Published in final edited form as:
Pflugers Arch. 2013 May ; 465(5): 643–654. doi:10.1007/s00424-013-1266-4.

Sex Differences in Neuroadaptation to Alcohol and Withdrawal Neurotoxicity

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Summary

Recent work suggests that sex differences exist with regard to both the nature of neuroadaptation to alcohol during the development of dependence, and possibly, the neurodegenerative consequences of alcohol dependence. Volumetric studies in human samples show that females may demonstrate increased volumetric brain loss with equal or lesser dependence histories than males. Further, animal studies demonstrate sex differences in glutamatergic, GABAergic, and adenosinergic receptor signaling and endocrine responses following prolonged alcohol exposure. These differences may influence the development of dependence, neuronal function and viability, particularly during alcohol withdrawal. The present review discusses the current state of knowledge in this regard. It is concluded that there exists a clear need for more extensive examination of potential sex differences in neurodegenerative consequences of alcohol dependence in men and women, particularly with regard to the role that alterations in amino acid signaling and hypothalamic-pituitary-adrenal axis function may play. Further, we note the need for expanded examination of the unique role that alcohol withdrawal-associated neuronal activity may have in the development of dependence-associated neurotoxicity.

Keywords

gender; alcohol dependence; detoxification; brain; glutamate; γ-aminobutyric acid; hippocampus; corticosteroid

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Introduction

Alcohol abuse and dependence occur are observed at high rates throughout the World, despite numerous deleterious health-related, psychosocial, and occupational consequences. Findings of the 2011 National Survey on Drug Use and Health suggest that nearly 23% of those in the United States engaged in binge drinking, defined as intake of 5 or more drinks on the same occasion during the prior 30 day period, while 6.2% engaged in "heavy drinking", defined as engaging in binge drinking 5 or more time during the recent 30 day period [143]. Similar findings of widespread binge and heavy alcohol use in adolescents and adults are found worldwide. Ramsoomar and Morojele [117] reported rates of binge alcohol intake in a South African sample that were similar or even exceeded those observed in American studies and comparable findings were reported with examination of Brazilian, Chinese and Western European samples [56,81,160]. Notably, marked regional differences in alcohol intake, particularly binge alcohol intake, do exist. For example Valencia-Martin et al. [149] reported much lower rates of binge drinking and "heavy" drinking in Spanish adults than are commonly seen in the United States. Further, while South American youth were reported to binge drink at relatively high levels [160], Brazilian adults [78] report levels of abstinence from alcohol use that are greater than those reported in North America [143] and the European Union [157]. The importance of understanding binge drinking as a risk factor for subsequent development of alcohol dependence and related pathological outcomes is clear, as is the likelihood that binge drinking itself may be directly neurotoxic.

In the United States, recent evidence suggests that the lifetime prevalence of alcohol dependence was nearly 13% while the 12-month prevalence was nearly 4% [59]. Rates of alcohol abuse and dependence in Asia, South Africa and the European Union have been widely reported to be similar, though rapid and recent increases in the prevalence of dependence in some Asian cultures have been documented [eg. 55,108,120]. A critical aspect of the studies discussed above is the common observation that binge drinking and dependence are more prevalent in males of all age groups and are either stable or declining in males. However, the prevalence of binge drinking, if not dependence as well, is increasing in females worldwide [143, reviewed in 163]. Thus, sex differences in alcohol-related tissue pathology, such as neuropathology, may become more easily identified if additional studies directly testing related hypotheses are conducted.

A Brief Overview of Brain Atrophy in Alcohol-Dependent Males and Females

Evidence presented above may lead to the logical assumption that central nervous system pathology associated with alcohol dependence would be most evident in alcohol dependent males, as binge drinking and dependence are more common in males. There is clear evidence of atrophy of several cortical, subcortical, and cerebellar structures following chronic and excessive alcohol intake in both males and females, effects that are correlated with behavioral abnormalities [reviewed in 20,64,99,144]. Further, there is a vast literature demonstrating brain morphological abnormalities observed in both males and females following fetal alcohol exposure, including reduced total brain volume, abnormalities in volume of many structures including cerebellum, corpus collosum and some subcortical regions, as well as, abnormalities in white matter volume (reviewed elegantly in 124). However, a limited body of compelling work suggests that the female brain may be more sensitive to the degenerative effects of prolonged alcohol dependence or even binge alcohol intake . Piazza et al. [111] described an "accelerated progression of alcoholism", or "telescoping" phenomenon, more than 20 years ago in detailing evidence that alcohol-dependent females experienced alcohol-related problems and sought entry into treatment earlier in their drinking history than did alcohol-dependent men. Both Schuckit et al. [132]

