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Line differences in ethanol consumption and immobility in the forced swim test, but not in stress reactivity, in alcohol preferring (P) and nonpreferring (NP) rats

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LINE DIFFERENCES IN ETHANOL CONSUMPTION AND IMMOBILITY IN THE FORCED SWIM TEST, BUT NOT IN STRESS REACTIVITY, IN ALCOHOL PREFERRING (P) AND NONPREFERRING (NP) RATS

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ABSTRACT

There is evidence to support a relationship between stress, depression, and alcoholism. Animal models have been developed to ascertain the impact of stress on depressive-like symptoms and ethanol intake. The swim test susceptible (SUS) line of rats selectively bred for enhanced susceptibility to stress-induced immobility in the forced swim test (FST) also show high voluntarily ethanol intake comparable to intake by P rats, suggesting that SUS rats may drink to alleviate a negative affective state. However, P rats spend less time immobile than NP rats in the FST, suggesting that depressive-like symptoms are not associated with ethanol preference, but P rats elevate drinking in response to stress. Thus, it is unclear whether high ethanol intake is related to stress, depressive-like symptoms, or both. To investigate this interaction, ethanol-naive male NP (n = 32) and P (n = 35) rats were divided into four stress groups: footshock, white noise, restraint, and no stress. Stressors were 30 minutes in duration and ended 10 minutes prior to the FST in which time spent immobile and struggling was observed for 10 minutes. Forty-eight hours following the FST, all rats (including additional groups of FST-naive rats, n = 9/line) were given continuous access to 10% ethanol and water. After 2 weeks to acclimate to the ethanol rats received their respective stress or no stress treatments at weekly intervals for 3 weeks. P rats drank more ethanol than NP rats, but both lines increased their intake over the 6-week access period. No main effects of stress were evident when compared on stress days. Analysis of the final week of ethanol drinking revealed that P rats exposed to footshock showed elevated drinking compared to unstressed and FST-naive rats, and restrained and unstressed NP rats tended to drink more than FST-naive rats. Consistent with prior research, NP rats spent more time immobile than P rats in the FST. A trend for an effect of stress on immobility appeared to be mediated by the ability of restraint stress to *reduce* immobility. While these data demonstrate stress-related increases in drinking, no stress-induced enhancement of immobility was evident in the P and NP lines. Further, while both ethanol intake and immobility differed between the P and NP lines, stress did not appear to mediate these behaviors in a line-dependent manner. Subsequent research using the SUS and swim test resistant (RES) lines in comparison to the P and NP line may reveal how selective breeding for different phenotypes converges on alcohol-related behaviors. Supported by AA07462 and AA015512

INTRODUCTION

Epidemiological studies have shown that anxiety and mood disorders are commonly comorbid with alcoholism. For example, the prevalence of depression among those with substance abuse disorder is twice that of the general population (Grant et al., 2006). This finding suggests that these disorders may share a common underlying mechanism. Animal models of depressive-like symptoms and the propensity to consume alcohol are particularly useful. Ethological models of depression such as the forced swim test (FST) implicate aversive or stressful stimuli in increasing helpless behavior and have been shown to be high in predictive validity (Borsini & Meli, 1988; Porsolt et al., 1977).

Models of alcoholism have also been developed by the use of selective breeding for high ethanol intake. This selective breeding strategy has also been implemented to develop lines of rats that are susceptible (SUS) or resistant (RES) to stress-induced enhancement of immobility in the FST (Scott et al., 1996). Interestingly, the SUS rats were shown to consume quantities of alcohol similar to those of the selectively bred alcohol-preferring (P) rat (West & Weiss, 2006). It was therefore suggested that the SUS rats drink significant amounts of alcohol to alleviate a negative affective state. However, P rats are less immobile in the FST than their nonpreferring (NP) counterparts, which did not support the link between depressive-like symptoms and alcohol drinking in the P rat (Godfrey et al., 1997; Viglinskaya et al., 1995).

Nonetheless, the P rat has been shown to elevate its ethanol intake in response to stress (Chester et al., 2004; Vengeliene et al., 2003). As such, it could be that stress represents the underlying mechanism that is associated with both depressive symptoms and alcohol drinking. The purpose of the present study was to determine whether or not differences between the P and NP rat in forced swim tank behavior and ethanol drinking would emerge following exposure to stress.

