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Review

Sex and Age Effects on Neurobehavioral Toxicity Induced by Binge Alcohol

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Abstract. Historically, most alcohol neurotoxicity studies were conducted in young adult males and focused on chronic intake. There has been a shift towards studying the effects of alcohol on the adolescent brain, due to alcohol consumption during this formative period disrupting the brain's developmental trajectory. Because the most typical pattern of adolescent alcohol intake is heavy episodic (binge) drinking, there has also been a shift towards the study of binge alcohol-induced neurobehavioral toxicity. It has thus become apparent that binge alcohol damages the adolescent brain and there is increasing attention to sex-dependent effects. Significant knowledge gaps remain in our understanding of the effects of binge alcohol on the female brain, however. Moreover, it is unsettling that population-level studies indicate that the prevalence of binge drinking is increasing among American women, particularly those in older age groups. Although study of adolescents has made it apparent that binge alcohol disrupts ongoing brain maturational processes, we know almost nothing about how it impacts the aging brain, as studies of its effects on the aged brain are relatively scarce, and the study of sex-dependent effects is just beginning. Given the rapidly increasing population of older Americans, it is crucial that studies address age-dependent effects of binge alcohol, and given the increase in binge drinking in older women who are at higher risk for cognitive decline relative to men, studies must encompass both sexes. Because adolescence and older age are both characterized by age-typical brain changes, and because binge drinking is the most common pattern of alcohol intake in both age groups, the knowledge that we have amassed on binge alcohol effects on the adolescent brain can inform our study of its effects on the aging brain. In this review, we therefore cover the current state of knowledge of sex and age-dependent effects of binge alcohol, as well as statistical and methodological considerations for studies aimed at addressing them.

Keywords: Sex differences, adolescence, aging, neuroplasticity, hippocampus, cortex, neurodegeneration

INTRODUCTION

Excessive alcohol consumption has a long and documented history of eroding human health by damaging all organs, including the brain. Long-term heavy consumption is linked to brain atrophy [1–3], particularly in the frontal lobe, hippocampus and cerebellum [1, 4–6], with accompanying cognitive impairments including alcohol-related dementia and Wernicke-Korsakoff's syndrome [1, 7]. Sex differences in the neural and cognitive effects of chronic alcohol consumption have long been of interest, and excellent reviews have been published on this topic [8–10]. It is becoming increasingly apparent, however, that heavy alcohol intake need not be long-term in order to be brain-damaging, and therefore this review addresses neurotoxicity induced by a binge pattern of alcohol consumption.

Binge drinking by definition involves sporadic episodes in which a large number of drinks are consumed in a short period of time. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) defines binge drinking as bringing the blood alcohol concentration (BAC) to 0.08 g/dL or above. Defined

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Table 1
Drinking Patterns and Definitions

Drinking Pattern	Youth Girls	Youth Boys	Adult Women	Adult Men	Older Adults
Moderate			1 drink/d	2 drinks/d	1 drink/d
Heavy			**>8 drinks/wk**	**>15 drinks/wk**	
			≥5 binges/mo	≥5 binges/mo	
Binge episode	≥3 drinks/2 hr	≥3–5 drinks/2 hr	≥4 drinks/2 hr	≥5 drinks/2 hr	≥**4 drinks/2 hr** (17)

Definitions/guidelines in black were found at www.niaaa.nih.gov; those in bold were found at www.cdc.gov or in the referenced publication.

in terms of drinks, this involves consumption of 4+ (women) or 5+ (men) alcoholic drinks in a 2-hour period [11]. Binge drinking is becoming increasingly common in the United States, with one in six Americans reporting that they binge drink [12]. Frequency and intensity of binge episodes are high - a recent study indicates that binge drinking Americans do so once weekly, 7 drinks per binge [13]. There is, however, increasing data attesting to binge episodes with much higher consumption (15+ drinks) among adolescents and adults in their twenties, with reported frequencies ranging from within the past year to every two weeks [14]. Regardless of the number of drinks consumed per episode, binge pattern drinking is clearly distinguishable from moderate drinking, defined as no more than 1 drink per day for women and 2 for men. Although this recommendation is put forth by NIAAA, these guidelines are from the *2015-2020 Dietary Guidelines for Americans* [15], published jointly every 5 years by the US Department of Health and Human Services and the US Department of Agriculture. Notably, at the time of this writing, the 2020-2025 guidelines are being drafted and the recommendation for daily moderate intake for men is likely to decrease to no more than 1 per day. Relevant to the current topic of sex differences, this would mean that there are no longer gender-based guidelines for moderate drinking. Moreover, as NIAAA and the Substance Abuse and Mental Health Services Administration (SAMHSA) recommend that adults over age 65 drink no more than 1 drink per day (https://pubs.niaaa.nih.gov/publications/arh26-4/308-315.htm), there would also be no age-specific guidelines.

With respect to binge pattern intake, age and sex both factor into the definition. For example, a recent NIAAA publication notes that the number of drinks required to raise the BAC above. 08 g/dL in the youth population is lower than that for adults [16]. To date, NIAAA does not have a specific binge definition for older adults, although a 1998 publication from SAMHSA provides 4 or more drinks per episode as a definition of binge intake in older adults of both sexes [17]. Table 1 delineates current definitions and guidelines disseminated by NIAAA and other government agencies for different patterns of alcohol intake.

The discrete epidoses of high alcohol intake interspersed with abstinence that characterize binge drinking prevent physiological adaptations [18], with the result that most binge drinkers are not dependent [19]. The brain is unable, therefore, to buffer itself against the high BAC achieved by binge consumption. In both human neuroimaging studies and animal models, binge alcohol has been shown to damage regions of the brain essential for executive functioning, learning and memory, including the hippocampus and cortex [20–38]. Animal models have revealed much information regarding the mechanisms underlying binge damage [24, 39] and also recovery, particularly with respect to the hippocampus [40], but most of these studies have been conducted in males. Fortunately, sex-dependent effects of binge alcohol are a growing focus of both preclinical and clinical research, because binge drinking is increasing among women [41, 42]. Moreover, sex is a key factor for the development of personalized medicine, and the study of sex-dependent alcohol neurotoxicity has generated concrete targets for sex-specific approaches to treatment of alcohol use disorder (AUD) [43]. In this review, we cover the current state of knowledge on sex differences in the brain effects of binge alcohol.

