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Female rats exposed to stress and alcohol show impaired memory and increased depressive-like behaviors

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HIGHLIGHTS

- Alcohol intake after a stressor impairs memory in female rats.
- Depressive-like behaviors were increased by alcohol treatment.
- Corticosterone response was increased by stress and alcohol treatment.
- Results for female rats differ from those previously found in males.

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ABSTRACT

Exposure to daily life stressors is associated with increases in anxiety, depression, and overall negative affect. Alcohol or other psychoactive drugs are often used to alleviate stress effects. While females are more than twice as likely to develop mood disorders and are more susceptible to dependency than males, they are infrequently examined. In this study, female rats received no stress/no alcohol control (CON), alcohol alone (ALC), stress alone (STR), or stress plus alcohol (STR + ALC). Stress consisted of restraint for 6 h/day/7 days, and alcohol was administered immediately following restraint via gastric gavage at a dose of 2.0 g/kg. Dependent measures included tests utilizing object recognition (OR), Y-maze, elevated plus maze (EPM), forced swim (FST), blood alcohol content, corticosterone levels, and body weights. ALC, STR + ALC, but not stress alone, impaired memory on OR. All treatments impaired spatial memory on the Y-maze. Anxiety was not affected on the EPM, but rats treated with alcohol or in combination with stress showed increased immobility on the FST, suggestive of alcohol-induced depression. Previously, we found alcohol reversed deleterious effects of stress on memory and mood in males, but current results show that females reacted negatively when the two treatments were combined. Thus, responses to alcohol, stress and their combination suggest that sex specific treatments are needed for stress-induced behavioral changes and that self-medicating with alcohol to cope with stress maybe deleterious in females.

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1. Introduction

Recent research shows robust neural and behavioral sex differences in responses to physiological stimuli such as stress and to pharmacological agents like psychoactive drugs and alcohol [1,2,10]. Compared to men, women are more susceptible to the effects of alcohol due to differences in metabolism, hormones, and physiology (review, [38]). For example, when tested with the same dose (0.3 g/kg), women produce higher blood alcohol levels than men [19]. Furthermore, the time from onset of alcohol use to acquiring chronic drinking behaviors or entering treatment due to complications is shorter for women than for men [25].

Alcohol is often used as a form of self-medication for coping with stressors [31], and attempts of self-medication are often gender specific. Berger and Adesso [4] tested emotional effects of alcohol on groups of depressed and non-depressed men and women and found that only depressed men and non-depressed women reported reduction in depressive feelings after consuming alcohol. A comparison of stressed versus non-stressed individuals found that, only in men, not in women, was stress a mediating factor for the amount of alcohol consumed, with greater reported stress associated with higher intake [16]. Despite the differences in use and effects between genders, neurological consequences are often similar. Men and women show comparable cortical shrinkage and ventricle enlargement after chronic alcohol abuse [39]. However, a comparison of

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hospitalized alcoholic women and men found that the magnitude of corpus callosum shrinkage was greater in women than men even after correction for cranial size [26], suggesting greater sensitivity to alcohol-induced brain damage in women.

With the use of animal models, researchers have confirmed some but not all sexual dimorphisms in response to alcohol or stress in humans. After 20 weeks of ethanol treatment, both male and female rats show the same frontal cortex shrinkage, but different impairment in the delayed matching-to-position task [57]. After alcohol treatment, females showed a delay-*dependent* impairment while males showed a delay-*independent* impairment, suggesting a sex specific effect of alcohol on cognitive function [57]. In response to an acute administration of alcohol, both male and female rats show a reduction in anxiety-like behaviors on the plus maze; however, corticosterone was increased in females but not in males following an intraperitoneal injection of 1.5 g/kg of alcohol [65]. Thus, administered alcohol appears anxiolytic, but females were subject to increased circulating stress hormones, suggesting a tradeoff when self-medicating with alcohol.

The link between stress and alcohol use is of much interest, especially when and why alcohol may be used as a form of self-medication. It has been noted that both exposure to chronic stress or withdrawal from alcohol increases anxiety [8]. Although an individual may start drinking alcohol after a stressor for its anxiolytic effects, abuse may ensue as a way of reducing negative affect associated with alcohol withdrawal [33]. Furthermore, during abstinence, relapse has been shown to be directly related to the number of experienced stressors [58]. The specific effects of stress-induced increases in drug seeking behavior during abstinence are thought to be mediated by corticotropin-releasing factor (review, [62]). However, the interactions between stress and alcohol are not limited to anxiety and stress hormones.

