd = subdominant, Sb = subordinate) and the pre- to post-stress test periods (*) were determined by the Bonferoni test with significance set at $P = .0167$.

the subdominant rats was higher than that of the dominant and subordinate rats ($P=.0018$ and $P=.0026$, respectively). By contrast, after the elevated plus maze stressor the percent ethanol preference was lower in the subdominant rat compared to the dominant rats ($P<.0001$). Finally, the modified resident–intruder stressor had a significant effect on the percent ethanol preference ($F1,39=47.199$, $P<.0001$). Although rank status had no effect on percent ethanol preference, the interaction of test period and rank status was significant ($F2,39=8.932$, $P=.0006$) (Fig. 5C). Specifically, the modified resident–intruder stressor increased the overall percent ethanol preference in both the dominant and the subdominant rats ($P<.0001$ and $P=.0207$, respectively). While prior to this stressor rank status had no effect on the percent ethanol preference, after this stressor the percent ethanol preference was elevated in the dominant rat compared to the subdominant and subordinate rats ($P=.0079$ and $P=.0163$, respectively).

4. Discussion

The evidence presented here demonstrates the importance of psychosocial factors in determining the effect stressors have on the intake ethanol by rats. The ingestion of ethanol by the triad-housed rats was found to vary with the level of aggression within a triad as well as with the characteristics of the novel stressor. Importantly, the impact of these variables on the ingestion of ethanol was highly dependent on the subject's rank status.

Previous studies had provided evidence that ethanol ingestion by group-housed rodents differed with rank status however this evidence was not consistent. For example, non-dominant group-housed animals were found to ingest less (Van Erp et al., 2001; Blanchard et al., 1993; 1987; Wolffgramm, 1990; Crowley and Andrews, 1987), more (Ellison, 1987; Pohorecky et al., 2008) or similar amounts (Keeney and Hogg, 1999; Higley et al., 1991) of ethanol compared to their dominant counterparts. In our study, irrespective of triad aggression, the overall basal (pre-stressor) intake of ethanol by the dominant rats was less compared to its cage-mates. Significantly, triad aggression was found to alter the rank-related differences in ethanol intake. Specifically, basal ethanol intake was higher in aggressive compared to non-aggressive triads. Prior to the first two stressors (open field and elevated plus maze tests) the dominant rats in aggressive triads ingested more ethanol compared to their counterparts in non-aggressive triads. Focusing on non-aggressive triads, we note that basal ethanol intake was the lowest in dominant rats, except for the modified resident–intruder test. At the last stressor test the dominant rats in non-aggressive triads ingested the most ethanol while the subordinate rats ingested the least ethanol. By contrast, at this final stressor test, basal intake in non-aggressive triads was lower in dominant rats compared to its cage-mates. Whether this change in the pattern of basal ethanol intake was a consequence of the exposure to the prior two stressors, or to an interaction of triad aggression with the previous stressors remains to be determined. For the non-dominant rats, particularly the subordinate rats, triad aggression differences in ethanol intake prior to the first novel stressor tended to be magnified by the experience of subsequent novel stressors. Whether this chronological alteration in ethanol intake reflects the development of sensitization of the implicated neurobiological mechanisms (Geerse et al., 2006) is at present an open question.

We have confirmed our hypothesis that acute stressors can have strikingly different consequences on ethanol intake. The pre-/post-stress ratio for ethanol intake indicates that the elevated plus maze stressor decreased ethanol intake of the non-dominant rats (ratio of less than 0.50). However, only the modified resident–intruder stressor had a pronounced differential effect on ethanol intake. Compared to its baseline ethanol intake, this stressor increased intake in the non-aggressive triads irrespective of rank, with the largest increase in intake was noted in the non-dominant rats. By contrast, ethanol intake of the non-dominant counterparts in aggressive triads was lower after the modified resident–intruder stressor. These findings lend support to Roske et al. (1994) who indicated that the "quality" of a stressor is likely to affect its impact on ethanol intake. Therefore, social instability generated by the modified resident–intruder test resulted had the largest impact on ethanol intake, while the open field stressor had only a minor effect on intake.

