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ORIGINAL INVESTIGATION

# Social stress and escalated drug self-administration in mice I. Alcohol and corticosterone

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**Abstract**
*Rationale* Stress experiences have been shown to be a risk factor for alcohol abuse in humans; however, a reliable mouse model using episodic social stress has yet to be developed.
*Objectives* The current studies investigated the effects of mild and moderate social defeat protocols on plasma corticosterone, voluntary alcohol drinking, and motivation to drink alcohol.
*Methods* Outbred Carworth Farms Webster (CFW) mice were socially defeated for 10 days during which the intruder mouse underwent mild (15 bites: mean=1.5 min) or moderate (30 bites: mean=3.8 min) stress. Plasma corticosterone was measured on days 1 and 10 of the defeat. Ethanol drinking during continuous access to alcohol was measured 10 days following the defeat or 10 days prior to, during, and 20 days after the defeat. Motivation to drink was determined using a progressive ratio (PR) operant conditioning schedule during intermittent access to alcohol.
*Results* Plasma corticosterone was elevated in both stress groups on days 1 and 10. Ethanol consumption and preference following moderate stress were higher (13.3 g/kg/day intake) than both the mild stress group (8.0 g/kg/day) and controls (7.4 g/kg/day). Mice with previously acquired ethanol drinking showed decreased alcohol consumption during the moderate stress followed by an increase 20 days post-defeat. Moderately stressed mice also showed escalated ethanol intake and self-administration during a schedule of intermittent access to alcohol.
*Conclusion* Social defeat experiences of moderate intensity and duration led to increased ethanol drinking and preference in CFW mice. Ongoing work investigates the interaction between glucocorticoids and dopaminergic systems as neural mechanisms for stress-escalated alcohol consumption.

**Keywords** Stress · Social defeat · Drug abuse · Corticosterone · Mice · Self-administration · Animal models · Ethanol

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## Introduction

Ostensibly aversive stress experiences can increase the pursuit and use of alcohol and stimulants in humans and animals (Becker et al. 2011; Koob 2008; Koob and Le Moal 1997; Leventhal and Cleary 1980; Tomkins 1966). Murine models promise to enhance our understanding of the genetic and neurobiological mechanisms underlying escalated intake and increased motivation for alcohol after stressful episodes. It has been challenging to reliably and adequately characterize the stress-alcohol relationship in animal models (Becker et al. 2011; Noori et al. 2014). Along with its companion paper on cocaine (Han et al. 2014), the current report identifies several key social determinants linking episodic social defeat stress and its effect on alcohol drinking behaviors.

Mild episodes of social defeat constitute an ethologically relevant stressor that triggers sympathetic activation and the release of glucocorticoids (Koolhaas

et al. 1997; Marti-Carbonell et al. 1992; Meerlo et al. 1996; Miczek and Tidey 1989) as indicated by elevated plasma concentrations of adrenocorticotropic hormone (ACTH) and corticosterone. Social defeat stress is unique in that rodents do not habituate to repeated episodes of this stressor, evidenced by long-lasting enhancement of corticosterone and Fos expression in the hypothalamus and central amygdala (Martinez et al. 1998; Nikulina et al. 2004; Raab et al. 1986) and brain-derived neurotrophic factor (BDNF) in the nucleus accumbens (NAc) and ventral tegmental area (VTA) (Berton et al. 2006). Episodic exposure even to brief, intermittent social interactions can have extensive long- and short-term cardiovascular, thermoregulatory, and behavioral changes in rodents (Tornatzky and Miczek 1993).

Glucocorticoid release resulting from social defeat stress may contribute to the reinforcing effects of alcohol (Deroche et al. 1993; Piazza et al. 1993). Alcohol consumption results in the release of corticosterone, suggesting that alcohol can be a stressor under some conditions (Koob et al. 1998). However, alcohol has also been shown to have anxiolytic effects in rodents as, for example, in the elevated plus maze task (Lister 1987). Different types of stress, such as chronic subordination in a social colony, social isolation, and episodic social defeat stress alter plasma corticosterone levels in rodents (Raab et al. 1986; Tornatzky and Miczek 1993; Uschold-Schmidt et al. 2012; Weiss et al. 2004). Systemic administration of corticosterone increases self-administration of ethanol in rodents (Deroche et al. 1993; Piazza et al. 1993). Here, we confirm previous findings that demonstrated that social stressors induce a rise in plasma corticosterone levels as well as begin to investigate the precise, temporal relationship between corticosterone and episodic social stressors of different intensities.

Stress-induced glucocorticoids in blood plasma may mediate the activation of dopaminergic mesolimbic neurons (Rougé-Pont et al. 1998). Repeated exposure to stressors results in a long-term enhancement of dopamine release in the mesoaccumbens pathway in response to a stimulant challenge (Sorg and Kalivas 1991; Wilcox et al. 1986). Social defeat stress increases extracellular dopamine release in the shell of the NAc and affects the sensitivity of dopamine receptors in mice and rats (Han et al. 2014; Piazza and Le Moal 1998; Puglisi-Allegra et al. 1991; Tidey and Miczek 1997). These findings have led to the proposal that vulnerability to drug use may be exacerbated by neuroplastic changes in the hypothalamic-pituitary-adrenal (HPA) axis and mesolimbic dopaminergic pathway as a result of social defeat stress. Changes in vulnerability have previously been measured using locomotor sensitization to acute drug challenges (Phillips et al. 1997). The present study attempted to predict a stress-induced escalation of ethanol intake by testing for sensitization of locomotor activity to an acute ethanol challenge (Fish et al. 2002). The exposure to a social stressor and the subsequent release of glucocorticoids may affect drug-taking behaviors by modifying dopamine release in the NAc (Han et al. 2014; Covington, III et al. 2005).

