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Female Rats Exposed to Stress and Alcohol Show Impaired Memory and Increased Depressive-like Behaviors

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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 6hr/day/7days, 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 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 may be deleterious in females.

Keywords

Female; Stress; Alcohol; Memory; Anxiety; Depression

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 (Barry and Petry, 2009; Bangasser et al., 2010; Buffalari et al., 2012). Compared to men, women are more susceptible to the effects of alcohol due to differences in metabolism, hormones, and physiology (review, Mancinelli et al., 2007). For example, when tested with the same dose (0.3 g/kg), women produce higher blood alcohol levels than men (Frezza et al., 1990). Furthermore, the time from onset of alcohol use to acquiring

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chronic drinking behaviors or entering treatment due to complications is shorter for women than for men (Hernandez-Avila et al., 2004).

Alcohol is often used as a form of self-medication for coping with stressors (Khantzian, 1997), and attempts of self-medication are often gender specific. Berger and Adesso (1991) tested emotional effects of alcohol on groups of depressed and non-depressed men and women and found 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 women, was stress a mediating factor for the amount of alcohol consumed, with greater reported stress associated with higher intake (Cooper et al., 1992). 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 (Mann et al., 1995). However, a comparison of 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 (Hommer et al., 1996), 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 (Savage et al., 2000). 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 (Savage). 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 males following an intraperitoneal injection of 1.5 g/kg of alcohol (Wilson et al., 2004). 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 (Breese et al., 2004). 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 (Koob and LeMoal, 2008). Furthermore, during abstinence, relapse has been shown to be directly related to the number of experienced stressors (Sinha, 2001). The specific effects of stress-induced increases in drug seeking behavior during abstinence are thought to be mediated by corticotropin-releasing factor (review, Stewart, 2000). 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 (Bowman et al., 2002; Beck and Luine, 2002; Conrad et al., 2004; McLaughlin et al., 2005). 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/d) impairs performance on the Morris water maze (Sircar et al., 2009) and 6-weeks (0.7 g/kg/d) impairs performance on the Barnes maze (Ranney and Petro, 2009). 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 (Haney et al., 1995), amphetamine (Piazza et al., 1990), morphine (Marks-Kaufman and Lewis, 1984), and alcohol (Lê et al., 1999). Bisagno

et al. (2004) found 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 (Gomez et al., 2012), an effect which supports the concept of self-medicating for the adverse effects of stress (Sinha, 2008). 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 (Gomez et al., 2013). Thus, the alcohol appeared to have beneficial effects on some adverse behavioral effects to stress. In the current study, we investigated whether alcohol treatment to stressed female rats would have the 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 (Breslin, et al., 1994), 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 (Gomez et al., 2013). 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.

Methods and Materials

Subjects

Intact cycling adult female Sprague-Dawley rats (Weight $\approx$ 180 g, Age $\approx$ 3 months, N = 32) obtained from Harlan Sprague-Dawley, Inc. (USA) were pair-housed and kept on a 12hr light cycle with lights *on* at 09:00. Standard rat chow and water was 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.

Procedure

A time-line of the procedures has been provided in figure 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 (1m, 40m, 1h, and 2h). Independent of OR, at two points during habituation, rats were administered 1cc of saline via gastric gavage to reduce stress associated with the procedure to 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-hours each day from 10:00–16:00. 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 (30–40min. 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-hours). 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.

Stress and Alcohol Administration

Rats were restrained, not immobilized, for 6hr/day/7days (10:00–16:00) in a Plexiglas restrainer measuring 21.5cm long × 6.3cm 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 water. Previous studies have used water for dilution (Livy et al, 2003; Griffin et al, 2009), and this difference in gavaging fluids may have contributed to the pattern of the current results. Gavage was performed using a blunted feeding needle (18G × 2") affixed to a 10cc syringe (VWR). All rats were gavaged with a dose of 2.0 g/kg of alcohol or saline in a counterbalanced fashion at the same time every day (~16:15). To measure blood alcohol concentration, 30-minutes after the final alcohol administration (day-7), blood samples were taken via tail-nick and blood alcohol content (BAC) was analyzed using 5µl of sera in an Analox GM7 micro-stat machine. Using the Analox GM7, after calibration with alcohol reagents (GMRD-113, Analox Instruments LTD), ethanol was oxidized by alcohol oxidase and the Analox measures the rate of oxygen used by samples as an indirect measure of BAC.