It is noteworthy that the overall changes in ethanol preference produced by the acute stressors did not necessarily mirror the changes in ethanol intake. The most significant changes in ethanol preference were noted with the modified resident–intruder stressor. This stressor increased ethanol preference in dominant and subdominant rats. Because the water intake of the dominant rats showed a decline after the stressor, while total fluid intake increased, the increase in ethanol preference most likely reflects the specific increase in ethanol intake of these rats, but does not explain the smaller increase in the subdominant rats. The elevated plus maze stressor primarily affected the subdominant rats. This group of rats showed a decrease ethanol intake and a decline in ethanol preference. This decline in ethanol preference can be explained by the substantial concomitant increase in water intake in these rats.

Among the possible mechanisms that may have contributed to observed novel stressor-induced differences in ethanol intake one can mention the role played by individual differences in coping style (Ebner et al., 2005; Koolhaas et al., 1999) and/or in trait anxiety (see below). Based on consistent behavioral and neuroendocrine characteristics investigators have defined two basic stress coping styles, referred to as proactive and reactive coping styles (for reviews see Koolhaas et al., 1999; Benus et al, 1991). Prior research has indicated that dominance status influences the level of physical and psychosocial stress in group-housed subjects, while lack of control generates psychosocial stress (Sapolsky, 2005). More aggressive rats, such as the aggressive dominant rats, have a proactive coping style that is characterized by escape from threatening and/or aversive stimuli, coupled with high defensiveness of the home territory against intruders. These animals have high plasma levels of testosterone and a high reactivity of the sympatho-adrenomedullary system (Pohorecky, 2006; Sgoifo et al., 1996; Fokkema et al., 1995; Cools et al., 1993; Peterson and Pohorecky, 1989; Fokkema et al., 1988). In the present study the dominant rat essentially controlled a triad's "stress level" by initiating offensive aggression towards its cage-mates. Social defeat on the other hand induces a shift toward more passive and immobile forms of defense that characterizes submissive behavior (reactive coping) (Wommack and Delville, 2003; Blanchard et al., 2001a). In our triads the subdominant rats had a limited degree of control over the triad aggression level, responding to the challenges of the dominant rats, and sometimes attacking the subordinate rats. Although the subordinate rats had no control over the level of aggression in a triad, after an initial confrontation they were generally ignored by the dominant rats. The distinctions in the described agonistic behavior of the non-dominant rats are supported by previously reported physiological and behaviors differences, including functional differences of the hypothalamo–pituitary–adrenal axis, the sympatho–adrenomedullary system, differences in responsiveness to stress (Walker et al., 2009; Blanchard et al., 2001b; Pohorecky et al., 2004a; 2004b; Virgin and Sapolsky, 1997; Shively et al., 1997; Fokkema et al., 1988; Ellison et al., 1987), and behavioral differences (Pohorecky, 2008; Blakley, Pohorecky, 2006; Wommack and Delville, 2003). Whether some of these neurobiological differences may have contributed to the observed differences in ethanol ingestion reported here remains to be determined by future research.

Compared to single-housed rats, a greater number of variables can influence the ingestion of ethanol of triad-housed animals. The current findings indicate that triad aggression indeed has a significant and consistent influence on the ingestion of ethanol of triad-housed rats. Furthermore, the effect of the novel stressors on the ingestion of

ethanol was dependent on the level of triad aggression, particularly in the subordinate rats. Recent evidence indicates that enhanced aggressiveness may be associated with a number of specific neurobiological alterations in a variety of species; for example, differences in cardiovascular and thermoregulatory activity (Caramaschi et al., 2008), in social impulsivity (Fairbanks et al., 2004) and in gene expression in certain brain areas (Feldker et al., 2003). These neurobiological alterations most likely play a role in ethanol intake of the triad-housed rats. While our data point to the importance of housing related aggression on ethanol ingestion, previous research on this issue has been controversial. For instance, enhance aggression in mixed gender colonies did not alter the intake ethanol (Adams and Oldham, 1996; Bergvall et al., 1996), while chronic food-restriction increased the intake of ethanol but did not change aggression (Bergvall et al., 1996). Interestingly, Duncan and associates (2006) reported that "alcohol-drinking colonies failed to establish dominance hierarchy and displayed little aggression" in the visible burrow model of group housing. In our study the *ad libitum* access to ethanol did not influence the maintenance of dominance rank status, probably because, in contrast to the study by Duncan and associates, ethanol became available after the triads had been established.

