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MODERATE DRINKING? ALCOHOL CONSUMPTION SIGNIFICANTLY DECREASES NEUROGENESIS IN THE ADULT HIPPOCAMPUS

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Abstract—Drinking alcohol in moderation is often considered a health-conscious behavior, associated with improved cardiovascular and brain health. However, ''moderate'' amounts of alcohol include drinking 3–4 alcohol beverages in a day, which is closer to binge drinking and may do more harm than good. Here we examined how daily drinking of moderate-high alcohol alters the production of new neurons in the adult hippocampus. Male and female adult Sprague–Dawley rats were provided free access to a liquid replacement diet that was supplemented with either 4% ethanol or Maltodextrin for a period of 2 weeks. Proliferating cells were labeled with 5-bromo-2-deoxyuridine (BrdU) and the number of BrdU-positive cells in the hippocampus was assessed after the final day of drinking. A subset of rats was also exposed to a motor skill or associative learning task to examine the functional effects of alcohol consumption. The drinking regime resulted in an average blood alcohol concentration of approximately 0.08%, which is comparable to the human legal driving limit in many countries. This level of intoxication did not impair motor skill learning or function in either sex, nor did the alcohol consumption disrupt associative learning 2 days after drinking. Therefore, moderate alcohol consumption did not disrupt basic sensory, motor or learning processes. However, the number of cells produced in the dentate gyrus of the hippocampus was reduced by nearly 40%. Thus, even moderate consumption of alcohol for a relatively short period of time can have profound effects on structural plasticity in the adult brain.

Key words: alcohol, adult neurogenesis, trace eyeblink conditioning, BrdU, hippocampus, sex differences.

INTRODUCTION

Light-moderate alcohol consumption is associated with improved cardiovascular and brain health (Ngandu et al., 2007; Ronksley et al., 2011). As such, some guidelines for health and wellness advocate drinking one or two alcoholic beverages each day. Conversely, it is widely accepted that large amounts of alcohol are detrimental to our health. This information has been gleaned, in part, from animal studies, which tend to focus on the consumption of large amounts of alcohol in models of addiction and binge drinking. Still, many individuals are more likely to consume alcohol at a level and frequency between the two categories of ''light/moderate'' and ''chronic binge drinking''. Moderate drinking defined as having as many as 3–4 drinks per day and no more than 7–14 drinks per week (depending on your sex) is generally socially acceptable and considered safe. Some research even suggests that moderate levels of alcohol intake are beneficial to our health. However, the definition of ''moderate consumption'' varies greatly across social circles, cultures, and most importantly, scientific literature. The reported effects of moderate consumption on brain and mental health vary as much as does the definition. For example, moderate alcohol consumption in middle-aged men and women is associated with improved cognitive function later in life (Ngandu et al., 2007), but also correlates negatively with brain volume in both sexes (Verbaten, 2009). Thus, it is not clear whether moderate drinking is beneficial or harmful to overall brain health and function.

Some of the effects of alcohol on brain health depend on sex/gender differences. For example some studies report a connection between alcohol consumption and cognitive performance in women but not in men (Dufouil et al., 1997; Edelstein et al., 1998; Au Yeung et al., 2012). Stampfer et al. (2005) found that elderly women drinking up to one portion of alcohol per day outperformed nondrinkers in cognitive tasks and also had a reduced risk for cognitive decline. In a similar manner, Dufouil et al. (1997) reported a positive correlation between alcohol consumption and cognitive performance in elderly women but not in men. Moderate alcohol intake in elderly women has also been associated with a 50% reduced risk of dementia (Espeland et al., 2005). On the contrary, yet another study reported a negative correlation between alcohol consumption and memory performance specifically in women (Edelstein et al., 1998). In summary, alcohol differentially affects males and females, but whether the effects are positive or negative remains unclear, particularly in females.

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Abbreviations: ANOVA, analysis of variance; BAC, blood alcohol concentration; BrdU, 5-bromo-2-deoxyuridine; CR, conditioned response; CS, conditioned stimulus; EMG, electromyography/electromyographic; GCL, granule cell layer; US, unconditioned stimulus.

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A crucial process in maintaining brain plasticity and healthy cognitive function is adult neurogenesis (Shors et al., 2012; Curlik and Shors, in press). New neurons are produced in the hippocampus of the adult mammalian brain throughout the entire lifespan. The production of new neurons is sensitive to environmental influences and the ingestion of chemicals (van Praag et al., 1999; Glenn et al., 2007; Hodes et al., 2009; Leuner et al., 2010). In turn, adult neurogenesis is crucial for certain types of learning, such as trace conditioning in which two events, separated in time, have to be associated (Shors et al., 2001; Nokia et al., in press). Moreover, in the adult brain, most new neurons die unless the individual is exposed to an effortful but successful learning experience (Curlik and Shors, 2011). Whereas convincing reports of deleterious effects of consuming large amounts of alcohol (i.e. binge drinking) on adult neurogenesis exist (Nixon and Crews, 2002; He et al., 2005), the effects of moderate consumption have not been studied to a large extent, particularly with regard to sex differences.