Behavioral Tests

The object recognition task (OR) is a prefrontal cortex – hippocampal working memory task comprised of a sample trial (T1) and a retention trial (T2). The procedure for habituation and testing were identical, except different objects were used each time and the intertrial delay was gradually increased (Habituation: 1m, 40m, 1h, 2h; Test: 4h delay). During T1, two identical objects were placed on one side near the corners of an open field (Plexiglas: 70 × 70 × 30 cm) and the rat was allowed to explore for 3-minutes. Exploring was determined as touching, whisking, or sniffing the objects from no more than 2cm away. Following the intertrial delay, the objects and field were cleaned and one of the objects was replaced with a new object and the rat was reintroduced and allowed to explore for 3-minutes. Increased exploration for the new object is indicative of intact recognition memory (Ennaceur et al., 1997). Placement and new/old objects were presented in a counterbalanced fashion to reduce possible confounding effects of place or object preference. The task, as applied in our laboratory, was recently described in detail by Bowman et al. (2009) and Gomez et al. (2013).

The Y-maze is a hippocampal dependent – spatial memory task that requires rats to use external maze cues to navigate the identical internal arms. The Y-maze was chosen to reduce habituation time and provide a measure of spatial memory limiting stressful confounds such as food deprivation (radial arm maze) or forced swimming (water maze). The maze was constructed of wood coated with high-gloss black paint and placed in a room with high-

contrast external cues (bullseyes, checkerboards). The arms were (56 × 19 × 36 cm) connected at the center by an equilateral triangle with 60° angles. The floor was covered with cage bedding that was thoroughly mixed before every trial to reduce any potential odor cue trails (Conrad et al., 2003). During the training trial, one arm was blocked and the rat was allowed to freely explore the start and other arm for 15-minutes. During the 4-hour intertrial delay, the Y-maze was rotated 120° clockwise, to reduce possible marking effects (Conrad et al., 1996). Rotating the maze assure the rat is exploring the arms based on external cues presumably using spatial information to navigate the arms. After the delay, all arms were open and memory retention was assessed based on entries into the arm in the previously unexplored location (novel) compared to the other and start arms. An entry was designated as the rat crossing the threshold of the arm with both forepaws and half its torso. The novel, other, and start arms were counterbalanced between rats to reduce any possible confounding effects of place. All behavior was videotaped and analyzed off-line by an experimenter blind to the conditions.

The elevated plus maze (EPM) is designed to test general anxiety-related behaviors in rodents (Pellow et al., 1985) and consists of two open (50 × 12 cm) and two closed arms (50 × 12 × 40 cm). An entry was designated as the rat crossing the threshold of an arm with both forepaws and half its torso. Using a single trial, rats were placed in the center and allowed to explore for 5-minutes. All behavior was videotaped and scored off-line. Increased time or entries into open arms indicates decreased anxiety (Pellow et al., 1985).

The modified forced swim task (FST) is designed to measure general depressive-like behaviors in a rat model. Originally developed for mice by Porsolt et al. (1977), the FST measures swimming, immobile, and climbing behaviors with increases in immobility associated with increases in depressive-like symptoms. Rats were placed in a Plexiglas cylinder (30 cm diameter) filled with water (~25°C) to a level of 30 cm. Rats were tested for 5-minutes, and behavior was videotaped and scored off-line. Behaviors consisted of swimming (horizontal movement), climbing (upward thrashing), and immobility (erect floating). Behaviors were counted at 5-second intervals over the 5-minutes of testing for a total of 60 counts, and scores were converted into percentages and analyzed (Cryan et al., 2002).

Blood samples were taken from trunk blood 30–40 minutes after the forced swim task was complete. Samples were centrifuged at 3000g in 4°C for 5-minutes and sera was collected. Using a corticosterone ELISA kit (Neogen Corp., Lexington, KY), 100µl of plasma was dissolved in diethyl ether to separate the organic phase and allowed to evaporate for 48-hours. Extraction with diethyl ether allows for measurement of bound corticosterone. Corticosterone levels were expressed in ng/mL.