Previous reports suggested a correlation of trait anxiety-like behavior and the ability to cope with environmental change (Salomé et al., 2006; Veenema et al., 2003; Ho et al., 2002). Anxiety-like behavior was found to correlate negatively with aggression in selectively bred Wistar rats (Veenema et al., 2007). Compared to high anxious rats, low anxious rats also displayed a greater activation of the hypothalamo–pituitary–adrenal axis and of brain areas associated with aggression (Veenema et al., 2007). Basal anxiety-like behavior of subordinates was higher compared to the dominant rats, and anxiety-like behavior was also greater in defeated single-housed rats (Pohorecky et al., 2008; Haller et al., 2000; Ruis et al., 1999; Vivian et al., 1994; Heinrichs et al., 1992). In so far there is evidence of a negative association of aggression and trait anxiety-like behavior, one interpretation for the greater aggressiveness of the dominant rats is their lower level anxiety (Henniger et al., 2000).

Additionally, basal level of "anxiety" may also play a role in ethanol intake. However the relationship of ethanol intake and anxiety-like behaviors is also complex. For example, some evidence indicates a positive correlation between anxiety-like behavior and ethanol ingestion (Colombo et al., 1995; Spanagel et al., 1995), while other reports indicate a negative correlation (Gallate et al., 2003; Henniger et al., 2002), or no correlation (Langen and Fink, 2004; Overstreet et al., 1997). Confirming our previous findings in triad-housed rats, ethanol intake declined significantly after the elevated plus maze stressor (Pohorecky et al., 2008). Since we also reported that the anxiolytic effect of self-ingested ethanol was most prominent in the dominant rats (Pohorecky et al., 2008), enhanced anxiolysis may have contributed to the lower pre-stress intake of ethanol noted in the dominant rats. Moreover, because chronic ethanol ingestion can decrease anxiety-like behavior in rats (Pohorecky et al., 2008; Gallate et al., 2003; Blokland et al., 1992), the greater intake of ethanol by non-dominant rats may reflect both their greater anxiety-like behavior, and would depend on the frequency of their daily agonistic interactions with the dominant rats (Blanchard et al., 1993).

Indeed there is evidence for individual, anxiety-related differences in response to ethanol. In both male undergraduates and in experimental animals ethanol reduced anxiety only in highly anxious, but not in low anxious subjects (Zack et al., 2007; Henniger et al., 2002). Such underlying differences in trait anxiety may have indeed contributed to the differences the ethanol intake due to rank status and to triad aggression in our study. It has also been suggested that the relationship of ethanol and anxiety-like behaviors may not be monotonic (Henniger et al., 2002). These investigators found that the relationship of ethanol and anxiety-like emotional state was more like an inverted U-function, therefore ethanol drinking may only be enhanced at intermediate levels of anxiety (Henniger et al., 2002). Lastly, the development of differential sensitivity to ethanol's anxiolytic effects, and the possible development of novel stressor-induced sensitization (Geerse et al., 2006) may have contributed to the present findings.

In contrast to prior reports, we found no difference in the basal daily ethanol intake of triad- and single-housed rats. Other investigators had noted that basal ethanol intake of single-housed rats was higher than that of group-housed rats (Roske et al., 1994; Wolffgramm, 1990; Parker and Radow, 1974). Unlike the triad-housed rats, the single-housed rats did not show a significant change the ethanol intake over the course of study. Moreover, none of the acute stressors had a significant effect on ethanol intake of the single-housed rats. The differences in our findings from those of other investigators may be accounted by a number of procedural and housing related differences. A possible environmental factor worth mentioning is that the single-housed rats were housed in the same room as the triad-housed rats, in contrast to some of our other studies. While an effect of visual inputs was highly unlikely, whether pheromonal (Pohorecky et al., 2008) and auditory information from the triad-housed cages influenced the ingestion of ethanol of the single-housed rats is an open question.

A potential problem with our study that warrants mentioning is the testing sequence of the stressors. Based on the literature, the sequence of stressor presentation was in order of increased severity. In fact the obtained data on ethanol intake (open field vs modified resident–intruder stressors) tends to support our reasoning. Despite the extended time intervals between the stressors, an order and/or carryover effect between novel stressors on ethanol ingestion cannot be entirely dismissed. Furthermore, repeated handling and daily weighing of the rats, as well as potential long-term adaptive changes to the housing conditions, are issues that should be further explored.