We also cover the current state of knowledge on age-dependent effects of alcohol, as its neural effects differ across the lifespan [44], making age another key factor for personalized AUD treatment. Furthermore, with respect to binge alcohol, it has been shown that the underlying mechanisms vary by age, with adolescents more severely affected compared to adults [45–47]. Comparably little attention has been devoted to the effects of binge alcohol on the aged brain, however, so although the question of whether alcohol prematurely ages the brain is an old one [3, 48], it remains open. Aging itself is associated with

brain decline, although this is modifiable by lifestyle factors [49], and binge drinking is one such lifestyle factor, yet we know little about the effects of binge alcohol on the aging brain. This is perhaps because binge drinking has traditionally been associated with younger age groups, although a recent study of binge drinking in the United States reported several findings that run counter to that idea. First, although the prevalence was highest in 18–35 year olds, over half the binge drinks consumed in the United States in 2015 was consumed by people over the age of 35. Secondly, although the prevalence of binge drinking was lowest in the 65+ age group, binge drinkers in that age group binged more frequently than did those in any other age group [13]. It is therefore imperative that we better understand how binge alcohol impacts neuroplasticity in the aging brain. Moreover, it is critical that we investigate the interactive effects of age and sex. In a recent meta-analysis of alcohol use data from 6 national surveys (2000-2016) Grucza and colleagues examined past year prevalence of 1+ binge drinking episodes. Their results showed an increase among women, and in age groups 30 and above, with the most marked increase occurring in those 50 and older [50], consistent with other results showing an increase in binge drinking among older women [42, 51]. In a similar vein, a review of national survey data since 2008 showed that the gender gap in alcohol use has been narrowing in middle-aged and older adults because its incidence has been remaining stable in men, but increasing among women [41].

The increasing prevalence of binge pattern drinking has made apparent the need to investigate its potentially deleterious neural consequences. In this review, we therefore focus on binge alcohol and cover the existing evidence that its behavioral, structural and neurochemical targets vary by sex and/or age. Throughout, we identify knowledge gaps in our understanding of sex and age-dependent neural effects of binge alcohol. We conclude with statistical and methodological considerations relevant to studies aimed at addressing them.

THE NEUROBEHAVIORAL EFFECTS OF BINGE ALCOHOL VARY BY SEX AND AGE

Behavioral and functional effects

Binge alcohol effects have been studied most extensively in adolescents (for a recent comprehensive review see [52]), as binge pattern drinking is most prevalent in this age group, and because exposure to alcohol's neurotoxic effects alters brain development. Adolescents naturally undergo characteristic behavioral changes that complicate detection of the impact of binge alcohol [53], yet general patterns of neurobehavioral changes have been identified. For example, adolescent binge drinkers exhibit impaired semantic recall compared to non-drinkers, which matches the evidence showing reduced hippocampi among binge groups [54]. Changes in prefrontal cortex (PFC) have been broadly observed in adolescent binge drinkers, and substantial deficits in spatial working memory [55, 56] and executive functioning [57, 58] are evident if drinking persists in adulthood [59]. Similarly, preclinical rodent models show that binge doses of alcohol during adolescence impair PFC and hippocampus-dependent functions in young adulthood [60–63].

Studying sex-dependent effects of binge alcohol is made difficult by inherent sex differences in drinking behaviors between males and females. One way to circumvent this problem is to compare binge drinkers to same-sex non-drinkers, rather than comparing the sexes directly. In doing so, patterns of sex-specific pathologies can be detected. When looking at young college binge drinkers, Parada et al. reported that male binge drinkers performed worse on a working memory task than male controls and female binge drinkers [58]. Yet, in declarative memory tasks, they demonstrated in a similar group of subjects that there were no sex differences when subjects were challenged [53]. In a test of spatial working memory, Squeglia et al. found that female binge drinkers exhibited substantial deficits compared to same sex non-drinkers whereas male binge drinkers performed better than non-drinking males [55]. Thus, adolescents and young adults of both sexes seem to have some aspect of working memory impairments from binge drinking while damage to the hippocampus from adolescent binge drinking does not produce demonstrable long term memory impairments. In preclinical models, repeated binge alcohol treatments during adolescence has been shown to have lasting effects in adulthood [52]. Several studies have shown that binge alcohol during adolescence is associated with impairments in spatial working memory during young adulthood [64–66]. Most studies in preclinical models have used males, so it is still largely unknown if females are similarly affected. Only recently have efforts into examining cognitive impairment in male and female subjects in preclinical models begun. In a long term memory task, both male and female rats

exhibited recognition memory impairments in adulthood when treated with chronic intermittent alcohol during adolescence [61].

In addition to effects on cognition, neural activation in frontal cortex, hippocampus and striatum have been shown to be affected by adolescent exposure to binge alcohol. In recent years, it has also become evident that alcohol exposure during adolescence can affect neural pathway activation differently in male and female subjects. In human studies, functional MRI evidence shows that adolescent female binge drinkers exhibit decreased activation in frontal cortex and hippocampus compared to controls; a phenotype not observed in adolescent male binge drinkers [55]. However, PET scans showed lower dopamine release in the ventral striatum of male but not female adolescent binge drinkers [67]. Although some studies have shown main effects of alcohol in adolescent amygdala-orbital frontal cortex connectivity and parietal cortex activation, these studies did not reveal a sex-specific change [68, 69]. In adolescent rodent models, similar functional consequences of alcohol exposure are observed in frontal cortical and hippocampal brain regions. Acute doses of alcohol in adolescent male rats lead to alterations in prefrontal and parietal cortex electrophysiology [70–73]. Alterations in the γ-aminobutyric acid (GABA) inhibitory signaling could help explain alcohol's effects on these brain regions [74]. Moreover, recent studies have shown that repeated binge alcohol treatment has long term effects on hypothalamic-pituitary-adrenal (HPA) axis activation [75, 76]. More functional studies are needed to better understand the effects of binge alcohol on male and female adolescent brains.