Various animal models of alcohol drinking can be implemented in order to study the effects of social defeat stress on voluntary ethanol drinking, including continuous or intermittent access to alcohol and drinking in the dark (McBride and Li 1998; Rhodes et al. 2005). Intermittent access (IA) to alcohol in a two-bottle free-choice paradigm leads to voluntary, preferential, and dependence-inducing alcohol consumption (Hwa et al. 2011, 2013). We evaluated the reinforcing effects of ethanol in socially stressed rodents by measuring the rate of operant responding maintained by a progressive ratio schedule of ethanol reward (Czachowski and Samson 1999; Rodd-Henricks et al. 2003). Ethanol self-administration and voluntary drinking procedures provide a profile of the appetitive and consummatory aspects of regulated and escalated alcohol drinking. We implemented these models in order to study the effects of social defeat stress on voluntary ethanol consumption and motivation to drink in mice.

The present study used outbred Carworth Farms Webster (CFW) mice because of their genetic and phenotypic variation (Crabbe et al. 1994). Mice of this strain demonstrate a wide range of alcohol intake and thus provide evidence that can be translated to human conditions. The current study and its companion (Han et al. 2014) report closely similar elevated plasma corticosterone as well as dopamine release from the nucleus accumbens in response to two different types of social defeat stressors of distinct intensities and durations. Significantly, only those mice that were exposed to moderate but not mild social defeat episodes showed escalated and persistent ethanol intake, preference, and self-administration.

## Methods

### Animals

Adult male CFW mice ($n$=165; Charles River Laboratories, Wilmington, MA, USA) weighed 23–25 g upon arrival. Experimental mice were group-housed for 1 week in groups of six in large polycarbonate cages (48×26×16 cm) with corn cob bedding and unlimited access to standard rodent chow (Purina LabDiet 5001) and tap water. This allowed mice to habituate to the constant temperature (21±1 °C), 20 % humidity, and light/dark photocycle (lights off at 0700 and lights on at 1900) conditions of the vivarium. The mice were then housed individually in polycarbonate cages (28×17×12 cm) with ad libitum access to water and rodent chow. The guidelines of the "Guide for the Care and Use of Laboratory Animals" were followed for all procedures (2011) and were

approved by the Institutional Care and Use Committee of Tufts University.

### Social defeat stress

Upon arriving in the vivarium, each male "resident" CFW mouse was pair-housed with a female CFW mouse in a polycarbonate cage (28×17×12 cm). After at least 2 weeks, each male resident was assessed for aggression in confrontation with an intruder in the absence of the female cagemate for 5 min. The number of attack bites by the resident mouse was recorded. This procedure was performed for ten consecutive days. Mice that were determined to be reliably aggressive (greater than 30 bites in 5 min) were used as "resident" mice to socially defeat the experimental mice.

Experimental mice were randomly assigned to be in the moderate (being attacked 30 times) stress group, the mild (being attacked 15 times) stress group, or the non-stressed control group. Mice in the control group were weighed daily, while the mice in the stress groups were weighed and then socially defeated for ten consecutive days (days 1–10) using the following procedure, which consisted of the pre-defeat threat, defeat, and post-defeat threat phases (Yap et al. 2005). This procedure began 2–3 h after the beginning of the dark cycle every day. The female cagemate was removed before the pre-defeat phase and kept in a holding cage until the end of the threat phase. In the pre-defeat threat phase, an intruder mouse was placed into a perforated, protective cage (15 cm×7 cm×7 cm) and placed into the home cage of an aggressive "resident" mouse for 5 min. Intruders faced a different resident during each confrontation to prevent habituation of the resident to the intruder. During the defeat phase, the intruder mouse was removed from the perforated cage and placed into the resident's cage without protection. The defeat phase lasted until one of the following conditions was met: the intruder had received 30 or 15 bites, depending on experimental condition, 5 min had elapsed, or the intruder showed at least three consecutive seconds of defeat posture (Miczek et al. 1982). Mice that displayed defeat posture were removed from the resident's cage and immediately began the next phase (occurred in 3 % of confrontations). In the threat phase, the intruder was placed back into the perforated protective cage in the resident's cage for 5 min. Following the threat phase, the intruder was returned to its homecage for the remainder of the 24 h.

### Corticosterone measurements

Blood samples were collected from the submandibular vein 20 min after the start of the defeat phase of the social stress procedures on days 1 and 10 using disposable sterile lancets (MEDIpoint Inc., NY). Blood collection took less than 1 min per sample. Samples were centrifuged for 10 min at 4 °C, 3,000 revolutions per minute, and blood plasma was extracted. A corticosterone enzyme immunoassay kit (Arbor Assays, Ann Arbor, Michigan) was used to analyze the samples for corticosterone. Detection levels for corticosterone were 7.8125–1,000 ng/ml.