Statistics

An independent samples t-test was used to test BAC between the ALC and STR+ALC groups. Univariate ANOVAs with treatment, alcohol or stress, as fixed factor variables were used to analyze corticosterone, object recognition (T1), Y-maze (total entries), and the elevated plus maze. Repeated measures ANOVAs were used to analyze body weights, object recognition (T2), Y-maze (arm entries), and the forced swim task with post-hoc LSDs for significant findings. All statistical analyses were run on PASWStatistics v.18 (SPSS IBM, USA).

Results

Physiological Effects

Blood alcohol concentration was measured 30–40 minutes after the final alcohol gavage administration. No difference in BAC was found between groups [$t_{(14)}=0.70$, $p=0.49$], suggesting stress did not affect alcohol metabolism (Figure 2A).

Corticosterone was measured as a response to a novel stressor, forced swim, 30–40 minutes following the task. Univariate ANOVA found an effect of stress [$F_{(1,28)}=6.06$, $p=0.02$]. Post-hoc analysis found the ALC, STR, and STR+ALC groups had higher levels of corticosterone than the CON group [$p<0.04$] (Figure 2B), but there were no differences among the treatment groups.

Body weight, a physiological marker of chronic stress, was affected by treatments. Repeated measures ANOVA ($7\times 2\times 2$, day $\times$ drug $\times$ stress) found an interactive effect [$F_{(6,168)}=3.92$, $p=0.001$]. No difference among groups was found from days 1–3, but from days 4–7, rats in the CON group weighed more than those in the ALC, STR, or STR+ALC groups [$p<0.04$] (Figure 2C), but there were no differences among the treatment groups.

Working memory using object recognition

The object recognition task tests visual working memory. During the sample trial (T1), no significant difference was found in exploration of identical objects between groups [$F_{(3,28)}=1.48$, $p=0.24$] (Figure 3A). In the retention trial (T2), exploration times were compared between the old and new objects. Repeated measures ANOVA ($2\times 2\times 2$, object $\times$ drug $\times$ stress) found a significant interaction between alcohol and stress [$F_{(1,28)}=4.07$, $p=0.05$]. Post-hoc analysis found the CON and STR groups had greater exploration of the new object when compared to the old object [$p<0.02$]. This difference in object exploration was not seen in the ALC and STR+ALC groups, suggesting impaired memory attributed to alcohol treatment (Figure 3B).

Spatial memory using the Y-maze

The Y-maze assesses hippocampal dependent spatial memory by analyzing exploration of familiar or novel arm location(s). No significant difference in total entries was found between groups [$F_{(3,28)}=2.30$, $p=0.099$], suggesting treatment did not alter locomotion or motivation to explore (Figure 3C). Arm entries during the probe trial were analyzed between groups using a repeated measures ANOVA ($3\times 2\times 2$, arm $\times$ drug $\times$ stress). An arm by alcohol by stress interaction was found [$F_{(2,56)}=5.45$, $p=0.04$]. To examine the interaction post-hoc analyses tested the exploration of the novel arm compared to the other and start arms within groups. The CON group had greater exploration of the novel arm compared to the other and start arms [$p<0.03$] (Figure 3D). No difference in arm exploration was found in the ALC, STR, or STR+ALC groups, suggesting impairment in spatial memory linked to alcohol, stress, or the combined treatment.

Anxiety and Depression Measures

Open and closed arm exploration on the plus maze assessed anxiety-like behaviors. No differences in percent of open arm entries (Figure 4A) or percent of time spent in the open arms (Figure 4B) were found between groups [$p=0.99$; $p=0.46$, respectively]. Thus, no observable effects of stress, alcohol, or combination treatment were found on the elevated plus maze for anxiety-like behaviors.

To measure depressive-like symptoms, we assessed swimming, immobile, and climbing behaviors on the forced swim task. Behavioral counts were converted into percentages and

analyzed with a repeated measures ANOVA ($3 \times 2 \times 2$, behavior x drug x stress) (Figure 4C). There was an effect of behavior [$F_{(2,56)}=32.69$, $p=0.0001$], whereby, the swim was greater than immobile, which was greater than climbing behavior [$p<0.0001$]. There was a behavior by alcohol interaction [$F_{(2,56)}=7.33$, $p=0.001$] and each behavior was compared within groups. Post-hoc analysis found the STR+ALC group displayed less climbing than the CON and STR groups [$p<0.02$]. An increase in immobility was found in the ALC and STR+ALC groups compared to the CON and STR groups [$p<0.01$], suggesting an alcohol induced increase in depressive like behaviors. Swimming behavior was not altered by any treatment [$p>0.05$] (Figure 4C).