Alcohol use can significantly influence affective behaviors in adolescent drinkers, and these changes persist into young adulthood and possibly beyond [77]. Rodent models of social interaction have enabled the study of sex-dependent effects of adolescent binge alcohol on social behaviors in adulthood. Binge alcohol during adolescence increased social anxiety-like behavior in adulthood in males, but not females. Interestingly, this effect was also age-dependent, as the adulthood deficits were only seen when alcohol was administered between postnatal days 25–45 (early adolescence), but not postnatal days 45–65 (late adolescence). Binge exposure during both time periods, however, was associated with changes in social behavior as a function of acute alcohol exposure in adulthood, but again, only in males [78]. Age and sex-dependent effects were also found in adulthood on other measures, with both males and females showing anxiety-like behavior in the elevated plus maze when exposed to alcohol early, but only males affected when exposed to repeated binge alcohol later in adulthood [79]. Moreover, social anxiety-like behaviors and impairment in behavioral flexibility were seen only in late-exposed males.

While much remains to be done, it is clear from human and rodent studies that bingeing during adolescence disrupts ongoing brain maturational processes in a sex-dependent manner. Studies of sex-dependent effects of binge alcohol in adulthood are less common than studies of its effects in adolescence, although hippocampus-dependent and frontal cortex-dependent functions have been examined. In the standard Morris water maze task, a classic test of hippocampus-dependent spatial navigation, females, but not males, showed a slight but significant acquisition deficit after binge exposure [30]. This difference was also associated with increased hippocampal damage in females (see next section). As the frontal lobes are also vulnerable to binge alcohol damage, we compared the performance of males and females in a rewarded alternation task, beginning 4 days into abstinence after a single 4-day binge. We found that both males and females had fewer correct trials compared to same-sex controls, indicating a working memory impairment in both sexes [80]. The absence of sex differences may be due to aspects of the task itself. It may also relate to the comparative resilience of the fully formed adult brain, which may make sex-dependent effects of binge alcohol more difficult to detect in adulthood. Given the deterioration that occurs even with healthy aging, older brains may, like juvenile brains, be comparably vulnerable to the effects of binge alcohol.

Compared to the surfeit of work addressing alcohol effects on the adolescent brain, however, few studies have addressed the other end of the lifespan and very few studies to date have included females (Table 2). Furthermore, the NIAAA has yet to set binge drinking guidelines for older adults and considering 40% of this population consumes alcohol, more research into the effects of risky drinking is warranted. Factors like body mass reduction and comorbities will likely play a role in determining these guidelines for older adult drinkers. There are some studies that have examined acute alcohol effects in older adults that do not have AUD. Compared to young adults, older adults show impairments in psychomotor, set-shifting and work-

Table 2
Studies examining neurobehavioral effects of alcohol in aged subjects

Subjects	Age	Assessments	Major findings	Authors
Humans F & M	age-matched placebo vs young drinkers (25–31 yr) vs older drinkers (55–70 yr)	Working memory: Trail Making, face/scene recognition	Working memory deficits in older drinkers. Not sex-dependent.	Boissoneault J. et al., 2014 [81].
Humans F & M	age-matched placebo vs older drinkers (55–70 yr)	Working memory: Trail Making, face/scene recognition	Alcohol improved working memory. Not sex-dependent.	Hoffman LA. et al., 2015 [82].
Humans F & M	AUD subjects (mean age 48 yrs) vs age-matched controls	MRI	Alcohol accelerated aging in frontal cortical regions. Not sex-dependent.	Sullivan EV.et al., 2018 [87].
Humans F & M	AUD subjects (mean age 53 yrs) vs age-matched controls	MRI, working memory tasks, executive, functioning, visiospatial tests	Alcohol accelerated aging in hippocampal Ammon's horn. Not sex-dependent.	Zahr NM. et al., 2019 [88].
Sprague Dawley rats M	adolescent (30–43 d), adult (58–120 d), older adults (∼19 mo)	Open field, righting reflex test 30 min after acute i.p. injection, *in vivo* recording, western blotting	Increased alcohol sensitivity in older rats with depressed Purkinje neuron firing and increased PKCγ expression in cerebellum.	Van Skike CEV. et al., 2010 [83].
Sprague Dawley rats M	adolescent (28–30 d), adult (70 d), aged (∼18 mo)	Aerial righting reflex, rotarod, LORR	Alcohol impaired motor reflexes in aged rats. Not Sex-dependent.	Ornelas LC. et al., 2015 [85].
Fischer rats F & M	adult (4–5 mo), aged (19–20 mo)	Social Interaction, LORR	Social investigation increased in older F but decreased in older M with alcohol. Increased LORR in aged rats.	Perkins AE. et al., 2018 [86].
Fischer rats F & M	adult (2–3 mo), aged (18–24 mo)	LORR, cytokine RNA analysis, plasma hormones	Alcohol increased LORR and IL-6 and IL-β in hippocampus with age; Not sex-dependent. Increased Cort and PROG in females with ethanol.	Gano A. et al., 2017 [89].
C57Bl/6N mice F & M	adult (2–3 mo), middle-aged (15 mo), aged (18+ mo)	Iba-1 densitometry/counts	Alcohol and age decreased microglial density in hippocampi. Not sex-dependent.	Grifasi IR. et al., 2019 [90].

ing memory tasks in response to a moderate dose (0.05 g/dL BAC) of alcohol [81], although a subsequent similar study showed that a low dose of alcohol enhanced working memory in older adults compared to placebo [82]. The effects of acute alcohol have been studied in aged animals; aged male rats perform similarly to adolescents and adults at baseline on motor tasks, but are more severely affected by acute ethanol injection, despite similar blood ethanol concentration (BEC) [83, 84]. A similar pattern of results was obtained on a spatial learning task, again with no BEC differences between age groups [84]. Older animals are also more susceptible to the sedating properties of alcohol, as they slept longer than adolescent and adult rats after a 3 g/kg intraperitoneal injection (i.p.) [85]. One of the few studies to address sex differences in alcohol effects in older animals looked at social interaction, as well as ethanol sensitivity as a function of age and sex, by examining latency and duration of the loss of righting reflex (LORR) following i.p. injection of ethanol [86]. In adult males and females, injection of 0.5 or 0.75 g/kg of ethanol decreased social behavior. This was also the case in aged males, however, the 0.5 g/kg dose increased social behavior in aged females. On measures of ethanol sensitivity, females had a shorter duration of LORR and middle-aged and older animals had a longer one. Moreover, BEC at recovery of righting reflex were lower in middle-aged and older animals, indicating increased sensitivity to the sedating effects of ethanol. Since BECs did not differ between young and older adult animals, behavioral sensitivities are likely due to changes in neuronal signaling. In male Sprague Dawley rats, moderate doses of alcohol enhanced age-related depression of Purkinje neurons concomitantly with increased PKCγ levels in the

cerebellum [79]. Further, cognitive deficits attributed to frontal cortical and hippocampal volume loss may be due to altered neuroinflammation in older drinkers [87, 88].