The data presented here documents the consequences of chronic psychosocial stress on the ingestion of ethanol in rats. It would be of further interest to determine whether rank status also influences the time frame for the development of the changes in ethanol intake, as well as their persistence when psychosocial stress is terminated. For example, the increase in ethanol preference and intake of mice subjected to five consecutive daily defeats had a slow onset, but no changes were noted after a single defeat, or in mice given a once weekly defeat for four weeks (Croft et al., 2005). Others have reported persistence of the defeat-generated neurobiological effects (reviewed by Buwalda et al., 2005). For example, defeated Syrian hamsters displayed conditioned defeat that lasted at least 33 days (Huhman et al., 2003), and defeated rats that were subsequently single-housed had impairments of reward- and cognition-related behaviors for up to three months (Von Frijtag et al., 2000). Also neuronal sensitization and changes in *zif268* mRNA expression have been also reported for as long as 60 days after the last defeat (Covington and Miczek, 2005; Miczek et al., 2004). However, it is noteworthy that the persistence of impairments generated by social stress is strongly influenced by the housing environment after the experience of defeat (Nakayasu and Ishii, 2008; Buwalda et al., 2005).

In conclusion, the daily ingestion of ethanol by triad-housed rats was found to be highly influenced by stress. This effect of stress on ethanol intake was dependent both on the subject's rank status and the level of housing stress. Furthermore, stressor specificity is an important consideration in research on the effect of novel stressors on ethanol intake. These results have significant implications for studies on drug and stress studies.

Acknowledgments

This research was supported in part by funds from the National Institute of Alcoholism and Alcohol Abuse, Grant 1RO1AA10124 and by funds from the Center of Alcohol Studies and Rutgers, The State University of New Jersey. The excellent technical assistance of Mrs. Chuan Chao is gratefully acknowledged.

References

Adams N, Oldham TD. Seminatural housing increases subsequent ethanol intake in male Maudsley reactive rats. J Stud Alcohol 1996;57:349–51.

Benus RF, Bohus B, Koolhaas JM, van Oortmerssen GA. Heritable variation for aggression as a reflection of individual coping strategies. Experientia 1991;15 (47):1008–19.

Bergvall AH, Fahlke C, Hansen S. An animal model for type 2 alcoholism? Alcohol consumption and aggressive behavior following lesions in the raphe nuclei, medial hypothalamus, or ventral striatum–septal area. Physiol Behav 1996;60:1125–35.

Björkqvist K. Social defeat as a stressor in humans. Physiol Behav 2001;73:435–42.

Blakley G, Pohorecky LA. Psychosocial stress alters ethanol's effect on open field behaviors. Pharmacol Biochem Behav 2006;84:51–61.

Blanchard RJ, Hori K, Tom P, Blanchard DC. Social structure and ethanol consumption in the laboratory rat. Pharmacol Biochem Behav 1987;28:437–42.

Blanchard RJ, Yudko EB, Blanchard DC. Alcohol, aggression and the stress of subordination. J Stud Alcohol Suppl 1993;11:146–55.

Blanchard RJ, Yudko E, Dulloog L, Blanchard DC. Defense changes in stress nonresponsive subordinate males in a visible burrow system. Physiol Behav 2001a;72:635–42.

Blanchard RJ, McKittrick CR, Blanchard DC. Animal models of social stress:effects on behavior and brain neurochemical systems. Physiol Behav 2001b;73:261–71.

Blokland A, Prickaerts J, Raaijmakers W. Reduced level of anxiety in adult Lewis rats after chronic ethanol consumption. Physiol Behav 1992;51:245–8.

Booth C, Hasking P. Social anxiety and alcohol consumption: the role of alcohol expectancies and reward sensitivity. Addict Behav 2009;34:730–6.

Boyce-Rustay JM, Janos AL, Holmes A. Effects of chronic swim stress on EtOH-related behaviors in C57BL/6J, DBA/2J and BALB/cByJ mice. Behav Brain Res 2008;186: 133–7.

Buwalda B, Kole MH, Veenema AH, Huininga M, de Boer SF, Korte SM. Long-term effects of social stress on brain and behavior: a focus on hippocampal functioning. Neurosci Biobehav Rev 2005;29:83–97.