### Locomotor sensitization

On day 20, locomotor activity of each experimental mouse was recorded in an open field (53 cm×38 cm×46 cm) using EthoVision tracking software (Version 2.4.19). Mice were given a daily injection of saline during the 3 days prior to this testing in order to habituate to the intraperitoneal (i.p.) injection. Experimental mice were first placed into the open field and allowed to habituate for 30 min. Mice were then given an injection (i.p.) of saline, and locomotor activity was recorded for 15 min. In the final phase, mice were given an ethanol challenge (2 g/kg, i.p.), and locomotor activity was recorded for 45 min. The ethanol dose and injection-test interval were chosen based on previous work (Fish et al. 2002; Middaugh et al. 1989).

### Ethanol drinking

Twenty percent ethanol solutions (*w*/*v*) were prepared by diluting 95 % ethyl alcohol (Pharmaco-AAPER, Brookfield, CT) with tap water. At least 2 days before the ethanol challenge, mice were given access to two 50 mL centrifuge tubes (Nalgene) with water. Centrifuge tubes, equipped with no. 5 rubber stoppers and stainless steel nozzles with ball bearings, were presented to the mice through the metal wire cage lid. On the day following the ethanol challenge (day 21), mice were given either continuous or intermittent access to a two-bottle choice of 20 % ethanol and water, for 3 weeks or for 5 weeks, respectively (Hwa et al. 2011). Bottles were weighed daily in order to determine the average daily fluid consumption. The ethanol and water bottles switched sides every day to control for side preference. Mice were weighed each morning to calculate ethanol intake (grams of ethanol per kilogram of body weight). Ethanol evaporation and spillage due to experimenter bottle weighing were determined by weighing a pair of ethanol and water bottles that were held on a cage without an animal. The fluid "drip" measurements were subtracted from the daily ethanol and water bottles' weights to calculate the volume of fluid consumption.

### Ethanol reinforcement during fixed ratio and progressive ratio schedules

The present experiment studied ethanol reinforcement while using an operant conditioning panel that was inserted into the home cage (Miczek and de Almeida 2001). For operant responding under the control of schedules of ethanol

reinforcement, all events were monitored and controlled by a computer using MED-PC for Windows (MedAssociates, St. Albans, Vermont).

Two different schedules of reinforcement were used: fixed ratio (FR-1) and progressive ratio (PR). The operant conditioning panel was affixed to the home cage at the start of the dark cycle, and each response was reinforced with the presentation of 0.05 mL of 20 % ethanol or tap water. After 30 min, the FR-1 session was terminated, the panel was removed, and the two-bottle presentation was resumed. It was not possible to implement a continuous access protocol due to the confound of having water and alcohol concurrently present under independent PR contingencies. An IA procedure was used as it offered the advantage of preventing periods of alcohol deprivation. During the progressive ratio performance, the operant conditioning panels contained only one nose-poke receptacle. The side at which the removable receptacle was presented alternated every other session, matching the IA procedure. The type of fluid reinforcement alternated each session to match those presented in the IA protocol. The progression of the ratio of responses to 0.05 mL fluid reinforcement followed an exponential series (Richardson and Roberts 1996). Thus, the response requirement increased progressively according to the following schedule: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, and 95 for reinforcements 1 to 150, respectively. Break point was defined as the maximum response requirement achieved before the session's termination (Hodos 1961). Sessions were terminated when an animal did not complete the following ratio requirement within 30 min. At the end of the sessions, the two-bottle IA protocol was resumed for the remainder of the 24 h.

Experiment 1

The timeline for experiment 1 is shown in Fig. 1a. Mice were handled daily for the 3 days prior to the first day of social defeat stress. Mice then underwent ten consecutive days of either moderate or mild social defeat stress. On the first and last days of stress (days 1 and 10), blood was collected 20 min after the beginning of the defeat phase. Non-stressed controls were weighed daily during the 10-day protocol. Blood plasma was later analyzed for corticosterone levels using the Corticosterone Enzyme Immunoassay kit.

Experiment 2

The timeline for experiment 2 is shown in Fig. 1b. During experiment 2, mice underwent ten consecutive days of social defeat stress, as previously described. On day 20, mice were tested for locomotor activity following an ethanol challenge. For the next 20 days, mice were given continuous access to both a bottle of 20 % ethanol and of water. After 20 days, mice were categorized as either "high drinking" or "low drinking" based on their ethanol intake. Controls drank approximately 5 g/kg/24 h; thus, high drinking was considered to be twice that value. Mice that drank 10 g/kg/24 h for 10 or more days during the 20-day period were designated "high drinkers".

Experiment 3

The timeline for experiment 3 is shown in Fig. 1c. During experiment 3, mice were given access to a 20 % ethanol bottle and water bottle during the duration of the experiment.