In the present study, we aimed to clarify the effects of moderate alcohol consumption on hippocampal neurogenesis and learning. Male and female rats consumed alcohol via a liquid diet for 14 days according to a regime that resulted in blood alcohol levels approximate to the legal driving limit in many countries including the United States, the United Kingdom and Canada (0.08%). The consequences for neurogenesis, motor skill learning, and associative learning were determined.

EXPERIMENTAL PROCEDURES

Subjects

Male and female Sprague–Dawley rats were bred at Rutgers University in the Department of Psychology. At 28 days after birth, animals were housed in groups of 2–3 for males and 2–4 for females in a plastic box style cage (44.5 cm long × 21.59 cm wide × 23.32 cm high) and kept on a 12-h light–dark cycle, with the lights turning on at 7 am. Before and after the experiment, animals were provided free access to water and solid food pellets. All experiments were conducted with full compliance with the rules and regulation specified by the PHS Policy on Humane Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals.

Diet

Adult rats (60 days or older) were handled, singly housed and randomly assigned to either an alcohol diet or non-alcohol diet. Animals assigned to the alcohol-drinking group were provided alcohol through a liquid diet for 2 weeks. The alcohol diet (approximately 4% w/v) was mixed in batches that contained 132.2 g of powder diet (Premix for Fisher's Suspendable Rat Diets, Research Diets, Inc., New Brunswick, NJ, USA), 45.3 g of 100% ethanol, and 823 g of water, which supplied 848 kcal per 1 kg consumed. An iso-volumetric and iso-caloric non-alcohol liquid diet containing 132.2 g of the same powder diet, 80 g of fine ground Maltrose Dextrin (Research Diets, Inc., New Brunswick, NJ, USA), and 788 g of water per batch was given to the no-alcohol-drinking group. Both diets were mixed and blended 1–3 days in advance, however, the alcohol was added fresh daily to prevent fermentation. Diets were mixed in a blender again immediately before administering it to the animals. Animals were given free access to approximately 150 ml of their assigned diet in a plastic bottle with a rubber stopper with ball bearing tipped sippers at 10 am (light portion of the cycle) daily. The sipper bottles containing the diet were weighed (total weight including the bottle, the diet and the sipper top) before and after to calculate the daily mass consumed for each animal. Consumption for 37 animals (male no alcohol $n = 7$, male alcohol $n = 7$, female no alcohol $n = 11$, female alcohol $n = 12$) in experiment 1 and 28 animals (female no alcohol $n = 14$; female alcohol $n = 14$) for experiment 2 was obtained and used in the analysis. Animals received all nutritional needs through this diet. Animals were weighed before starting the diet and twice more, 1 and 2 weeks after starting the liquid diet. Consumption was expressed as grams of the diet consumed per kilogram of the average body weight. The overall health of the animals was also monitored. Animals ($n = 4$) that did not acclimate well to the liquid diet were immediately removed from the study and returned to solid food. Overall, animals did not experience significant changes in weight or overall health during these experiments. Rats were provided with a surplus of diet each day. In a few instances (less than five), all of the diet given was consumed in 1 day.

BrdU and immunohistochemistry

In experiment 1, the effect of alcohol on the number of new cells produced in the hippocampus was assessed. All 37 rats were injected intraperitoneally with 5-bromo-2-deoxyuridine (BrdU, 200 mg/kg, Sigma, Atlanta, GA, USA) in physiological saline solution 1 week after the onset of liquid diet consumption. BrdU is a thymidine analog that incorporates into the DNA of a dividing cell during the S-phase of the cell cycle and thereby marks cells that are actively proliferating at the time of the injection. Animals were sacrificed 1 week after the BrdU injection, at the end of the liquid diet regime. With this timeline, new neurons labeled with BrdU were born under the influence of alcohol and then exposed to another week of alcohol before the rats were euthanized. Rats were deeply anesthetized with sodium pentobarbital (100 mg/kg) and intracardially perfused with 4% paraformaldehyde, in 0.1 M phosphate buffer, to preserve the tissue structure. Trunk blood was collected from animals immediately before the perfusion. Brains were extracted and fixed in 4% paraformaldehyde for 24 h and then transferred to 0.1 M PBS until sectioning.

A vibratome was used to obtain 40-μm coronal sections through an entire extent of the hippocampus in one hemisphere. The hemisphere used was counterbalanced in all groups. Using one hemisphere is standard practice in our laboratory and no differences have been observed in neurogenesis in animals where samples were taken from the left versus right hemisphere (Dalla et al., 2007; Anderson et al., 2011). Tissue from one animal in the "male alcohol" group was lost due to excessive damage during sectioning, and therefore, not included in the BrdU analysis. Every 12th slide was placed onto a superfrost glass slide (Fisher, Suwanne, GA, USA) and allowed to air dry. Once dry, slides were stained with immunoperoxidase staining to visualize the cells that incorporated BrdU as described previously (Anderson et al., 2011). In summary, the tissue was pretreated with a heated 0.1 M citric acid solution (pH 6.0) for 15 min, a trypsin solution for 10 min and finally 2 N HCl for 30 min. Slides were kept overnight at 4 °C in primary mouse anti-BrdU (1:200, Becton–Dickinson, Franklin Lakes, NJ, USA), then biotinylated anti-mouse antibody (1:200, Vector Labs, Burlingame, CA, USA) for 1 h, followed by avidin–biotin–horseradish peroxidase (1:100,