Caramaschi D, de Boer SF, Koolhaas JM. Is hyper-aggressiveness associated with physiological hypoarousal? A comparative study on mouse lines selected for high and low aggressiveness. Physiol Behav 2008;95:591–8.

Chester JA, Blose AM, Zweifel M, Froehlich JC. Effects of stress on alcohol consumption in rats selectively bred for high or low alcohol drinking. Alcohol Clin Exp Res 2004;28:385–93.

Colombo G, Agabio R, Lobina C, Reali R, Zocchi A, Fadda F, et al. Sardinian alcohol-preferring rats: a genetic animal model of anxiety. Physiol Behav 1995;57:1181–5.

Cools AR, Rots NY, Ellenbroek B, de Kloet ER. Bimodal shape of individual variation in behavior of Wistar rats: the overall outcome of a fundamentally different make-up and reactivity of the brain, the endocrinological and the immunological system. Neuropsychobiology 1993;20:100–5.

Croft AP, Brooks SP, Cole J, Little HJ. Social defeat increases alcohol preference of C57BL/10 strain mice; effect prevented by a CCKB antagonist. Psychopharmacology (Berl) 2005;183:163–70.

Crowley TJ, Andrews AE. Alcoholic-like drinking in simian social groups. Psychopharmacology (Berl) 1987;92:196–205.

Doremus TL, Brunell SC, Rajendran P, Spear LP. Factors influencing elevated ethanol consumption in adolescent relative to adult rats. Alcohol Clin Exp Res 2005;29: 1796–808.

Duncan EA, Tamashiro KL, Nguyen MM, Gardner SR, Woods SC, Sakai RR. The impact of moderate daily alcohol consumption on aggression and the formation of dominance hierarchies in rats. Psychopharmacology (Berl) 2006;189:83–94.

Ebner K, Wotjak CT, Landgraf R, Engelmann M. Neuroendocrine and behavioral response to social confrontation: residents versus intruders, active versus passive coping styles. Horm Behav 2005;47:14–21.

Ehlers CL, Walker BM, Pian JP, Roth JL, Slawecki CJ. Increased alcohol drinking in isolate-housed alcohol-preferring rats. Behav Neurosci 2007;121:111–9.

Ellison G. Stress and alcohol intake: the socio-pharmacological approach. Physiol Behav 1987;40:387–92.

Fairbanks LA, Newman TK, Bailey JN, Jorgensen MJ, Breidenthal SE, Ophoff RA, et al. Genetic contributions to social impulsivity and aggressiveness in vervet monkeys. Biol Psychiatry 2004;55:642–7.

Feldker DE, Datson NA, Veenema AH, Meulmeester E, de Kloet ER, Vreugdenhil E. Serial analysis of gene expression predicts structural differences in hippocampus of long attack latency and short attack latency mice. Eur J Neurosci 2003;17:379–87.

Fernandes C, File SE. The influence of open arm ledges and maze experience in the elevated plus-maze. Pharmacol Biochem Behav 1996;54:31–40.

Fidler TL, LoLordo VM. Failure to find postshock increases in ethanol preference. Alcohol Clin Exp Res 1996;20:110–21.

Fokkema DS, Smit K, Van der Gugten J, Koolhaas JM. A coherent pattern among social behavior, blood pressure, corticosterone and catecholamine measures in individual male rats. Physiol Behav 1988;42:485–9.

Fokkema DS, Koolhaas JM, van der Gugten J. Individual characteristics of behavior, blood pressure, and adrenal hormones in colony rats. Physiol Behav 1995;57:857–62.

Funk D, Harding S, Juzytsch W, Lê AD. Effects of unconditioned and conditioned social defeat on alcohol self-administration and reinstatement of alcohol seeking in rats. Psychopharmacology (Berl) 2005;183:341–9.

Gallate JE, Morley KC, Ambermoon P, McGregor IS. The consequences of beer consumption in rats: acute anxiolytic and ataxic effects and withdrawal-induced anxiety. Psychopharmacology (Berl) 2003;166:51–60.

Geerse GJ, van Gurp LC, Wiegant VM, Stam R. Individual reactivity to the open-field predicts the expression of cardiovascular and behavioural sensitisation to novel stress. Behav Brain Res 2006;175:9-17.