Although alcohol exacerbates neuroinflammation in aged brains [89], there appears to be a diminsihed microglial presence, as Grifasi et al. observed decreased microglial populations in aged male and female C57BL/6N mice after binge alcohol treatments [90]. Acute and chronic binge alcohol-induced neuroinflammation has consistently shown to be a strong driver of neuronal death which could accelerate neurodegeneration in aged Alzhiemer's and Parkinson's disease patients [91]. Together, these studies suggests that acute and chronic binge alcohol enhance hypnotic effects and motor deficits in older humans and preclinical rodent models. The challenge of elucidating alcohol-dependent pathologies is modeling deficits in aged rodents as a majority of older drinkers do not start binge drinking at 65 years and older. Furthermore, research into sex-specific effects of alcohol on cognitive domains and neuropathologies is needed as a reduction in sex hormones with age likely diminishes their protective roles in the brain. The importance of studying these populations have already begun with a recent special issue compiling clinical and preclinical articles addressing alcohol associated pathologies in aged subjects [92].

Cellular and structural plasticity

Adult hippocampal neurogenesis is a form of brain plasticity that is profoundly influenced by experience. It has consistently been shown to be stimulated by environmental enrichment, exercise and antidepressants while stress, chemo/radiotherapy, aging and neurodegeneration decrease neuron production [93–101]. Several studies have also demonstrated that binge alcohol negatively impacts adult hippocampal neurogenesis [32, 45, 102]. In a 4-day model of binge alcohol exposure, male rats exhibited decreased proliferation and survival of neural stem cells [32] in the hippocampal dentate gyrus, although both rebound with abstinence [103]. This decline in hippocampal neurogenesis has been suggested to underlie binge-induced hippocampal degeneration [45], particularly as binge alcohol increases neural inflammation and decreases brain-derived neurotrophic factor (BDNF) levels in the hippocampus (see section below on neurotrophic factors) which can in turn reduce neurogenesis. Thus, in conjunction with cell death, a binge-induced failure of cell genesis or maturation in the dentate gyrus, could contribute to overall cell loss, and the magnitude of these effects could be sex-specific. Therefore, using this same 4-day model of binge alcohol exposure, we quantified remaining granule neurons in the hippocampal dentate gyrus. We found a significant decrease in adult females, but not males [30, 104]. Interestingly, this cell loss persisted for at least 5 weeks, unless animals exercised, in which case the granule neuron layer was repopulated [105]. This suggests that, in females, binge alcohol induces a long-term hostile microenvironment within the dentate gyrus that does not support neurogenesis.

We also examined cell proliferation and cell death after binge alcohol in the dentate gyrus of male and female rats. We found that both sexes showed a significant binge-induced decrement in cell genesis, but that females showed a more significant increase in cell death [30], suggesting a possible contributing factor to the sex difference in remaining granule neurons described above. Because the hippocampus is one of the few brain regions in which adult neurogenesis occurs, binge-induced cell loss in non-neurogenic regions (such as the cortex) would have to be due to negative effects on other forms of plasticity. We therefore examined binge alcohol effects on the number and size of neurons in the medial prefrontal cortex (mPFC), a frontal region vulnerable to alcohol damage [65, 106–108]. We found that the size of mPFC neurons was decreased immediately after the end of binge exposure (before withdrawal) in both males and females, but we did not find neuronal loss in this region in either sex. Moreover, after nine days of abstinence, there was no longer a decrease in neuronal size, consistent with gains in brain volume seen with cessation of alcohol consumption [109, 110]. Thus, with abstinence, cellular recovery in the cortex of both sexes is likely due at least in part to normalization of cell size.

Although much is known about the effects of binge alcohol on adult neurogenesis, there is still more to learn, particularly about female subjects, since a majority of previous studies were performed in male rodents. Sex hormones influence adult hippocampal neurogenesis [111] and sex hormones are affected by alcohol (see section below on steroid hormones), making it highly probable that the effect of binge alcohol on neurogenesis will be sex-specific. Like sex, age is almost certain to influence the effect of binge on neurogenesis. This latter is particularly important to study, because neurogenesis decreases with age [112], yet bingeing behavior persists into older age, raising the possibility that binge effects on neuro-

genesis may contribute to age-related hippocampal neurodegeneration.

Like neurogenesis, dendritic arborization is a form of neuroplasticity that is profoundly affected by experience. Decades of work attest to the ability of alcohol to remodel dendritic arbors in alcohol-vulnerable regions of the brain, including the cerebellum, hippocampus and cortex, in both humans with AUD and in preclinical animal models. In the cerebellum, chronic alcohol reduces Purkinje cell dendritic networks [113, 114], while in the cortex there is decreased arborization of layer III pyramidal neurons compared to age and sex-matched controls [115]. It has also been reported that humans with AUD show loss of dendritic spines on dendrites of layer V pyramidal neurons from Brodman's area 6 [116]. An early study in rats found no chronic alcohol-induced effects on dendrites in this region, but did find that basal dendritic arbors decreased in abstinence [117]. Later studies have reported chronic alcohol-induced dendritic changes in the mPFC of rats, accompanied by alterations of synaptic and glial plasticity [106, 107, 118]. In the hippocampus, an early study in mice showed decreased spine density on CA1 basilar dendrites with chronic intake [119]. Also, on CA1 pyramidal cells, there was a loss of basilar dendrites with 5 months of chronic intake, which normalized with 2 months of abstinence [120].