and Randall et al. [118] reported comparable findings. However, a more recent investigation did not find evidence of the phenomenon. Keyes et al. [73] recently reported on a lack of a clear “telescoping” phenomenon in alcohol-dependent females with regard to age at initiation of alcohol use and development of dependence. In fact, these authors suggest that male subjects developed alcohol dependence more rapidly than females, which may contradict initial findings suggesting the presence of a “telescoping” effect in females. While it is unclear as to why there may be such contradiction in this literature, Keyes et al. suggest that sex differences in drinking topography have possibly changed since the work of Piazza and colleagues, with more recent subject samples showing either no evidence of the phenomenon or an attenuated “telescoping” effect. A similar conclusion is presented by Zilberman et al. [163]. Such effects may be influenced by changes in the age of onset of drinking in females and/or the increased prevalence of binge drinking in females. This is quite clearly a critical issue with important clinical relevance that requires further study. For example, evidence of sex differences in the trajectory of alcohol dependence would suggest that sex-specific neuroadaptations occur in response to chronic alcohol intake.

Despite the lack of a cohesive body of literature confirming a telescoping effect in alcohol-dependent females with regard to drinking history and development of dependence and treatment seeking, there is some evidence of a possible telescoping effect with regard to brain atrophy. Hommer et al. [65] reported that alcohol-dependent females had a proportionally greater loss of brain volume and increased sulcal and ventricular cerebrospinal fluid volume than did alcohol-dependent males, when either group was compared to their non-dependent same-sex cohorts after 3 weeks of abstinence from drinking. More recent work has been consistent the existence of a telescoping phenomenon in females, in a sample 158 male and female subjects [93]. These findings demonstrated the development of significant and comparable brain atrophy, reflected in a “global atrophy index”, in alcohol-dependent females and males, despite evidence that women had a shorter history of dependence. Notably, atrophy was partially reversed with several weeks of abstinence, though global brain volume did not return to control levels in both males and females. The work of Tapert and colleagues suggests that possible sex differences in the neuropathological response to alcohol use may be observed without a prolonged history of excessive use, as these are seen in adolescents with alcohol use disorders (AUD). For example, they reported that despite similar alcohol use histories, older adolescent females with AUD showed deficits in prefrontal cortex (PFC) volume when compared to same-sex controls, while males with AUD had larger PFC volume [96]. These findings may suggest that adolescent females are more likely than males to experience cognitive deficits associated with AUD with equal or lesser drinking histories. Indeed, this group has also reported that adolescent female binge drinkers showed lower levels of spatial working memory-associated activation of many brain regions and worse cognitive function than males, when both are compared to their same-sex controls [140]. Conversely, Pfefferbaum et al. [110] did not observe evidence of sex differences in alcohol-associated cortical atrophy in a sample of 86 alcohol-dependent men and women. In fact, alcohol-dependent men in this sample showed deficits in cortical gray and white matter, as well as sulcal and ventricular enlargements that were not observed in alcohol-dependent women. Demirakca et al. [38] also reported the lack of sex differences with regard to global gray and white matter volume, as well was voxel-based morphometry in the cingulate gyrus and insula. While the reasons for these discrepancies are not entirely clear, there are several variables, such as age of the subjects, duration of abstinence and cigarette smoking history that moderate the research findings. Given the very high rate of nicotine dependence in alcohol-dependent individuals [94] and the possibility that nicotine dependence may be a risk factor for relapse to drinking [62]; increase the severity of alcohol withdrawal [68]; and may also protect against many distinct insults including alcohol withdrawal [112, for review, see 48], smoking history in particular may be a key moderator of alcohol effects on brain atrophy.

Demirakca et al. [38] identified what may also be a key moderator of putative sex differences in brain volume in alcohol dependence, length of alcohol use/abuse in female patients. Female subjects studied in this report had a drinking history that was more extensive than those reported by others who found evidence of sex differences [65,93]. An additional and critical variable may be the specific brain region examined in such studies. It has been suggested previously that distinct brain regions or components are differentially sensitive to the neurotoxic effects of alcohol and/or alcohol withdrawal. For example, Ruiz et al. [126] recently demonstrated that alcohol-dependent men may be more susceptible than females to white matter losses in the corpus collosum, whereas females may be more susceptible to white losses in frontal and temporal lobes with a history of prolonged heavy drinking, a finding that may well be influenced by blood alcohol level differences in males and females. Notably, white matter volume was found to recover more quickly with abstinence in females, than in males. Thus, more clarification is needed to determine if sex differences in CNS injury in response to alcohol, whether it be loss of white matter or gray matter volume, may be highly region-specific.