Hall FS, Huang S, Fong GW, Pert A, Linnoila M. Effects of isolation-rearing on locomotion, anxiety and responses to ethanol in Fawn Hooded and Wistar rats. Psychopharmacology (Berl) 1998: 139:203–209.

Haller J, Halasz J, Makara GB. Housing conditions and the anxiolytic efficacy of buspirone: the relationship between main and side effects. Behav Pharmacol 2000;11:403–12.

Heinrichs SC, Pich EM, Miczek KA, Britton KT, Koob GF. Corticotropin-releasing factor antagonist reduces emotionality in socially defeated rats via direct neurotropic action. Brain Res 1992;581:190–7.

Henniger MS, Ohl F, Hölter SM, Weissenbacher P, Toschi N, Lörscher P, et al. Unconditioned anxiety and social behaviour in two rat lines selectively bred for high and low anxiety-related behaviour. Behav Brain Res 2000;111:153–63.

Henniger MS, Spanagel R, Wigger A, Landgraf R, Holter SM. Alcohol self-administration in two rat lines selectively bred for extremes in anxiety-related behavior. Neuropsychopharmacology 2002;26:729–36.

Higgins GA, Tomkins DM, Fletcher PJ, Sellers EM. Effect of drugs influencing 5-HT function on ethanol drinking and feeding behaviour in rats: studies using a drinkometer system. Neurosci Biobehav Rev 1992;16:535–52.

Higley JD, Hasert MF, Suomi SJ, Linnoila M. Nonhuman primate model of alcohol abuse: effects of early experience, personality, and stress on alcohol consumption. Proc Natl Acad Sci U S A 1991;88:7261–5.

Ho YJ, Eichendorff J, Schwarting RK. Individual response profiles of male Wistar rats in animal models for anxiety and depression. Behav Brain Res 2002;136:1-12.

Huhman KL, Solomon MB, Janicki M, Harmon AC, Lin SM, Israel JE, et al. Conditioned defeat in male and female Syrian hamsters. Horm Behav 2003;44:293–9.

José BS, van Oers HA, van de Mheen HD, Garretsen HF, Mackenbach JP. Stressors and alcohol consumption. Alcohol Alcohol 2000;35:307–12.

Juárez J, Vázquez-Cortés C. Alcohol intake in social housing and in isolation before puberty and its effects on voluntary alcohol consumption in adulthood. Dev Psychobiol 2003;43:200–7.

Keeney AJ, Hogg S. Behavioural consequences of repeated social defeat in the mouse: preliminary evaluation of a potential animal model of depression. Behav Pharmacol 1999;10:753–64.

Koolhaas JM, Korte SM, De Boer SF, Van Der Vegt BJ, Van Reenen CG, Hopster H, et al. Coping styles in animals: current status in behavior and stress-physiology. Neurosci Biobehav Rev 1999;23:925–35.

Kvetnansky R, Sabban EL, Palkovits M. Catecholaminergic systems in stress: structural and molecular genetic approaches. Physiol Rev 2009;89:535–606.

Langen B, Fink H. Anxiety as a predictor of alcohol preference in rats? Prog Neuropsychopharmacol Biol Psychiatry 2004;28:961–8.

Lynch WJ, Kushner MG, Ralweigh JM, Fiszdon J, Carroll ME. The effects of restraint stress on voluntary ethanol consumption in rats. Exp Clin Psychohopharmacol 1999;368: 9-16.

Matthews DB, Morrow AL, O'Buckley T, Flanigan TJ, Berry RB, Cook MN, et al. Acute mild footshock alters ethanol drinking and plasma corticosterone levels in C57BL/6J male mice, but not DBA/2J or A/J male mice. Alcohol 2008;42:469–76.

McEwen BS. The neurobiology of stress: from serendipity to clinical relevance. Brain Res 2000;886:172–89.

Miczek KA, DeBold JF, van Erp AM. Neuropharmacological characteristics of individual differences in alcohol effects on aggression in rodents and primates. Behav Pharmacol 1994a;5:407–21.

Miczek KA, Weerts E, Haney M, Tidey J. Neurobiological mechanisms controlling aggression: preclinical developments for pharmacotherapeutic interventions. Neurosci Biobehav Rev 1994b;18:97-110.