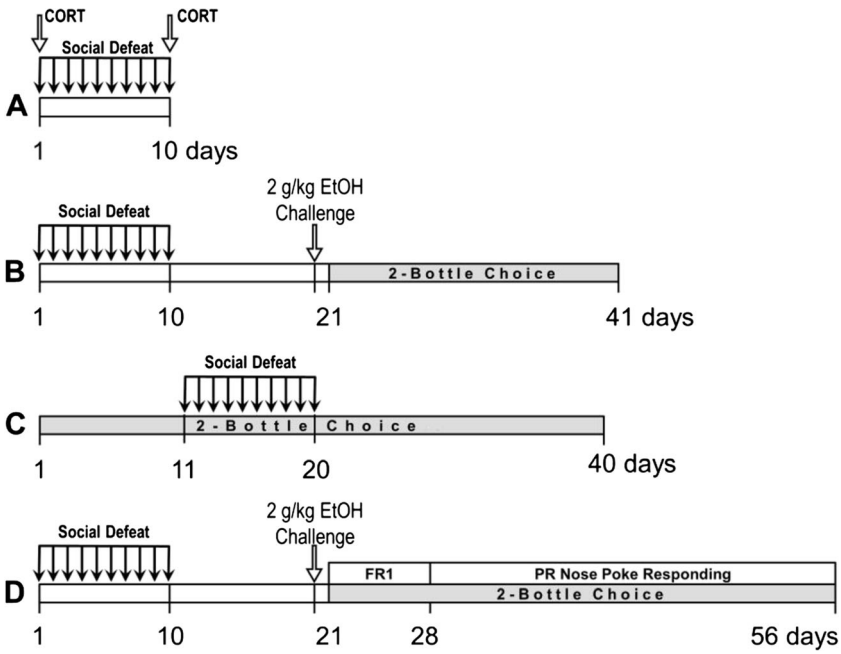


**Fig. 1** The experimental timelines of social defeat stress and **a** blood plasma corticosterone, **b** ethanol intake during continuous access, **c** previously acquired ethanol drinking during continuous access, **d** operant conditioning schedules during intermittent access

Baseline ethanol and water drinking were established during the first 10 days of the experiment. Mice were assigned to the moderate stress and non-stress groups and matched for their baseline ethanol drinking. In this experiment, mice were socially defeated on consecutive days during days 10 to 20. Fluid intake was measured daily during the social defeat procedure as well as 20 days afterward.

### Experiment 4

The timeline for experiment 4 is shown in Fig. 1d. During experiment 4, mice underwent the same social defeat and locomotor procedure as in experiment 2. Immediately following the locomotor test on day 20, mice were exposed to the FR-1 schedule of ethanol reinforcement for 1 week. After 1 week of FR-1 performance, mice were subjected to the PR schedule for 4 weeks. Throughout both schedules, mice were given water and intermittent access (i.e. every other day) to 20 % ethanol. Break points and 24-h fluid intake were measured each day.

### Statistical analysis

SigmaStat 11.0 software (Systat Software, San Jose, California) was used to analyze the data statistically. Two-way repeated measure (RM) analyses of variance (ANOVA) were performed to analyze the effect of the two stress procedures on weekly, voluntary ethanol consumption, and preference during continuous access to ethanol and water in experiment 2. This test was also used to determine the effect of time and stress experience on voluntary, daily ethanol consumption, and self-administration, defined as break point (Hodos 1961). One-way RM ANOVA was used to determine that stress had a similar effect on several cohorts of mice within the same group before data were merged. The effect of stress on blood plasma corticosterone on day 1 and day 10 of stress was analyzed using two-way RM ANOVA. Two-way RM ANOVA was used to determine the effect of the moderate stress group on previously acquired ethanol drinking behavior. Holm-Sidak post hoc *t* tests were used to identify significant treatment differences, as shown by $p<0.05$. A Wald chi-squared test was used to analyze the proportions of high drinkers in the stress groups.

## Results

### Effect of moderate and mild social defeat stress on blood plasma corticosterone

The moderate social defeat procedure (mean=227±5.9 s; 23±0.4 bites) was shown to be longer in duration than the mild social defeat procedure (mean=90±5.1 s; 15±0.1 bites). Both socially defeat groups showed elevated corticosterone on day 1 and day 10 of the social defeat procedure (Fig. 2). Two-way RM ANOVA revealed a main effect of stress on blood plasma corticosterone measurements [$F(2, 29)=15.789$, $p<0.001$]. The interaction between stress and time showed a trend [$F(2, 29)=2.789$, $p=0.078$]; however, there was no significant main effect of time.

Holm-Sidak post hoc analysis revealed that on day 1, both mild and moderate stress groups showed significantly increased corticosterone compared to the control group ($p<0.05$). Mice in the mild stress group had a significantly smaller rise in plasma corticosterone on day 10 when compared to day 1 [$t=2.052$, $p<0.05$]. In contrast, plasma corticosterone concentration in the moderate stress group did not significantly change from day 1 to day 10.