Discussion

The present experiment tested the physiological and behavioral effects of alcohol treatment given daily via gavage to female rats for 7 days immediately following a 6 h bout of restraint stress. Blood alcohol levels in subjects receiving treatment after the stress session were not different from subjects receiving alcohol alone, suggesting that stress did not alter alcohol metabolism. Thus, differences in physiology and behavior between the ALC and STR+ALC groups were most likely due to interactive effects and not to blunting or enhancing effects due to differences in metabolism. In response to the forced swim task, all groups elicited a stress response, but the ALC, STR, and STR+ALC groups had an elevated response compared to CON (i.e. a ~20% increase in serum CORT). The same pattern was reflected in body weight, where, by day-4 and continuing to day-7, the ALC, STR, and STR+ALC groups gained weight at a lower rate. As expected, working memory on the object recognition task was intact in the CON and STR groups (Bowman et al., 2009) but was impaired in the ALC and STR+ALC groups. In the Y-maze, only the CON group displayed intact spatial memory. Conversely, treatments had no effect on anxiety-like behavior on the elevated plus maze. Alcohol treatment increased immobility, a sign of depressive-like behavior, in the forced swim task; however, the response to the combination treatment was a complex interaction with the combined treatment group showing significantly more immobility than the stressed group but not the control group. It should be noted that the pattern of behavioral effects found may be due to the order of testing following treatments or time of testing following the treatments. These novel interactive effects between alcohol and stress in females are further discussed below.

First, alcohol concentration was confirmed in blood samples, and stress did not alter concentrations 30–40 minutes after administration. The blood alcohol levels attained here were comparable to a human female consuming about 3–5 drinks (dependent on weight). Studies have reported differences in blood alcohol concentration in response to an alcohol challenge between men and women (Breslin et al., 1994), but rats often show similar BACs between male and females (Savage et al., 2000; Wilson et al., 2004). These results suggest that the effects found in our study may be due to alcohol altering the effects of stress and not stress altering alcohol metabolism.

Corticosterone was measured after the forced swim as it is a novel stressor and could determine if the stress response was altered in rats exposed to stress and/or alcohol compared to controls. Females generally show an elevated CORT response to both stressors and alcohol consumption (Kant et al., 1983; Rivier, 1993), and non-stressed female rats generally have a mean daily CORT level of 50 ng/ml (Windle et al., 1998). Although we did not measure basal CORT levels to compare CORT levels within subjects, the levels of CORT were elevated in the experimental groups following the FST compared to the controls, showing that exposure to chronic alcohol, stress, or both may sensitize the stress response to a novel stressor. The significance of this finding needs further examination to determine if an increased CORT response may be associated with an increase in voluntary

alcohol consumption when subjects are exposed to a novel stressor. Additionally, a regular sampling of blood corticosterone levels would help to determine how treatments altered HPA function and how possible alterations in corticosterone might contribute to the behavioral differences.

Similar to the CORT response, body weight was altered by treatments, with stress or alcohol treated females as well as the combination showing a decrease in weight gain over the 7-days of treatment. The difference in body weight supports the findings of others that show a decrease in weight gain over periods of stress treatment (McLaughlin et al., 2010; Conrad et al., 2012; Huynh et al., 2011). The weight differences were only seen while treatments were administered. Rats were weighted immediately before the forced swim task and no differences were found between groups (data not shown). This result suggests a quick recovery from the physiological effects of chronic alcohol and/or stress. However, the effects on CORT levels and weight change do show that the treatments were effective in altering physiology. Thus, the fact that group differences were apparent for some tasks and not others, suggests that exposure to chronic alcohol and/or stress alters physiological and behavioral processes differently; however, effects seen in specific tasks may be dependent on timing of tests following stress cessation or on order of testing (see below).