The Role of Withdrawal in Alcohol Dependence

Alcohol dependence is often associated with a predictable pattern of alcohol consumption in that periods of intoxication are followed by periods of abstinence, during which withdrawal symptoms are commonly observed [98]. This seminal work reported that in alcohol-dependent individuals who were required to complete a simple operant task to obtain alcohol in an inpatient setting, the subjects worked for periods of several days to achieve blood alcohol levels of approximately 100-250 mg/dl, but typically chose to abstain from alcohol intake for shorter periods of 2-3 days during which signs of withdrawal were evident. In humans, the alcohol withdrawal syndrome is often associated with myriad effects reflecting neuronal over activity including craving, anxiety, tremor, and dysphoria [116]. Rodent models of withdrawal support the hypothesis that symptoms are reflective of an imbalance between inhibitory and excitatory neurotransmission and involve perturbations of multiple neurotransmitter systems and ion channels [4,10,18,19,23,24,32,34,43,57,58,70,82,83,85,89,95,125,146,148,159,162; for review see 36,37,45,88,114].

Decades ago, Ballenger and Post [8] demonstrated a significant positive correlation between the number of years of alcohol consumption in human males and the severity of withdrawal-induced physiological manifestations, particularly seizures. A growing body of literature supports the suggestion that not is only alcohol withdrawal a reflection of the severity of dependence, but it increases the likelihood for subsequent withdrawal-induced seizures. For example, in a study examining more than 400 inpatient records (350 male, 60 female) a significant positive correlation between history of previous detoxifications and occurrence of withdrawal-induced seizures in male and female patients was reported more than 20 years ago [79]. Similar findings have been reported by others examining withdrawal severity of both males and females and it has been hypothesized to reflect a “kindling”-like phenomenon in limbic structures [2,15,134,158]. Studies employing rodent models support the “kindling” nature of multiple alcohol withdrawals [11,151] and suggest that male rodents may be more sensitive to this effect. Collectively, these studies demonstrate the number of previous detoxifications may be predictive of withdrawal severity in both male and female alcohol-dependent individuals. However, it is not clear if sex differences may exist with regard to the manifestation of severe alcohol withdrawal or the “kindling-like” phenomenon observed with repeated alcohol withdrawals. While animal studies suggest a greater susceptibility to withdrawal seizure and greater duration of withdrawal in male rodents as compared to female rats or mice [6,150], studies of human samples suggest that either few sex differences exist [61,84] or that males display alcohol withdrawal of a greater severity

than females [139,155]. Further, a link between withdrawal seizure and overt neurodegeneration has not been established.

Importantly, there is evidence that while the severity of alcohol withdrawal may not be substantially different between men and women with similar drinking histories, the qualitative nature of withdrawal may indeed be divergent, suggesting that profound differences exist in neuroadaptation to alcohol dependence in men and women. For example, Deshmukh et al. [39] reported that more alcohol-dependent males endorsed anxiety criterion for withdrawal than females, even when matched on prior alcohol consumption variables. Further, others have demonstrated that in social drinkers [26,133] or alcohol dependent patients who also abuse cocaine [49], sexually dimorphic patterns of brain activation (measured using functional magnetic resonance imaging); hypothalamic-pituitary-adrenal axis responses; and alcohol craving were identified in response to emotional stress delivered via guided imagery. It will be intriguing to expand and extend such work in examining alcohol-dependent men and women during early or protracted abstinence as differences in stress response may have implications for therapeutic intervention during detoxification and abstinence. Collectively, these findings clarify the need to fully consider sex as a moderating variable in both human and animal studies of alcohol withdrawal-related phenomena.