### Weekly ethanol intake after moderate or mild social defeat

The distinct effects of moderate and mild social defeat stress were evident across several cohorts of mice, as revealed by one-way ANOVA; therefore, the data from the cohorts of each experimental group were merged (Figs. 3 and 4). During the 20 days of continuous ethanol access, the moderate stress group drank more ethanol during 24 h access than either the mild stress group or the control group (moderate: mean=11.3±0.80 g/kg; mild: mean=6.49±1.1 g/kg; control: mean=5.68±0.82 g/kg). Two-way RM ANOVA revealed main effects of stress [$F(2, 84)=10.408$, $p<0.001$] and time [$F(3, 251)=10.826$, $p<0.001$]. Post hoc tests showed that the ethanol drinking behavior of the moderate stress group was significantly elevated compared to both the mild stress and non-stressed control group over the course of the four 5-day periods of continuous access to ethanol. Animals in the moderate stress group also demonstrated elevated ethanol preference during the experiment, compared to the non-stressed

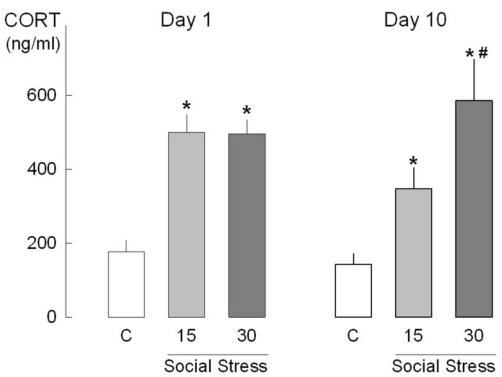


**Fig. 2** Blood plasma corticosterone (CORT, ng/ml) levels measured after day 1 or day 10 of being attacked 15 times (i.e. mild social stress, $n=11$) or 30 times (i.e. moderate social stress, $n=11$) daily. *$p<0.05$ compared to controls ($n=12$), #$p<0.05$ compared to the mild stress group

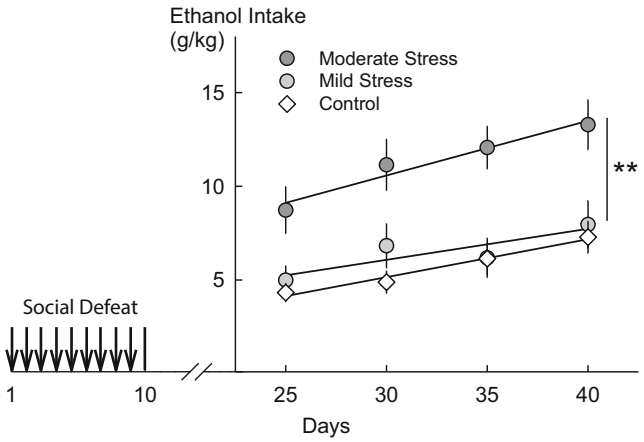


**Fig. 3** Twenty percent ethanol intake (g/kg/day) during continuous access two-bottle choice over the course of 20 days, starting 10 days after moderate ($n$=39) or mild ($n$=19) social defeat stress (control, $n$=29). Data points are 5-day averages±SEM beginning on the day indicated (i.e. 25 signifies days 25–29); **$p$<0.001 compared to controls

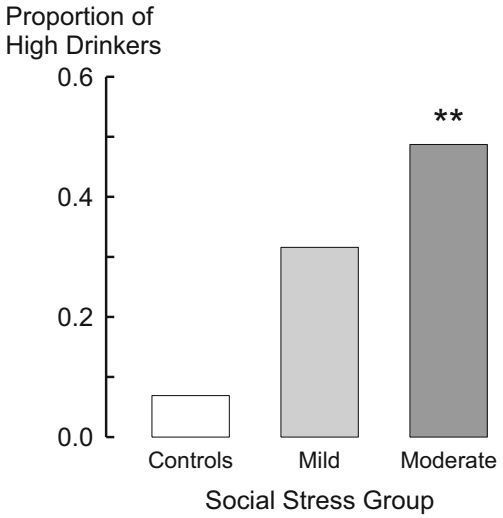


**Fig. 5** Proportion of high drinkers in the control ($n$=29), mild ($n$=19) and moderate stress ($n$=39) groups after continuous access to 20 % ethanol for 20 days. **$p$<0.001 compared to controls

control group. There was a main effect of time [$F$(3, 251)= 5.088, $p$<0.01] and stress [$F$(2, 84)=8.764, $p$<0.001] on ethanol preference during the 20 days of two-bottle free choice access to 20 % ethanol and water as demonstrated by a two-way RM ANOVA. The mild stress group demonstrated ethanol intake and preference similar to that of the non-stressed control group. A Wald chi-squared test revealed that the proportion of high drinkers in the moderate stress group but not the mild stress group was significantly higher than controls (Fig. 5). These data provide evidence for an initial and persistent increase in ethanol consumption in the moderate stress group.

Body weight may influence ethanol intake values that are measured as grams of ethanol per kilogram of body weight. Body weights of the stress groups and controls were analyzed during the social defeat procedure, and no significant differences were found. During ethanol consumption, there was a main effect of stress [$F$(2, 84)=3.673, $p$<0.05] and time [$F$(2, 252)=43.706, $p$<0.001]. Post hoc tests revealed that the moderate stress group had a significantly increased body weight compared to controls. All groups had significantly increased body weight after 5 days of ethanol intake when compared to their initial weight on day 1.