Previous studies in our lab have shown that chronic stress has no effect on the object recognition (OR) task in female rats (Beck and Luine, 2002; Bowman et al., 2002; Bowman et al., 2009), a result which is shown in the present study; however, when alcohol was given after each stressor, memory was impaired on the OR task. Additionally, chronic treatment with alcohol alone also impaired OR memory, suggesting a non-specific cognitive impairing action of chronic alcohol exposure in female rats. With regards to stress effects on spatial memory in the Y-maze, others have found that female subjects show no effect or enhancements in performance following chronic stress (Conrad et al., 2004; McLaughlin et al., 2005). Our results did not support the previous findings between stress and Y-maze performance because memory was impaired for the STR group. Only the control group showed a preference and increased exploration of the novel arm, suggesting intact spatial memory. Differences between stress effects on the Y-maze in previous studies and the current one may be due to the shorter duration of stress treatment, 7-days, as compared to 21-days in previous experiments (Conrad et al., 2004; McLaughlin et al., 2005). McLaughlin et al. (2008) have also suggested that females habituate or lose interest in the Y-maze task quickly, implying that the task is better suited for assessing spatial memory in male as compared to female rats.

In the current experiment, the Y-maze was used because other spatial memory tasks require habituation so its use reduced the elapsed time between habituation and testing. However, tests such as the object placement task may be a more suitable spatial memory assessment to use with female subjects (review, Luine et al., 2007). We support results of studies that show impaired memory in female rats exposed to alcohol doses ranging from 0.5 – 2.0 g/kg/d over a period ranging from 6d – 6wks (Sircar et al., 2009; Ranney and Petro, 2009). It is unknown how the combination treatment compares to other studies, as most studies that combine alcohol and stress only test effects in male subjects (Ryabinin et al., 1995; Hansson et al., 2006). Nonetheless, the memory tasks, associated with the prefrontal cortex (OR) and hippocampus (Y-maze) were differentially altered by alcohol, stress, and their combination. Thus, it would be important to assess how the combination of alcohol and stress alter neural function in these areas. For the current study we did assess hippocampal neurotransmitter receptor levels of GABA α 4 and GluNR2B, but found no differences between groups (data not shown). The lack of difference may be due to the time lapse between final treatment and tissue collection (Kalluri et al., 1998; Narita et al., 2000). In a previous experiment with male rats, we found that tissue collected following the last treatment (no behavior) showed

between group differences in receptor expression (Gomez et al., 2013). Thus, future studies will aim to collect tissue at earlier time points to determine if females show similar neuronal changes following chronic alcohol and/or stress treatments.

A cornerstone of alcohol research involves the effects of consumption on anxiety and depression, and there are multiple findings (taken from below). A recent survey has shown that close to 20% of individuals self-medicate with alcohol to deal with anxiety disorders (Robinson et al., 2009). Also, repeated exposure to withdrawal from alcohol has been shown to increase anxiety-like behavior in male rodents (Overstreet et al., 2002), which provides insight as a possible reason of negative reinforcement to continue drinking in alcoholics. On the elevated plus maze, in both sexes, low to moderate doses of alcohol have anxiolytic-like effects and increase open arm exploration (Wilson et al. 2004). Chronic intake leads to increases in anxiety and depression, and effects are often greater in females than males (Weinberger et al., 2009). There are multiple findings on anxiety as pertaining to stress or alcohol. Immobilization and variable stress regimens show no change in anxiety on the plus maze in female rats (Mitra et al., 2005), while restraint stress increases anxiety (Huynh et al., 2011). Usually, only after chronic alcohol intake or during withdrawal, does anxiety increase (Kliethermes et al., 2004). Our results support studies showing no difference between control and alcohol treated groups. Withdrawal may have been a factor; however, the dose and duration used is believed to be insufficient to cause withdrawal or dependence (Becker and Lopez, 2004; Finn et al., 2007).

