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Binge ethanol effects on prefrontal cortex neurons, spatial working memory and task-induced neuronal activation in male and female rats

Rebecca K. West[a], Mark E. Maynard[a,1], J. Leigh Leasure[a,b,*]

[a] Department of Psychology, University of Houston, Houston, TX 77204-5022, United States
[b] Department of Biology & Biochemistry, University of Houston, Houston, TX 77204-5022, United States

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ABSTRACT

Excessive alcohol intake is associated with a multitude of health risks, especially for women. Recent studies in animal models indicate that the female brain is more negatively affected by alcohol, compared to the male brain. Among other regions, excessive alcohol consumption damages the frontal cortex, an area important for many functions and decision making of daily life. The objective of the present study was to determine whether the medial prefrontal cortex (mPFC) in female rats is selectively vulnerable to alcohol-induced damage. In humans, loss of prefrontal grey matter resulting from heavy alcohol consumption has been documented, however this volume loss is not necessarily due to a decrease in the number of neurons. We therefore quantified both number and nuclear volume of mPFC neurons following binge alcohol, as well as performance and neuronal activation during a prefrontal-dependent behavioral task. Adult male and female Long-Evans rats were assigned to binge or control groups and exposed to ethanol using a well-established 4-day model of alcohol-induced neurodegeneration. Both males and females had significantly smaller average neuronal nuclei volumes than their respective control groups immediately following alcohol binge, but neither sex showed a decrease in neuron number. Binged rats of both sexes initially showed spatial working memory deficits. Although they eventually achieved control performance, binged rats of both sexes showed increased c-Fos labeling in the mPFC during rewarded alternation, suggesting decreased neural efficiency. Overall, our results substantiate prior evidence indicating that the frontal cortex is vulnerable to alcohol, but also indicate that sex-specific vulnerability to alcohol may be brain region-dependent.

1. Introduction

Excessive alcohol intake contributes to at least 30 different diseases affecting nearly all systems of the body [1]. Globally, among people ages 15–49, alcohol use is the leading risk factor for premature death and disability [2]. In addition to the well-known short-term health risks, excessive alcohol use can also lead to the development of serious problems such as heart disease, stroke, cancer, cognitive impairment, depression, anxiety, unemployment, social problems, alcohol dependence, and brain damage [3]. For women especially, there is an increased risk of negative consequences from alcohol consumption, including alcohol-related incident fatalities, heart disease, stroke, suicide, liver cirrhosis, and death [4]. Although women tend to consume less alcohol and have a shorter duration of intake than men, long term heavy consumption is more likely to harm a woman's health and, potentially, damage the brain [4]. Both human and animal studies suggest that females may be uniquely vulnerable to the harmful neurological effects of alcohol [5–12], although not all studies show this [13,14].

Using a rodent model of alcohol-induced neurodegeneration, we have recently shown sex differences in hippocampal damage and cognitive impairment [15]. Like the hippocampus, the medial prefrontal cortex (mPFC) is alcohol-vulnerable, and loss of prefrontal grey matter has been well-documented in human alcoholics [16–18]. Some volume recovery occurs with abstinence [19,20], and this combined with evidence showing that volume loss is not equivalent to neuronal loss [21,22], suggests that the primary issue is with cell shrinkage, not loss. In the present study, we used archived tissue from our study of sex differences in alcohol-induced hippocampal damage and quantified the number and volume of neuronal nuclei in the mPFC. The karyoplasmic ratio, or the ratio between cell body and nucleus volume, is maintained at a constant value within the cell [23–25]. Therefore, we hypothesized that binged female rats, but not male, would show a decrease in nuclear volume in the mPFC when compared to same-sex controls.

We have also recently shown that sex differences in hippocampal

* Corresponding author at: Department of Psychology, University of Houston, Houston, TX 77204, United States.
E-mail address: jlleasure@uh.edu (J.L. Leasure).
[1] Present address: Department of Neurobiology & Anatomy, University of Texas Health Science Center, PO Boxes 20,708, Houston, TX 77225–0708.

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damage after binge alcohol are associated with spatial learning impairment in female rats, but not males [15]. This finding prompted us to investigate whether binge alcohol would result in sex differences in a prefrontal cortex-mediated cognitive task. The frontal cortex is critical for a wide variety of tasks, including motor function, emotional processing, problem solving, spontaneity, memory, language, spatial orientation, judgment, impulse control, working memory, and social behavior [26–28]. To test for sex differences in mPFC functioning following alcohol exposure, we tested rewarded alternation (RA) performance with a short inter-trial interval to target working memory [29], in a second cohort of male and female rats. We predicted that binged females, but not males, would show impairment compared to same-sex controls.

Cognitive efficiency is another consideration when evaluating alcohol-related damage. Even in the absence of observed cognitive or behavioral deficits, brain damage may still have occurred, as is evident in other forms of brain injury like asymptomatic traumatic brain injury or stroke [30–32]. Increased frontal activation has been observed in human frequent binge drinkers during tests of working memory [33], cognitive control [34], and spatial attention [35], indicating the recruitment of more neuronal resources to achieve behavioral performance comparable to controls. The neural efficiency hypothesis is that more intelligent individuals require less neural activation to achieve the same performance outcome [36,37]. Following this line of logic, increased neuronal activation during a cognitive task would be indicative of decreased efficiency. In the present study, we analyzed activation of mPFC neurons in animals that underwent the RA task. It was predicted that binged females, but not males, would show increased c-Fos expression compared to control animals during the rewarded alternation test, indicating decreased cognitive efficiency resulting from binge alcohol.