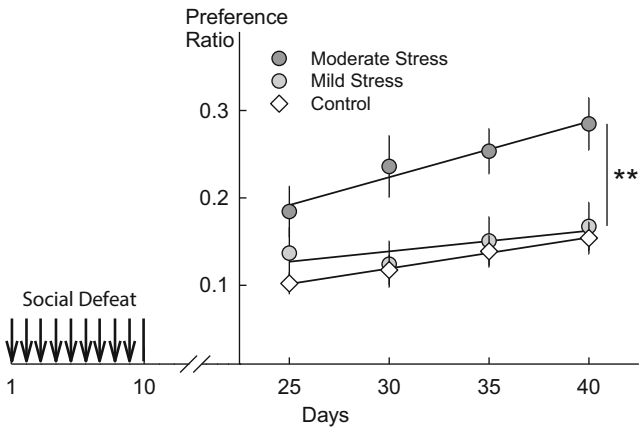


**Fig. 4** Preference for 20 % ethanol (ethanol intake/total fluid intake in 24 h) during continuous access over the course of 20 days, starting 10 days after moderate ($n$=39) or mild ($n$=19) social defeat stress (control, $n$=29). Data points are 5-day averages±SEM beginning on the day indicated (i.e. 25 signifies days 25–29); **$p$<0.001 compared to controls

## Locomotor sensitization to ethanol

Mice in the moderate stress (pre-drug: 94±5 m post-drug: 78± 9 m), mild stress (pre-drug: 59±3 m post-drug: 61±8 m), and control (pre-drug: 79±5 m post-drug: 61±8 m) groups did not show a significant change in locomotor activity in response to an i.p. ethanol injection. There was no significant difference in the percent of mice that showed locomotor sensitization to the ethanol challenge. There was no correlation between the average daily ethanol intake (g/kg) of the moderate stress, mild stress, or non-stressed control groups and the change in locomotor activity in response to an ethanol injection.

## Effect of moderate social defeat on previously acquired ethanol drinking

The baseline (pre) average for all mice during the 5 days before the start of social defeat stress was 6.50 g/kg (Fig. 6). Two-way RM ANOVA revealed a significant main effect of time [$F$(7, 129)=8.951, $p$<0.001] and a significant interaction between stress and time [$F$(7, 59)=4.105, $p$<0.001]. The moderately stressed mice showed significantly decreased ethanol drinking during the social defeat stress procedure compared to their baseline ethanol intake. After 40 days of ethanol drinking, these mice showed a significantly increased ethanol

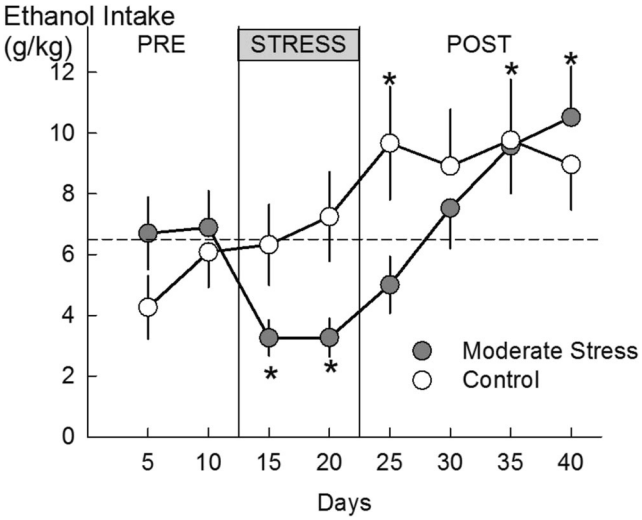


**Fig. 6** Twenty percent ethanol intake (g/kg/day) during continuous access over the course of 40 days starting 10 days before moderate social defeat stress. *PRE*: intake during the first 10 days. *STRESS*: intake during 10 days of moderate social defeat stress. *POST*: intake during 20 days after social defeat stress. The *dotted line* indicates average of control ($n$=18) and moderately stressed ($n$=16) mice 5 days before the social defeat stress procedure. Data points are 5-day averages±SEM. *$p$<0.05 compared to 5 days before the defeat stress procedure

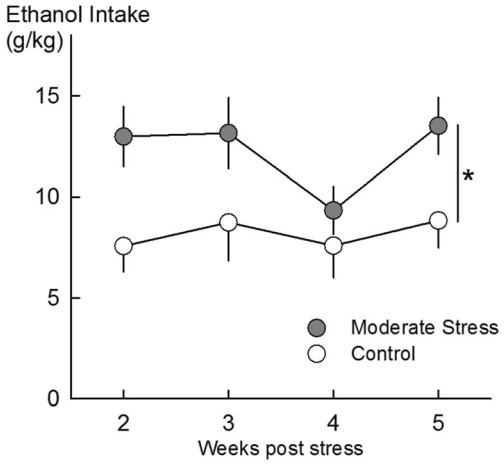


**Fig. 7** Twenty percent ethanol intake (g/kg) during intermittent access over the course of 4 weeks, starting 17 days after moderate social defeat stress ($n$=22). Data points are averages of three alcohol drinking days per week±SEM; *$p$<0.05 compared to controls ($n$=12)

intake compared to their baseline. Control mice demonstrated significantly increased ethanol intake relative to their baseline 25 and 35 days after the start ethanol drinking.