METHODS

Subjects

- Ethanol-naive male NP (n = 32) and P (n = 35) rats were divided into 4 stress groups:
  - footshock - 0.8 mA shock, 0.5 s duration, on a VI60 s schedule
  - white noise - 90-95 dB in a novel cage
  - restraint - transparent plastic tubes 22.3 cm in length, 6.4 cm in diameter
  - no stress - handled, transported similar to stressed rats
- An additional group of rats (n = 9) were neither stressed nor given FST ("naïve"), but were given ethanol access along with the FST groups

Stress-related behavior in the FST

- Stressors were 30 minutes in duration and ended 10 minutes prior to the FST
- The FST tank was 62 cm high, 30 cm in diameter, filled with water at 26ºC to a depth of 48 cm
- Time spent immobile, struggling, and engaged in "other" behaviors (e.g., swimming) were recorded during the 10-minute test

Stress-related drinking behavior

- 48 hours following the FST, all rats were given continuous access to 10% ethanol and water
- Rats received 2 weeks of acclimation to ethanol, followed by exposure to their respective stressors at weekly intervals for 3 weeks, ending with a post-stress drinking week

Statistics

- Time spent immobile and struggling were separately analyzed using factorial ANOVAs
- Ethanol consumption (g/kg) on each stress day was analyzed using 3-way (stress type, day, line) mixed factorial ANOVAs

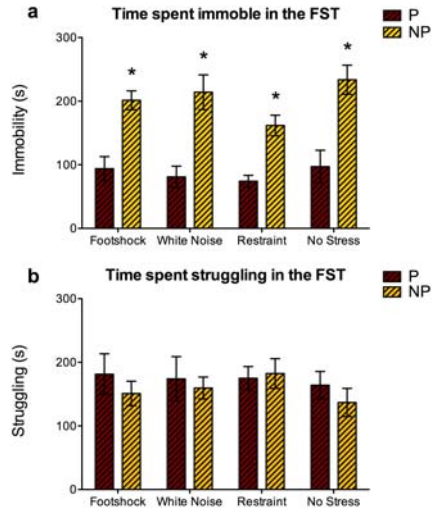

Figure 1: Mean ± SEM time spent immobile (a) and struggling (b) during the 10-minute forced swim test. NP rats spent significantly more time immobile than P rats. No line difference was evident in struggling. A nonsignificant trend for an effect of stress was found in NP rats only. This appeared to be mediated by differences in the restraint and no stress groups.

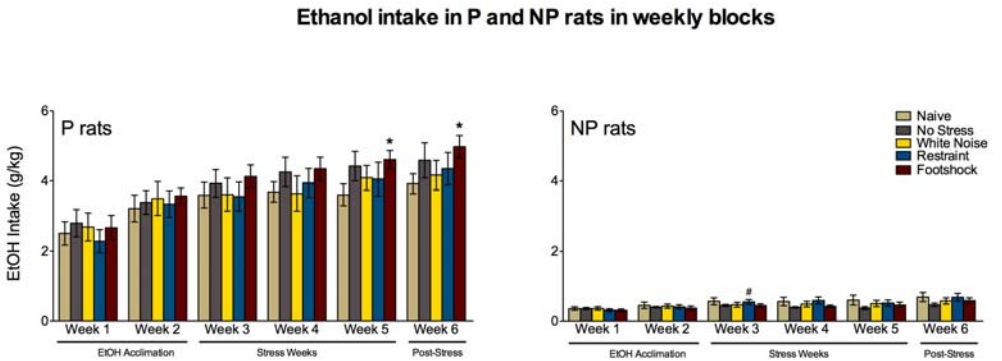

Figure 2: Mean ± SEM ethanol intake in P and NP rats in weekly blocks. P rats drank significantly more ethanol than NP rats, but both lines increased their intake across the 6 weeks of drinking. Weeks 1-2 were used for ethanol acclimation. On the first day of weeks 3-4 rats were exposed to their respective stressors. Week 6 was used to measure post-stress ethanol intake. During the final 2 weeks, P rats exposed to footshock stress drank significantly more ethanol than their naïve counterparts. In NP rats, there was a trend for naïve rats and rats exposed to restraint stress to increase their drinking compared to their unstressed counterparts during the final 3 weeks.

DISCUSSION

- Consistent with previous findings, P rats drank significantly more ethanol, but spent significantly less time immobile in the FST, than NP rats
- This does not support the hypothesis that the P rat drinks to alleviate negative mood states as is suggested for the SUS rat
- No overall main effects of stress type were found for either ethanol drinking or activity in the FST
- Exploratory post hoc comparisons on the final 2 weeks of drinking revealed that footshock stressed P rats showed elevated drinking compared to stress naïve controls
- A similar but nonsignificant trend was found for NP rats wherein rats exposed to restraint stress drank more ethanol than their unstressed counterparts
- Exploratory post hoc comparisons also revealed that NP rats exposed to restraint stress spent less time immobile than their unstressed counterparts
- As such, subsequent research using either footshock or restraint stress could yield stronger results concerning the effect of stress on drinking and depressive-like behaviors
- In addition, direct comparison between the P and SUS as well as NP and RES selected lines could further elucidate how selective breeding for different phenotypes converges on alcohol-related behaviors
- Based on these findings, the P rat and the SUS rat may represent different "subtypes" of alcoholics

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