Dendritic complexity varies as a function of age [121] and also sex [122], and therefore the effects of alcohol on dendritic complexity are likely to vary by age and sex. Indeed, alcohol effects on cerebellar Purkinje neurons have been shown to be age-dependent, as 10 weeks of alcohol consumption decreased Purkinje dendritic networks of 12-month old, but not 5-month old rats [114]. Whether alcohol effects on dendrites vary by sex has not been investigated. Moreover, most of the available research concerning alcohol effects on dendrites has been on chronic alcohol exposure, with binge effects on dendrites largely unstudied. A recent diffusion MRI study in humans, however, examined dendritic complexity in young adult binge drinkers. They reported decreased dendritic complexity in frontal regions, and increased dendritic complexity in parietal and striatal regions, as measured by orientation dispersion index [123]. Much more work is needed to understand how in particular binge alcohol affects dendrites, and, ultimately, behavior, as the binge-associated executive functioning impairments noted above could stem in part from dendritic remodeling and synaptic alterations.

Neurochemical effects

Neurotransmitters

Alcohol (ethanol) is a simple molecule that has multiple complex neurochemical actions across the central nervous system (CNS) as described in various reviews [124–126]. Rather than reiterating the information provided in the prior excellent reviews in this paper, we sought to emphasize systems associated with neurotoxic effects, including decreased neurogenesis, that are induced by chronic alcohol administration, withdrawal, and binge exposure. We specifically emphasize those systems that may change across the lifespan or are sexually dimorphic. These systems include GABA, glutamate, opioids, and the biogenic amines, norepinephrine (NE), dopamine (DA), and serotonin (5-HT). Notably, all of these systems interact with or are affected by stress hormones. While much research documents that acute alcohol administration increases NE, DA, and 5-HT levels, little is known about the neurotoxic consequences of chronic alcohol administration or its withdrawal in these systems. Thus for the purpose of this paper, we focus on GABAergic, glutamatergic, and opioidergic systems as well as provide a discussion of prominently studied neurotrophic and neuroimmune factors all of which have been shown to be altered by chronic alcohol administration.

The GABA system is a family of ligand-gated chloride channels found throughout the CNS and is the major inhibitory system of the brain. Alcohol is associated most strongly with actions at the $GABA_A$ receptor complex, a fast-acting ion channel system that when stimulated, leads to hyperpolarization. Chronic alcohol exposure reduces GABA hyperpolarization causing a decrease in neural inhibition and contributing to the shift in balance of neural excitation and inhibition along with glutamatergic effects described below [127–129]. Upon withdrawal from chronic alcohol exposure, this system can become hyper-excitable due to rebound effects. Such effects enhance the susceptibility to seizure activity and its associated neurotoxic sequelae. Sex and gender differences have been found in GABAergic tone and in alcohol withdrawal-induced seizures. Male mice are more prone to and experience more severe withdrawal-induced seizures than female mice [130, 131] consistent with gender differences reported in humans [132, 133]. $GABA_A$ receptor functioning is modified by the neurosteroid, allopregnanolone (3α-hydroxy-5α-pregnan-20-one; ALLO), that is synthesized *de novo* from proges-

terone [134]. Accordingly, ALLO levels will fluctuate across the estrous/menstrual cycle, differ between males and females, and will be age-dependent. ALLO has anxiolytic actions and is marketed for treatment of anxiety and depression. Recent research suggests that ALLO can reverse decreases in neurogenesis as well as impaired performance in cognitive tasks in mouse models of Alzeheimer's disease [135]. Thus, the direct and indirect actions of GABA likely contribute to sex- and age differences in the neurotoxic effects of chronic alcohol exposure and withdrawal.

Glutamate is the major excitatory system in the brain that consists of at least three types of receptors affected by alcohol: N-methyl-D-aspartate (NMDA) receptor channels, alpha amino-3-hydyroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and metabotropic glutamate (mGluR) receptors. Although early research showed support for a selective role of NMDA receptors in alcohol effects [136], more recent studies demonstrate that AMPA [137] and mGluR [138, 139] receptors are also involved. Alcohol inhibits NMDA receptor effects [140–142] but chronic alcohol ingestion upregulates NMDA receptors and alters synaptic activation that likely contributes to withdrawal-induced seizures [143–145]. As discussed above, the increase in glutamatergic excitation coupled with the decreased GABA inhibition caused by chronic alcohol or its withdrawal results in the shift in balance of neural excitation and inhibition. Indeed, overactivation of NMDA receptor function has neurotoxic effects [146]. Chronic alcohol and its withdrawal show sex-specific effects on glutamatergic systems but whether these differences relate to neurotoxicity is not clear [147–149]. For example, seizure activity correlates with hippocampal density levels of one NMDA receptor type (GluN2) in male rats, but no such relation is seen in females [150]. Further, while the preclinical work is consistent with findings from post-mortem studies of human brain for alcoholic men, it is not for women. Yet, there is some evidence that alcohol withdrawal-induced neurotoxicity associates with NMDA receptor function differentially by sex and that this sex difference is modulated by age [151]. Both NMDA and AMPA receptor densities are lower in female rat hippocampus compared to males with AMPA receptor density found to be higher during diestrus compared to the estrus stage [152, 153]. Whereas estradiol regulates NMDA receptor binding with no effect on AMPA receptors in one study [154], estrogen agonists are found to increase AMPA receptors in another study [155]. Differences in techniques (autoradiography vs immunohistochemistry) may explain the discrepancies. Density of NMDA and AMPA receptors in hippocampus decline from early adulthood (3-months) to old age (29-months) in a study that tested male rats only [156]. The manner in which sex, sex hormones, and age interact with alcohol or its withdrawal on glutamatergic systems and its induction of neurotoxicity is not clear and is in need of more research.