Miczek KA, Covington III HE, Nikulina Jr EM, Hammer RP. Aggression and defeat: persistent effects on cocaine self-administration and gene expression in peptidergic and aminergic mesocorticolimbic circuits. Neurosci Biobehav Rev 2004;27: 787–802.

Nakayasu T, Ishii K. Effects of pair-housing after social defeat experience on elevated plus-maze behavior in rats. Behav Processes 2008;78:477–80.

Overstreet DH, Halikas JA, Seredenin SB, Kampov-Polevoy AB, Viglinskaya IV, Kashevskaya O, et al. Behavioral similarities and differences among alcohol-preferring and-nonpreferring rats: confirmation by factor analysis and extension to additional groups. Alcohol Clin Exp Res 1997;21:840–8.

Parker LF, Radow BL. Isolation stress and volitional ethanol consumption in the rat. Physiol Behav 1974;12:1–3.

Peterson JT, Pohorecky LA. Effect of chronic ethanol administration on inter male aggression in rats. Aggress Behav 1989;15:201–16.

Pohorecky LA. Interaction of ethanol and stress: research with experimental animals—an update. Alcohol Alcohol 1990;25(2–3):263–76.

Pohorecky LA. Stress and alcohol interaction: an update of human research. Alcohol Clin Exp Res 1991;15:438–59.

Pohorecky LA. Housing and rank status of male Long-Evans rats modify ethanol's effect on open-field behaviors. Psychopharmacology (Berl) 2006;185:289–97.

Pohorecky LA. Psychosocial stress and chronic ethanol ingestion in male rats: effects on elevated plus maze behavior and ultrasonic vocalizations. Physiol Behav 2008;9 (94):432–47.

Pohorecky LA, Skiandos A, Zhang X, Rice KC, Benjamin D. Effect of chronic social stress on delta-opioid receptor function in the rat. J Pharmacol Exp Ther 1999;290: 196–206.

Pohorecky LA, Baumann MH, Benjamin D. Effects of chronic social stress on neuroendocrine responsiveness to challenge with ethanol, dexamethasone and corticotropin-releasing hormone. Neuroendocrinology 2004a;80:332–42.

Pohorecky LA, Blakley GG, Kubovcakova L, Krizanova O, Patterson-Buckendahl P, Kvetnansky R. Social hierarchy affects gene expression for catecholamine biosynthetic enzymes in rat adrenal glands. Neuroendocrinology 2004b;80:42–51.