2. Material and methods

2.1. Animals

All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal protocol was approved by the Institutional Animal Care and Use Committee of the University of Houston (#14–013). To assess mPFC neuron number and volume directly after binge alcohol, archived tissue from 27 male and female rats (8 female binge, 6 female control, 7 male binge, 6 male control) from a prior study [15] was used (Cohort 1). To probe for sex differences in binge alcohol effects on spatial working memory, mPFC neuronal number and volume and task-induced activation, 39 male and female rats (9 female binge, 10 female control, 10 male binge, 10 male control) were used (Cohort 2). Except where noted, procedures were identical between the cohorts.

Upon arrival (from Harlan Sprague Dawley, Indianapolis, IN, USA) at 8 weeks of age, rats spent one week acclimating to vivarium conditions, which included same-sex housing (2–3 per cage) in clear Plexiglas cages, ad libitum rat chow and water and a reversed light/dark cycle (lights off at 9:00/ on at 21:00). During the acclimation period, rats were handled to make them accustomed to the experimenters. Next, rats were randomly assigned to the following groups: male control, male binge, female control and female binge. Rats then underwent 4 days of binge alcohol (or control diet) and were either sacrificed the day the binge ended (Cohort 1), or following 4 days of abstinence and 5 days of rewarded alternation testing (Cohort 2).

2.2. Binge ethanol administration

Food was removed from both control and experimental groups prior to the first dose of ethanol or control solution and replaced after the final dose, although water was always available. Animals received either the ethanol diet (25% ethanol w/v in nutritionally complete diet; vanilla Ensure Plus™) or isocaloric control diet (dextrose in vanilla Ensure Plus™) given every 8 h over 4 days via intragastric gavage, according to a paradigm modified from Majchrowicz [38] as previously described [8,39,40].

The initial loading dose of ethanol diet was 5 g/kg. Thereafter, animals were dosed according to their behavioral intoxication, based on the Majchrowicz scale (0, normal rat; 1, hypoactive; 2, ataxia; 3, ataxia with dragging abdomen and/or delayed righting reflex; 4, loss of righting reflex; 5, loss of eye blink reflex). Each of these scores has an accompanying dose of ethanol between 0 and 5 g/kg, such that a higher score (higher observed behavioral intoxication) corresponds to a lower dose. Behavioral intoxication was assessed immediately before each dose of alcohol was administered. This method maintains consistent intoxication relevant to an alcohol use disorder (AUD) while avoiding mortality [39,41]. Control animals were given the average volume of fluid that their same-sex alcohol group received for each dose. To determine blood ethanol concentration (BEC), blood was drawn from the lateral saphenous vein 90 min following the seventh dose of ethanol. Samples were immediately centrifuged, then extracted serum was stored at −80 °C until analysis. Samples were analyzed using an AM1 Analyzer (Analox, MA, USA), based on external standards.

Some evidence suggests that female rats may have increased ethanol sensitivity during certain phases of the estrous cycle [42,43]. Thus, in order to control for this variability in animals undergoing cognitive testing, all female rats in Cohort 2 received their first dose of control or alcohol diet when they were in the diestrus stage of their cycle. Vaginal smears of both female binge and control groups were taken between 8:00 and 9:00 daily, beginning 5–7 days prior to the first alcohol or control dose. Each sample was placed on a glass slide, stained with cresyl violet, and then coverslipped. Stage of estrous was determined by examining samples under a light microscope at 10× magnification.

2.3. Withdrawal

Because Cohort 2 was sacrificed 9 days post-binge, spontaneous withdrawal symptoms were monitored every 30 min for hours 10–26 after the last dose, which encompasses the peak withdrawal period [38]. Every 30 min, the most severe observed withdrawal symptom was scored using the scale created by Penland and colleagues [44]. Red lights were used during the dark phase of the light cycle so as not to disrupt the animals' circadian rhythms.

2.4. Spatial working memory task

The rewarded alternation (RA) task relies on rodents' natural inclination to alternate areas of exploration in a new environment and rewards them for correctly alternating choice arms. Working memory, a prefrontal cortex dependent function, is engaged by using a short inter-trial interval (ITI), as rats must recall which arm they most recently visited to obtain access to the food pellet reward [29,45]. Prior to the 4-day binge, all rats were habituated in home cage groups to the T-maze, which is an apparatus made of clear Plexiglas, with three guillotine doors secured in metal brackets which can be raised and lowered by the experimenter. During habituation, all doors were raised and unlimited food reward pellets (chocolate 45 mg Dustless Precision Pellets®, Bio-Serv, NJ, USA) were present in both food dishes located at the end of each choice arm. Also during this time, food pellets were placed into each home cage so rats could become accustomed to searching out pellets in a familiar environment. Throughout behavior testing, rats were food-restricted so that they were adequately motivated to seek out the food reward. Because all rats (controls and binged) lose body weight (9–20%) during the 4-day binge, afterwards, each animal was either food restricted or fed ad libitum to reach 85–90% of their pre-binge body weight by the start of the spatial working memory task. During behavior testing, each rat was weighed daily and given food