The endogenous opioid system that consists of the opioid peptides ß-endorphin, enkephalins, and dynorphins, contributes to the consummatory and reinforcing effects of alcohol [157–159]. While results from numerous studies in animals and humans consistently show that acute alcohol administration either increases mu opioid receptor activity or blockade of this system decreases alcohol effects, it is less clear how this system is affected by withdrawal from chronic alcohol exposure [160–164]. Some findings are consistent with the concept of a rebound in activity but there are discrepancies that may relate to variations in techniques, including alcohol administration, to brain regions assessed, or to species and strain utilized. Much evidence shows that exogenously administered opioid drugs decrease neurogenesis in hippocampus [165] or have other neurotoxic effects [166], but whether an increase in endogenous levels due to withdrawal from alcohol administration has similar effects is less well-studied. However, some research suggests that withdrawal from chronic binge alcohol drinking alters hippocampal neurogenesis through a mu opioid receptor mechanism [167]. Moreover, activation of mu receptors can inhibit GABA release leading to increased excitability of hippocampal cells in the dentate gyrus and possibly enhancing neurotoxic effects of alcohol withdrawal [168]. Sex and aging likely affect the capacity of alcohol withdrawal to alter mu activity in brain as well. The concentration of opiate receptors in brain regions such as hippocampus decrease with age [169] and endogenous opioid peptide levels are depressed by estrogen [170]. Clearly, there is a need for a better understanding of the contribution of the endogenous opioid system to neurotoxic sequelae of binge alcohol exposure and withdrawal with particular attention paid to how it is affected by age and sex factors.

Neurotrophic factors

In addition to the more classic neurotransmitter systems discussed above, binge alcohol exposure affects neurotrophic factors. Using a 4-day binge

exposure model, we have shown that binge alcohol in female and male rats downregulates expression of neurotrophic proteins in the hippocampus, including BDNF, insulin-like growth factor 1 (IGF-1) and their receptors [30]. Others have reported reduced BDNF expression in the hippocampus [171] and in the frontal cortex [172] immediately after the end of binge exposure in adult male rats. In our study, we observed sex differences in the pattern of decline in neutrophin levels during binge exposure with females downregulating BDNF and its receptor sooner than males. Furthermore, IGF-1 levels returned to baseline in males 8 hours after end of binge exposure. These sex differences were accompanied by female-specific deficits in remaining granule neurons in the dentage gyrus as well as in spatial learning. Sex-dependent effects of binge alcohol on trophic factor expression may be due in part to the effect of stress. For example, stress downregulates *bdnf* gene methylation [173]. In turn, decreased availability of BDNF is linked to stress-induced cell death and decreased cell genesis in the hippocampus [94, 174–177]. As discussed in the section on steroid hormones below, the female stress system reacts more strongly to stressors, including alcohol, which is a physiological stressor [178–180]. Indeed, there is evidence that stress induces a female-specific reduction in hippocampal BDNF mRNA expression [181] and gene methylation [182]. Reduced hippocampal BDNF expression has been shown to impair [183] and increased expression to enhance [184] spatial learning.

Neuroimmune system

There is a great deal of information available on alcohol-induced neuroimmune activation, and excellent reviews have been published on this subject [91, 185, 186]. There is not, however, a great deal of data available on sex-differences in binge-induced neuroimmune response. There is, for example, data from binge studies of toll-like receptors (TLRs), located on glial cells, which recognize molecular patterns of pathogens and activate the innate immune system leading to the synthesis and production of cytokines and other pro- and anti-inflammatory molecules. The most widely studied member of this family is the Toll-like receptor 4 (Tlr4). Tlr4 recognizes gram positive bacteria such as lipopolysaccharide (LPS) to initiate immune responses. In recent years, Tlr4 has been linked to binge alcohol drinking mediated within $GABA_A$ neurons of the central amygdala [187] and to consequences of chronic alcohol exposure [188] including cell death [189]. Innate immune system signaling and the Tlr system regulates neurogenesis and helps to integrate new neurons into functional circuitry [190, 191]. Thus, this neuroimmune factor likely contributes to the neurotoxic consequences of chronic alcohol exposure and withdrawal. There are sex differences in immune responsivity. Females show greater immune and inflammatory responses compared to males [192] that likely reflect the presence of sex hormones [193, 194]. While enhanced immune responsivity in females may be thought to have neuroprotective effects, it is also true that over-activation of the immune system can have untoward effects. For example, women are more prone to develop auto-immune diseases or chronic inflammatory conditions [195]. Thus, it is likely that binge alcohol exposure would have greater neurotoxic consequences acting through the innate immune system in females than males. Indeed, in a recent study of binge drinking young men and women visiting the emergency room, higher levels of Tlr4 activation were present in the women. Moreover, the same study examined Tlr4 activation in a mouse model of binge alcohol and found it to be higher in the serum and prefrontal cortex of female mice [196].

The presence and morphology of microglial cells is often used as a proxy measure of neuroimmune activity. While increased microglial presence in the brain is associated with perturbations of homeostasis, it does not necessarily indicate a negative state. Indeed, after binge alcohol exposure, microglia proliferate, preceeding a regenerative burst of hippocampal neurogenesis during abstinence [103, 197]. Binge alcohol does "prime" microglia, however, preparing them to mount an exaggerated response to a subsequent challenge (including a subsequent binge) [198]. Microglial presence and activity in the brain is both sex- [199] and age-dependent [200], and, given their response to binge alcohol, it is highly likely that they play a role in the differential effects of alcohol between the genders and across the lifespan [201]. We have found that, during abstinence after a 4-day binge exposure, microglial expression in alcohol-vulnerable regions of the brain is sex-dependent. In the mPFC, we found that binge alcohol decreased the total number of microglia in males, but not females, and increased the total number of primed (partially activated) microglia in females, but not males. In the hippocampus, binge alcohol increased total and primed microglia in females, but decreased them in males [202]. Notably, these were chronic changes seen during prolonged abstinence (11 days), suggest-

ing that the microglial response to binge alcohol is both lasting and sex-specific.