Pohorecky LA, Blakley GG, Ma EW, Soini HA, Wiesler D, Bruce KE, et al. Social housing influences the composition of volatile compounds in the preputial glands of male rats. Horm Behav 2008;53:536–45.
Racz I, Schürmann B, Karpushova A, Reuter M, Cichon S, Montag C, et al. The opioid peptides enkephalin and beta-endorphin in alcohol dependence. Biol Psychiatry 2008;64:989–97.
Richman JA, Flaherty JA, Rospenda KM. Perceived workplace harassment experiences and problem drinking among physicians:broadening the stress/alienation paradigm. Addiction 1996;91:391–403.
Roske I, Baeger I, Frenzel R, Oehme P. Does a relationship exist between the quality of stress and the motivation to ingest alcohol? Alcohol 1994;11:113–24.
Rospenda KM, Richman JA, Wislar JS, Flaherty JA. Chronicity of sexual harassment and generalized work-place abuse: effects on drinking outcomes. Addiction 2000;95: 1805–20.
Ruis MA, te Brake JH, Buwalda B, De Boer SF, Meerlo P, Korte SM, et al. Housing familiar male wildtype rats together reduces the long-term adverse behavioural and physiological effects of social defeat. Psychoneuroendocrinology 1999;24:285–300.
Salomé N, Landgraf R, Viltart O. Confinement to the open arm of the elevated-plus maze as anxiety paradigm: behavioral validation. Behav Neurosci 2006;120:719–23.
Sapolsky RM. The influence of social hierarchy on primate health. Science 2005;308: 648–52.
Schenk S, Gorman K, Amit Z. Age-dependent effects of isolation housing on the self-administration of ethanol in laboratory rats. Alcohol 1990;7:321–6.
Sgoifo A, de Boer SF, Haller J, Koolhaas JM. Individual differences in plasma catecholamine and corticosterone stress responses of wild-type rats: relationship with aggression. Physiol Behav 1996;60:1403–7.
Shively CA, Laber-Laird K, Anton RF. Behavior and physiology of social stress and depression in female cynomolgus monkeys. Biol Psychiatry 1997;41:871–82.
Siegmund S, Vengeliene V, Singer MV, Spanagel R. Influence of age at drinking onset on long-term ethanol self-administration with deprivation and stress phases. Alcohol Clin Exp Res 2005;29:1139–45.
Spanagel R, Montkowski A, Allingham K, Stohr T, Shoaib M, Holsboer F, et al. Anxiety: a potential predictor of vulnerability to the initiation of ethanol self-administration in rats. Psychopharmacology (Berl) 1995;122:369–73.
Steensland P, Blakley G, Nyberg F, Fahlke C, Pohorecky LA. Anabolic androgenic steroid affects social aggression and fear-related behaviors in male pair-housed rats. Horm Behav 2005;48:216–24.
Thorsell A, Slawecki CJ, Khoury A, Mathe AA, Ehlers CL. Effect of social isolation on ethanol consumption and substance P/neurokinin expression in Wistar rats. Alcohol 2005;36:91–7.
Van Erp AM, Tachi N, Miczek KA. Short or continuous social stress: suppression of continuously available ethanol intake in subordinate rats. Behav Pharmacol 2001;12:225–42.
Veenema AH, Meijer OC, de Kloet ER, Koolhaas JM. Genetic selection for coping style predicts stressor susceptibility. J Neuroendocrinol 2003;15:256–67.
Veenema AH, Torner L, Blume A, Beiderbeck DI, Neumann ID. Low inborn anxiety correlates with high intermale aggression: link to ACTH response and neuronal activation of the hypothalamic paraventricular nucleus. Horm Behav 2007;51: 11–9.
Vengeliene V, Siegmund S, Singer MV, Sinclair JD, Li TK, Spanagel R. A comparative study on alcohol-preferring rat lines: effects of deprivation and stress phases on voluntary alcohol intake. Alcohol Clin Exp Res 2003;27:1048–54.
Virgin Jr CE, Sapolsky RM. Styles of male social behavior and their endocrine correlates among low-ranking baboons. Am J Primatol 1997;42:25–39.
Vivian JA, Weerts EM, Miczek KA. Defeat engenders pentylenetetrazole-appropriate responding in rats: antagonism by midazolam. Psychopharmacology (Berl) 1994;116:491–8.
Von Frijtag JC, Reijmers LG, Van der Harst JE, Leus IE, Van den Bos R, Spruijt BM. Defeat followed by individual housing results in long-term impaired reward-and cognition-related behaviours in rats. Behav Brain Res 2000;117(1–2):137–46.
Walker FR, Masters LM, Dielenberg RA, Day TA. Coping with defeat: acute glucocorticoid and forebrain responses to social defeat vary with defeat episode behaviour. Neuroscience 2009;162:244–53.
Weisinger RS, Denton DA, Osborne PG. Voluntary ethanol intake of individually or pair-housed rats: effect of ACTH or dexamethasone treatment. Pharmacol Biochem Behav 1989;33:335–41.
Werme M, Lindholm S, Thorén P, Franck J, Brené S. Running increases ethanol preference. Behav Brain Res 2002;133:301–8.
Wolffgramm J. Free choice ethanol intake of laboratory rats under different social conditions. Psychopharmacology (Berl) 1990;101:233–9.
Wolffgramm J, Heyne A. Social behavior, dominance, and social deprivation of rats determine drug choice. Pharmacol Biochem Behav 1991;38:389–99.
Wommack JC, Delville Y. Repeated social stress and the development of agonistic behavior: individual differences in coping responses in male golden hamsters. Physiol Behav 2003;80:303–8.
Yang X, Wang S, Rice KC, Munro CA, Wand GS. Restraint stress and ethanol consumption in two mouse strains. Alcohol Clin Exp Res 2008;32:840–52.
Zack M, Poulos CX, Aramakis VB, Khamba BK, MacLeod CM. Effects of drink-stress sequence and gender on alcohol stress response dampening in high and low anxiety sensitive drinkers. Alcohol Clin Exp Res 2007;31:411–22.