Effect of moderate social defeat stress on motivation to drink ethanol

Moderately socially defeated mice showed elevated ethanol intake and self-administration during an IA schedule, compared to non-stressed controls. There was a main effect of moderate social defeat stress on daily voluntary ethanol consumption [$F$(1, 96)=4.198, $p$<0.05] (Fig. 7) during IA. There was also an effect of moderate stress on the break points (Fig. 8) achieved in the PR operant responding condition [$F$(1, 96)=8.530, $p$<0.01]. Holm-Sidak post hoc tests revealed that these effects were maintained for the first 4 weeks of IA.

## Discussion

This report identifies salient features of episodic social stress conditions that result in reliably escalated alcohol drinking in mice. The current experimental procedures attempt to capture the epidemiologically established stress-alcohol link in a mouse model (José et al. 2000; Richman et al. 1996; Rospenda et al. 2000). Exposure to social defeat stress of moderate intensity and duration (i.e. being attacked 30 times in 5 min) engendered an increase in ethanol intake and preference 10 days later; however, there was no relationship between locomotor sensitization to alcohol and escalated alcohol consumption. In contrast, a mild social stressor had no effect on alcohol drinking compared to non-stressed controls despite similar corticosterone and dopamine responses in both stress procedures (Han et al. 2014). Studies with cocaine have shown that a range of stress conditions escalate self-administration in rodent models (Boyson et al. 2011; Goeders and Guerin 1994; Haney et al. 1995; Tidey and Miczek 1996); however, increases in alcohol consumption are generated by more limited stress parameters (Funk et al. 2005; Van Erp et al. 2001). While other studies have investigated the stress-alcohol link in mice (Chester et al. 2008; Croft et al. 2005; Sillaber et al. 2002), this is the first to delineate conditions of social defeat stress that reliably lead to escalated voluntary

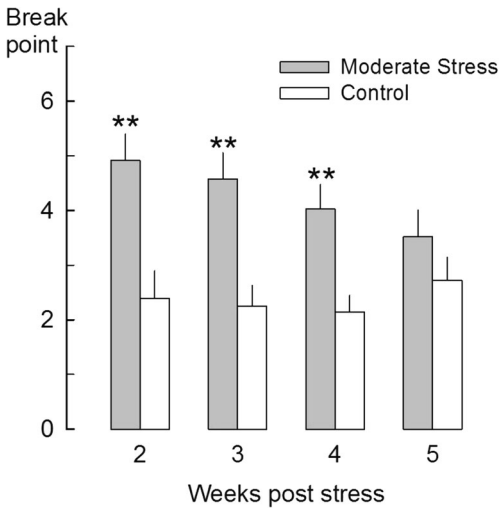


**Fig. 8** The maximal number of ethanol reinforcements achieved (break points) during a PR schedule starting 17 days after moderate social defeat stress ($n$=22). Bars are three session averages±SEM. **$p$<0.01 compared to controls ($n$=12)

ethanol consumption and self-administration in several cohorts of outbred mice.

In rodent models, social defeat stress protocols vary greatly in intensity and duration, defined respectively by the number of attack bites inflicted upon an intruder by a resident rodent. Many social defeat stress protocols combine lengthy episodes of severe stress with continuous exposure to sensory stimuli from a potential aggressor (Berton et al. 2006; Golden et al. 2011; Kudryavtseva et al. 1991). In some studies, mice were exposed to severe social stressors in which bouts ended only after an intruder received 100 attack bites and opioid-like antinociception became apparent (Miczek et al. 1982). It has been challenging to develop a social stress procedure which consistently escalates alcohol consumption in mouse strains (Croft et al. 2005; Sillaber et al. 2002). Here, we selected the conditions in which intruders received 15 or 30 attack bites to begin to identify the necessary and sufficient parameters of social defeat stress and stress hormones in the escalation of alcohol drinking. We have designated these two intensities as mild and moderate, respectively, in contrast to the more severe social stress procedures (Golden et al. 2011; Miczek et al. 1982).

Social defeat stress has been well characterized in terms of sympathetic and glucocorticoid activity in several mammalian species (Haller et al. 1997; Haller 2014). The present effects of social defeat stress on alcohol drinking appear to be paralleled with activation of the HPA axis; however, while both mild and moderate social stress resulted in increased corticosterone, only the latter resulted in escalated alcohol drinking. This dissociation of ethanol intake and plasma corticosterone elevation may be due to adaption of mildly stressed mice to the aggressive confrontations as evidenced by the decreased rise in corticosterone on day 10 of the social defeat procedure. The moderate stress group had a similar rise in plasma corticosterone on day 1 as day 10 suggesting no such adaptation. The measured concentration of basal plasma corticosterone (180 ng/mL) was consistent with some studies in DBA/2 J and albino mouse strains (Kakihana and Moore 1976; Gibson et al. 1979); however, other studies have found much lower basal plasma corticosterone levels (Finn et al. 2004). Plasma corticosterone may be altered by many factors such as handling (Irwin et al. 1986) or time of day (Barriga et al. 2001) which could account for the variance from study to study.