Steroid hormones

Sex and stress steroid hormones have well-documented effects on neurocognitive plasticity across the lifespan and are key contributors to sex differences in plasticity [203–205]. Despite this, there have been few experimental investigations of their roles as mediators of sex differences in binge alcohol-induced brain damage. Alcohol, including binge pattern exposure, is a physiological stressor which activates the HPA axis [206–208]. Stress exposure acutely activates the hypothalamus to secrete corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) which act on the pituitary gland to stimulate the release of adrenocorticotrophic hormone (ACTH) which, in turn, stimulates the release of glucocorticoids from the adrenal cortex into systemic circulation. This triggers a negative feedback loop wherein the glucocortoids, mainly corticosterone (CORT) in rodents and cortisol in humans, act on the hypothalamus and pituitary gland to downregulate CRH, AVP, and ACTH signaling. Chronically high levels of CORT are associated with reduced neurogenesis and enhanced risk of neurodegeneration in the hippocampus which is enriched with glucocorticoid receptors (GRs) in the dentate gyrus and pyramidal neurons [209]. In male rats, medium and high, but not low dose CORT replacement after adrenalectomy has been shown to mediate binge ethanol-induced degeneration of dentate gyrus granule neurons and pyramidal neurons in the entorhinal cortex via Type II glutamate receptors [206]. Like other stressors, alcohol elicits a greater stress response in females relative to males, reflected in a greater increase in levels of ACTH and CORT [178, 180] although at higher alcohol doses, this sex difference might be abolished or masked [210]. Thus, sex difference in HPA reactivity to binge alcohol may be a critical driver of sex differences in binge-induced neurodegeneration. Organizational and activational effects of sex hormones underlie sex differences in activation of the HPA axis by a stressor [211–213]. In general, these activational effects are such that circulating testosterone inhibits and circulating estradiol, the primary active estrogen, facilitates HPA activation in response to a stressor, including alcohol [178, 180]. There is also some evidence that the sex difference in the HPA response to acute alcohol exposure is independent of the organizational effects of sex hormones on the HPA axis in males and females [179]. Thus, sex differences in binge alcohol-induced neurodegeneration may be attributable, at least in part, to the differential activational effects of sex hormones on HPA reactivity via their direct actions at the level of the brain such as the hypothalamus and/or the periphery [10, 180, 212, 213]. Moreover, repeated alcohol exposure is reported to downregulate circulating testosterone and upregulate estradiol levels puportedly by aromatization of androgens to estrogen although these changes in gonadal hormone milieu appear to be alcohol dose and duration-dependent as well as sex- and age-dependent [214–216]. It is also possible that sex hormones distinctly modulate neurobehavioral effects of binge alcohol via their organizational and/or activational effects (like CORT discussed above) on alcohol-vulnerable brain regions such as the hippocampus [10, 217, 218]. Given that sex differences in stress responsivity extend into late life [219] and that brain vulnerability to stress increases with aging, particularly for the hippocampus [220], frequent binge drinking in the older population likely exacerbates the risk of neurodegeneration and cognitive impairment [221, 222] and this risk may be greater for females. The onset and history of alcohol use are also important factors to be taken into consideration when studying the contributions of steroid hormones to binge damage in the older population given that binge alcohol exposure during adolescence can alter the development of the HPA axis and its reactivity to alcohol in adulthood in a sex-specific manner [75, 215, 223–225].

METHODOLOGICAL AND STATISTICAL CONSIDERATIONS FOR STUDYING EFFECTS OF SEX AND AGING

Binge models

Because of the inherent sex differences in human drinking behaviors it is difficult to match drinking histories between men and women, complicating the investigation of sex-specific neural effects of binge alcohol. Animal models therefore provide an invaluable means by which to standardize alcohol between the sexes, including the total amount administered and the pattern of administration. Notably, however, voluntary intake models are not optimal for this purpose as there are also inherent sex differences in drinking behaviors between male and female rodents [226, 227]. Gavage and i.p. administration of ethanol both circumvent this problem and both have been used to study sex differences in binge ethanol effects.

Ideally, animal models of binge alcohol effects should reflect the frequency and intensity of human binge drinking, but the latter is particularly difficult to pinpoint. Retrospective reports of drinks consumed by human binge drinkers are distorted by the effects of alcohol on memory and by misunderstanding of what constitutes a standard drink, with the result that many people underestimate the number of drinks they consumed [228, 229]. This makes it difficult to estimate blood alcohol levels achieved by binge drinkers, but it can be safely assumed that they vary substantially. Animal models of binge alcohol exposure also vary, both in terms of how frequently the ethanol is administered (which would affect the influence of compensatory adaptations by the brain and liver) and the BEC achieved. For example, as outlined above, we found sex-dependent effects of a 4-day binge exposure on granule neurons in the dentate gyrus, with females more severely affected than males [30]. More recently, we have found that if binge exposure is distributed across 8 weeks (a single 4 g/kg binge dose per week), this sex difference is not present – both sexes show ∼20% loss of granule neurons (West, Rodgers & Leasure, manuscript under revision). Thus, it may be that sex-dependent effects are more easily detectable in models that induce dependence, as males may adapt physiologically more readily than females. Indeed, this is consistent with the wealth of data indicating that women's health is disproportionately harmed by alcohol.

In this review, we have included data from many rodent models of binge drinking, even though they may or may not induce dependence and even though the blood ethanol levels achieved may be very high. We have focused instead on the ability of all of these rodent models to reflect human bingeing in that a large amount of alcohol is administered in a short period of time, with accompanying high BEC. Nonetheless, as we strive to better understand the effects of binge alcohol in both sexes and across the lifespan, consideration of dependence and BEC will become increasingly important.

Pharmacokinetics

While the absorption, distribution, metabolism and elimination of alcohol is undoubtedly affected by both age and sex [230], pharmacokinetic differences cannot fully explain sex and age-dependent effects of alcohol. For example, as described above, older animals demonstrate a BEC not significantly different from young adults, yet their motor and/or cognitive performance is significantly impaired [84, 85]. For adolescent animals, their BEC was significantly higher than that of young adult animals, but their motor capacity was not different [85]. Using a 4-day binge model of ethanol exposure, we have found that BEC of males and females do not differ, yet females (but not males) show a significant increase in cell death and a significant decrease in remaining granule neurons [30]. Simply put, BEC does not predict behavioral impairment of neuronal damage between the sexes or between age groups. Therefore, we suggest that focusing research efforts on the contribution of pharmacokinetic differencs to sex- and age-specific neurobehavioral effects of binge alcohol would be of limited utility.