Cognitively, chronic stress impairs memory in male rats but either has no effect or enhances performance in female rats, depending on the task [3,6,14,41]. Unlike the sex differences in memory following stress, chronic alcohol consumption leads to memory impairments in both sexes. Memory impairment caused by ethanol depends on dose and duration, but in female rats, as short as 6 days (2 g/kg/day) impairs performance on the Morris water maze [60] and 6 weeks (0.7 g/kg/day) impairs performance on the Barnes maze [53]. The interactive effects of stress and alcohol have been sparsely studied in female subjects. Studies using other drugs find that stress increases self-administration of most drugs in both sexes, including cocaine [23], amphetamine [50], morphine [40], and alcohol [34]. Bisagno et al. [5] found that amphetamine impairs memory in female rats, but the impairment is not present in rats that were also exposed to chronic restraint stress, which shows an interactive effect.

We have previously shown that male rats increase alcohol intake following stress [20], an effect which supports the concept of self-medicating for the adverse effects of stress [59]. Moreover, we found that when alcohol was involuntarily administered to male rats following daily restraint stress, impairments in spatial memory and anxiety were reversed by the alcohol treatment [21]. Thus, alcohol appeared to have beneficial effects on some adverse behavioral consequences of stress. In the current study, we investigated whether alcohol treatment to stressed female rats would have similar effects as in previously studied males. Because females have a different pattern of alcohol use and abuse than males and show a different pattern of behavioral changes following stress [9], the effects of the combination treatments were not predictable based on male models. Dependent variables included tests for memory, anxiety, depression, and physiological responses for each treatment using the same design, as was previously conducted in male rats [21]. The behavioral and physiological results show that females respond robustly, but differently, than males to treatments, and consequently these changes may contribute to our understanding of sex differences in alcohol abuse and self-medication as well as to the greater incidence of mood disorders in females versus males.

2. Methods and materials

2.1. Subjects

Intact cycling adult female Sprague-Dawley rats (weight ≈ 180 g, age ≈ 3 months, N = 32) obtained from Harlan Sprague-Dawley, Inc. (USA) were pair-housed and kept on a 12 h light cycle with lights *on* at 09:00. Standard rat chow and water were available *ad libitum*. Rats were randomly assigned to one of four conditions (n = 8 per group): no stress/no alcohol control (CON), alcohol alone (ALC), stress alone (STR), or combination of stress plus alcohol (STR + ALC). All procedures were approved by Hunter College's Animal Care and Use Committee.

2.2. Procedure

A time-line of the procedures has been provided in Fig. 1. After arrival and acclimation to the animal facility, habituation for object recognition (OR) was conducted for five days. Habituation consisted of exposure to the testing arena alone, followed by consecutive days of exposure to objects with increasing intertrial delays (1 m, 40 m, 1 h, and 2 h). Independent of OR, at two points during habituation, rats were administered 1 cc of saline via gastric gavage to reduce stress associated with the procedure and prepare them for future administrations. Alcohol and stress treatment started after the last OR habituation trial. For seven consecutive days rats in the stress groups were restrained for 6 h each day from 10:00 to 16:00. Each day, post-stress, alcohol (ALC and STR + ALC) or saline (CON and STR) was administered via gastric gavage at a dose of 2.0 g/kg. Following treatments, rats were put through a battery of behavioral tests. Rats were tested on the elevated plus maze for anxiety and OR task for visual working memory on day-8, the Y-maze for spatial memory on day-9, and the forced swim task for depression on day-10. Following the completion of the FST (30–40 min post-swim) blood samples were collected for corticosterone analysis. The order of behavioral testing was chosen to provide optimal information regarding the interactive effects of stress and alcohol. Thus, the EPM was conducted on day-8 to test for anxiety and for any possible withdrawal effects of daily alcohol intake. It was expected that conducting the OR task immediately after the EPM would have no effect on performance. To confirm, correlation analyses were run and no significant correlation was found between behavior on the EPM and performance on the OP task (data not shown). The Y-maze was done alone on day-9, as it was the task that took the longest time given the N size (~6 h). To test for depressive-like behavior and limit any possible confounding effects of stress induced by swimming, the FST was run last. Additionally, the FST provided a novel stressor to assess possible changes in stress hormone release between groups. It should still be noted that order of testing and time of testing post treatments may have influenced the outcomes.

2.3. Stress and alcohol administration

Rats were restrained, not immobilized, for 6 h/day/7 days (10:00–16:00) in a Plexiglas restrainer measuring 21.5 cm long × 6.3 cm internal diameter (Harvard Apparatus). All non-stressed rats were handled daily but not restrained. The closed front was clear and contained ventilation holes, while the back had a sliding plastic plug, which was adjusted to fit the size of the rat. To assess physiological effects of stress, all rats were weighed while treatment was being conducted (7 days), just before being restrained or handled. Pure ethanol (200 proof, Sigma-Aldrich) was diluted in saline (0.9% NaCl, Fisher Scientific) to produce a concentration of 20% v/v ethanol. Although water is a more acceptable way of diluting ethanol for gavage, saline was used because it was believed to maintain a better salt balance for the volume given than that of water. Previous studies have used water for dilution [22,36], and this difference in gavaging fluids may have contributed to