In rat models of social defeat, treatment with stress-relevant levels of exogenous corticosterone increases alcohol intake above baseline values (Fahlke et al. 1995). Glucocorticoids can have intrinsic reinforcing effects, such that rodents will self-administer corticosterone to maintain physiologically high blood plasma concentrations (Piazza et al. 1993). Injections of the corticosterone synthesis inhibitor metyrapone to alcohol-preferring rats reduce their alcohol consumption to the level of alcohol-non-preferring animals (Fahlke et al. 1994). We hypothesize that stress-induced elevated corticosterone levels may interact with dopaminergic systems in the nucleus accumbens in order to engender these changes in alcohol drinking behaviors (Cabib and Puglisi-Allegra 1996; Rothschild et al. 1985); however, further investigation into the relationship between escalated alcohol intake and corticosterone is needed before a mechanistic explanation of this relationship may be proposed.

Here, we describe a social defeat stress procedure which escalates alcohol consumption in CFW mice under two different conditions of alcohol access, continuous and intermittent. While previous findings suggest that mice of this strain do not consume high levels of alcohol (Crabbe et al. 1999; Phillips and Crabbe 1991), exposure to bouts of moderate social defeat elevated alcohol consumption in these outbred mice to a level comparable to that of the ethanol-preferring mice (Hwa et al. 2011; Middaugh et al. 1999). While the moderate stress group demonstrated high levels of drinking, all stress groups, including controls, showed a gradual increase in ethanol intake over time. This effect is consistent with other studies that have found similar results in non-stressed mice (Hwa et al. 2011, 2013). The cause for the emerging drinking in control mice remains to be determined. We are currently determining how the high alcohol consumption of the inbred C57BL/6 J mouse strain may be altered by our moderate social defeat stress procedure.

Subgroups of outbred CFW mice are highly motivated to seek out and consume high amounts of ethanol (high drinkers), while others consume very little (low drinkers). The average daily alcohol consumption of high drinkers was 15 g/kg/24 h, while low drinkers consumed 5 g/kg/24 h. These data suggest that within the CFW strain of mice, there are some subjects that are susceptible to stress-induced escalated alcohol consumption while others are resilient to this stress effect. The present study found that the proportion of high drinkers in a population of CFW mice increases with increasing stress intensity. Since pre-clinical and clinical studies implicate genetic variation, rather than environmental differences, as especially important in defining an individual's drug abuse liability, most ethanol drinking studies use inbred strains of mice (Crabbe et al. 1994; Middaugh et al. 1999). Indeed, two-bottle choice procedures were introduced to highlight differences in alcohol consumption and preference as a function of strain (McClearn and Rodgers 1959).

Here, we began to investigate how social defeat stress interacts with previously established ethanol consumption. When exposed to 10 days of episodic social defeat stress, ethanol-drinking animals initially showed a decrease in alcohol consumption, consistent with previous studies in rats (Funk et al. 2005; Van Erp and Miczek 2001). Relative to the alcohol-naïve mice, the onset of drinking behaviors was delayed. Prior exposure to ethanol could possibly be blunting HPA activation in response to social defeat stress, leading to a suppression of systemic glucocorticoid release

and its effect on dopaminergic reward pathways (Lee et al. 2000a, 2000b).

The present study demonstrates high levels of ethanol self-administration during a PR condition in socially stressed outbred mice without the use of fluid restriction; however, motivation to self-administer alcohol decreased over time (Fig. 8) which is in contrast to the 24-h ethanol intake that remained elevated (Fig. 7). The basis for this dissociation of ethanol intake and the motivational effects of ethanol remains unknown and must be studied further. The PR schedule of reinforcement is particularly useful when measuring the relative strength of a reward (Hodos 1961). Rats that perceive alcohol as highly preferable will self-administer larger quantities of ethanol than alcohol non-preferring rats under a PR schedule of reinforcement (Samson et al. 1992; Waller et al. 1984). Though some inbred mouse strains will voluntarily drink significant quantities of ethanol, in CFW mice, fluid restriction is usually required for reliable ethanol self-administration under the control of several schedules of reinforcement (Faccidomo et al. 2012; Newman et al. 2012).

Pre-clinical and clinical evidence implicates social stress as a major trigger in the initiation and maintenance of drug-taking behaviors (Goeders 2003; Sinha 2008). However, not all social stressors will similarly affect drug use in these populations. Even when physiological consequences appear closely similar, stressors of different controllability and predictability can dissimilarly affect underlying neurobiological reward pathways (Miczek et al. 2008).

In conclusion, we present a social defeat stress procedure which reliably escalates alcohol consumption and self-administration in outbred CFW mice. Despite similar initial elevations in glucocorticoid and dopamine response (Han et al. 2014 Revision under review), only the social defeat experience of moderate intensity and duration led to increased ethanol drinking. The interaction between glucocorticoids and dopaminergic systems in the brain can be further explored after this identification of the salient parameters of social defeat stress that lead to increased drug taking.

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