Grappling with the Alcohol x Sex x Age interaction

One of the most interesting aspects of binge drinking in aged individuals is that both alcohol and aging are associated with brain dysfunction and cognitive decline. How then, does binge drinking impact brain aging in males and females? In order to systematically evaluate this question, it is essential to address the three-way interaction of alcohol, sex and age. Sex and gender bias in biological sciences and biomedical research as well as theoretical and methodological approaches to mitigate bias and integrate sex as a biological variable (SABV) in experimental research have been discussed extensively by experts [231–239]. Major funding organizations such as the European Commission, Canadian Institutes of Health Research, and the US National Institutes of Health (NIH) now mandate the integration of SABV in preclinical research and provide guidelines and resources on how to successfully accomplish this from concept development through experimental design and data analysis to publication. Here we discuss the potential challenges and considerations of including both sexes in rodent models of binge alcohol effects on the aging brain using what we have learned from studies on the comparatively younger brain. For instance, what are the limitations of indiscriminately extrapolating from alcohol models formalized in males to females? How best to account for potential confounds such as stress which vary as a function of sex and age [219, 240–243]? When is it appropriate to disaggregate the sexes versus compare them statistically?

In their 2018 paper "*The Telescoping Phenomenon: Origins in Gender Bias and Implications*

for Contemporary Scientific Inquiry," the authors discuss how generalizing a model of alcoholism formalized in males to females limits our ability to detect female-typical features of alcohol use and damage, which has critical implications for clinical research targeting therapeutic options [232]. Similarly, the Majchrowicz binge alcohol model [244] relies on a scale for scoring intoxication and withdrawal behaviors which was developed in male rats. In this model, after the first intragastric administration of 5 mg/kg ethanol, subsequent doses are titrated according to the degree of behavioral intoxication. Male rats score higher than female rats on the behavioral intoxication scale and thus receive lower ethanol doses and yet the sexes do not differ in blood ethanol concentrations and withdrawal severity [80]. Nonetheless, ideally, models of binge exposure should standardize ethanol amount, pattern and duration of administration between the sexes. Assessment of cognitive and affective changes poses a similar challenge as most test procedures have been developed and validated in young adult male rats. Yet, there are a number of physical and behavioral differences between the sexes and across the lifespan that need to be considered when adapting test procedures to females and to older animals. Body weight and size are obvious differences; females are smaller and older animals are larger than the typical young male rodent. This suggests that many apparatuses used to evaluate a variety of behavioral indices may be the wrong size. Furthermore, some apparatuses utilize information acquired from the rodent's movements that break infrared beams placed at predetermined locations and feed these data to software algorithms that calculate a variety of behavioral endpoints. Behavioral data obtained from various procedures are also likely affected by differences in baseline activity levels. Females are more active and older animals are less active than younger, adult males. Procedures that involve learning may be affected by sex differences in response strategies [245, 246] or aging-related increase in variability [247–249]. We have also seen sex differences in response strategies in operant drug and alcohol self-administration [250, 251]. Additional considerations include differences in response to the mild stress of being introduced into a novel test apparatus which could affect the endpoints obtained. Stress as a potential confound is particularly relevant in binge models using oral gavage as a means of ethanol delivery and cage control animals (non-gavaged) should be included.

NIH policy does not mandate statistical comparison of sexes and the decision to disaggregate the sexes or compare them statistically must be driven by study hypotheses and the constraints of statistical approaches. However, if a sex comparison is being conducted, within-sex variability must first be scrutinized. The most common concern expressed in this regard is the female estrous cycle as a major source of variability. We have found that estrous stage does not influence the extent of binge-induced brain damage [30, 105]. Moreover, relevant to studies of sex and age, estrous cycle would contribute minimally to variability within females near the beginning and end of the lifespan as prepubescent females are not yet cycling and aged females have entered reproductive senescence and are no longer cycling. Furthermore, meta-analyses of neuroscience research demonstrate that, on average, adult male and female rodents exhibit comparable variability on behavioral, electrophysiological, neurochemical, and histological measures [252–254] with some exceptions [252, 253]. In our own research with young adult rats, we have encountered this particular challenge of greater within-sex variability (females > males) in the initial acquisition of a complex, behavioral task known as the five-choice serial reaction time task which assesses several executive functions such as attentional processes, inhibitory control, and reaction time [239, 240]. If the goal is to train animals to criterion and then conduct the experimental manipulation, such a sex difference in acquisition is likely not a concern. However, if acquisition itself is of interest, then the sexes should be analyzed separately if using common parametric tests such as analysis of variance (ANOVA) tests, especially when there are unequal sample sizes.

Finally, in this section we have specifically addressed experimental approaches to study the *acute* neurobehavioral effects of binge drinking at different ages in both sexes as a first step towards extending the study of binge drinking to the aged population. Subsequent investigations must factor in the long-term consequences of early-life binge drinking as well as the cumulative effects of binge drinking at different stages of the lifespan.

CONCLUSIONS

Elucidating sex-dependent neural effects of binge alcohol throughout the lifespan is crucial, in order to develop personalized approaches to the prevention

and treatment of AUD. Extra attention to both adolescence and older adulthood is warranted, however, as both are characterized by sex-typical brain changes and because binge drinking is prevalent in both age groups. Attention to adolescent alcohol neurotoxicity has made it clear that adolescence is a period of vulnerability to binge alcohol, as it disrupts brain maturation. Older age, with its characteristic decline in neural function, may well represent a second vulnerable period, and we know little about binge alcohol effects on the aging brain, including whether they are sex-dependent. Because adolescence and older age are both periods of marked, sex-typical brain changes, we may be able to use the wealth of data we have obtained from the study of binge alcohol effects on the adolescent brain to inform the study of its sex-dependent effects at the other end of the lifespan. For example, the sex-specific pattern of brain maturation may be responsible for why binge drinking young females show thicker cortices compared to age-matched controls whereas males show thinner. In other words, it may be that alcohol disrupts the pruning process in females (resulting in thicker cortex) but enhances it in males (resulting in thinner cortex) [255, 256]. Similarly, there may be sex-specific patterns of brain aging differentially affected by binge alcohol. Also, as binge drinking is a risk factor for stroke and neurodegeneration [222, 257] it is critical that we learn more about whether it would confer sex-dependent vulnerability to brain disease. Although there are methodological and statistical considerations that complicate the study of sex and age differences in binge alcohol effects, they are neither unsurmountable nor unique to alcohol research. Most importantly, given the objective of personalized therapeutics for AUD, we advocate approaches, both scientific and intellectual, unconstrained by reflexive adherence to a battle of the sexes or ages.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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