The forced swim task tested depressive-like behaviors, mainly by assessing the amount of immobility. Much like anxiety, comorbidity exists between depression and alcohol abuse in humans (Conner et al., 2009). Previous studies have shown an increase of depressive-like behaviors in male rodents when exposed to either chronic alcohol (Walker et al., 2010) or chronic stress (Qi et al., 2008). In the current study, female rats in the ALC group showed increased immobility compared to the CON and STR groups and alcohol treatment after the stressor did not alter immobility compared to alcohol alone. However, the STR+ALC group was not statistically different from the CON group. This result suggests the combination had an interactive effect that alleviated alcohol-induced increases in depressive-like behaviors compared to CON. The former result supports others who have found an increase in depressive-like behavior following alcohol consumption (Olivier et al., 2008; Walker et al., 2010). The stressor alone was apparently not sufficient for alter immobility. However, others who have used longer periods of stress (21-days) have seen increases in depressive-like behaviors (Xu et al., 2006; Qi et al., 2008). The behaviors in the forced swim task have been associated with changes in neurotransmitter levels. Compounds that mediate norepinephrine alter climbing behavior and those that mediate serotonin alter swimming behavior (Page et al., 1999). We found, as a whole, rats showed more swimming, however, because treatments did not alter behavior, it is difficult to determine the specific effects of alcohol or stress. Morphological and neurochemical investigation of the limbic areas associated with depression, such as the prefrontal cortex and hippocampus (Venzala et al., 2012; Li et al., 2009) may elucidate the observed effects of combining stress and alcohol when compared to alcohol alone. It must also be considered that females react differently to stress and alcohol, and our study suggests that chronic alcohol increases depressive like behaviors in females, an effect not seen in males (Gomez et al., 2013).

The current results should be viewed within the context that depression is widespread, and about 21 million American adults, or about 9.5 percent of the population aged 18 and older, experience a mood disorder in a given year (Kessler et al., 2005). Moreover, the lifetime prevalence of depressive disorders in women is approximately twice that of men (Steiner and Young, 2008). Consistent with these data, alcohol administration to females increased depressive-like behavior on the Porsolt test and the combination of alcohol and stress led to

greater depressive-like behavior than in the stressed group. In contrast, when given the same treatments, males did not show increased depressive-like behavior on the Porsolt (Gomez et al, 2013). Males and females did respond similarly to alcohol in the memory tests; both object recognition and spatial memory on the Y-maze was impaired by alcohol. However, when males received both stress and alcohol, the memory impairments were alleviated (Gomez et al, 2013), but memory in females remained impaired after the combination treatment. Thus, in the current study, the behavioral responses to chronic alcohol, stress, or their combination may help explain why more depressive phenotypes are found in females as compared to males. However, the considerable sex differences noted in response to alcohol need further verification because the sexes were not run in a concurrent experiment. A number of previous stress studies, which have run the sexes concurrently, have verified the remarkable sex differences in response to stress for anxiety and cognition (Haney et al, 1995; Beck and Luine, 2002; Conrad et al, 2003, 2004; Luine et al, 2007; Bowman et al, 2009; Bangassari et al, 2010). Thus, possible sex differences in response to alcohol and stress should be taken into account for understanding the progression of alcohol abuse in women versus men, the greater incidence of self-medication in males and also for understanding how stress and alcohol contribute to mood disorders.

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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.

Figure 1.
Time-line for experimental procedures. Each tick mark represents one day.

Figure 2.

Physiological Measures: (A) Blood alcohol concentration, no significant difference. (B) Corticosterone levels; rats in the ALC, STR, and STR+ALC group had higher levels than the CON group, asterisk $|*| p < 0.05$. (C) Body weights; the CON group gained weight at a greater rate than the other groups from days 4–7, asterisk $|*| p < 0.04$. Values presented as mean $\pm$ S.E.M.

Figure 3.

Memory Tests: (A) Object Recognition: Exploration time during the sample trial (T1). No significant differences between groups. (B) Object Recognition: Exploration time (T2) of old and new objects during the retention trial; the CON and STR group explored the new object more than the old, asterisk $|*| p < 0.02$. (C) Y-Maze: Total entries into all arms during the training trial. No significant differences between groups. (D) Y-Maze: Entries into each individual arm in the retention trial; a significant difference in arm exploration was found only in the CON group, asterisk $|*| p < 0.03$. Values presented as mean $\pm$ S.E.M.

Figure 4.

Anxiety and Depression tests: (A) Elevated Plus Maze: Percent of entries into the open arms; no significant differences. (B) Elevated Plus Maze: Percent of time spent in the open arms; no significant difference found. (C) Forced Swim Task: Difference in observed behaviors; immobile greater than climb, asterisk $|*| p < 0.0001$; swim greater than both immobile and climb, double asterisk $|**| p < 0.0001$. Within-behavior differences observed; Climbing – STR+ALC group less than CON and STR groups $|a| p < 0.02$; Immobile – ALC greater than CON and STR $|b| p < 0.01$ and STR+ALC only greater than STR $|c| p < 0.01$. Swim – No differences.