Until relatively recently, the consequences of alcohol withdrawal, and in particular, a pattern of repeated alcohol withdrawal/detoxifications, for CNS function were not clear. Though alcohol produces overt injury to the developing and mature central nervous system via myriad mechanisms associated with the presence of high doses of alcohol [27,72,76,106,122,131,153], there exists substantial evidence from the study of rodents that alcohol withdrawal, and possibly the associated neuronal hyperexcitability that occurs during withdrawal, may produce excitotoxicity in brain regions that are enriched in glutamatergic receptors, such as the hippocampal formation [for review, see 21,37,114]. However, Collins and Neafsey [31] have recently suggested that neurodegeneration associated with alcohol dependence in the adult brain may be independent of withdrawal and that withdrawal-induced excitotoxicity is most relevant to the developing brain. This intriguing suggestion clearly warrants more extensive examination, particularly with regard to studies of human subjects. Perhaps most importantly, evidence from pre-clinical and clinical studies suggests that impaired neurological function is associated with limited alcohol withdrawal or multiple, prior episodes of alcohol withdrawal. For example, Thomas and colleagues [147] demonstrated , with use of a third-trimester rodent model of alcohol exposure, that administration of the NMDA receptor GluN2 subunit modulator eliprodil during alcohol withdrawal in neonatal rats reduced subsequent deficits in learning in male rats. Duka et al. [46] reported deficits in performance of cognitive tasks known to require inhibition of prepotent motor responses in alcohol-dependent subjects (both male and female) that were greater with a history of more, prior detoxifications, though the possibility of other moderating influences could not be discounted, including smoking history. Loeber et al. [86] recently reported that patients with a history of more than 2 prior detoxifications (hi-detoxification) were delayed in their cognitive recovery (at 3 months post detoxification) when compared to those with less than 2 prior detoxification (low detoxification). These findings support the contention that prior multiple detoxifications may adversely influence plasticity of frontal lobe circuitry. It is important to note that this delayed cognitive recovery was not associated with increased risk of relapse to drinking, suggesting distinct substrates for general cognitive function and risk for relapse to drinking. In contrast, Loeber et al. [87] did not find differences between hi- and lo-detoxification subjects on cognitive tasks that were distinct from those employed previously [46,86]. These findings, and those of Chanraud et al. [25] provide clear evidence that the adverse effect of multiple withdrawals on cognitive function is highly task specific and moderated by additional variables, possibly including age of onset of drinking, for example. Importantly, the moderating influence of

prior medical detoxifications (as opposed to unsupervised withdrawal) is unclear. Duka et al. [46] did attempt to examine this issue, however, the influence of this variable was possibly confounded by binge alcohol history in those who attempted prior unsupervised detoxifications. This important issue needs full characterization for the purpose of best estimating the influence of medical detoxification, or the lack thereof, on cognitive function and possible neurodegeneration, in addition to relapse prevention.

While sex differences are largely under examined in studies relating cognitive function to prior alcohol withdrawal, some work does implicate a role for withdrawal in alcohol-associated learning and memory deficits. Glenn et al. [52] did report an association between multiple withdrawals and memory impairment in a sample of 143 alcohol-dependent individuals (76 male, 67 female). Further, they provided the first evidence that this impairment may be greater in female subjects than in male. However, molecular influences on this putative sex difference remain largely uncharacterized, though De Witte et al. [37] have suggested that repeated withdrawal may produce neurotoxic effects in the frontal lobe that are dependent on over activity of glutamatergic systems. Similarly, Bleich et al. [14] suggest that the endogenous NMDA receptor ligand homocysteine is elevated in alcohol dependence and levels are positively correlated with withdrawal severity. Chanraud et al. [25] provide compelling evidence of possible anatomical substrates of the cognitive deficits observed following alcohol withdrawal. Using a morphometric magnetic resonance imaging approach, these authors reported negative correlations among performance on tasks of attention and executive function and volume of multiple brain regions including, temporal and frontal gray matter, hippocampus, thalamus, cerebellum, and others. However, these studies did not examine sex differences. Thus, the study of both animal and human subjects suggests the importance of studying alcohol withdrawal as a putative insult affecting cognitive function.

Neuroadaptation to Alcohol: Molecular Suspects for Sex Differences

Neurotransmitter Signaling

Effects of alcohol on the expression and function of neurotransmitter signaling and ion channels continue to be more fully characterized with evidence that alcohol induces changes in the expression, synthesis and/or trafficking of each. The development of dependence, including tolerance to alcohol and manifestations of alcohol withdrawal, clearly reflect coordinated alterations in the expression and/or function of multiple signaling cascades, yet few have been studied with regard to possible sex differences that may exist. In the most general terms, alcohol is known to affect each of the known neurotransmitter systems and nearly every ion channel. Acute alcohol exposure increases binding capacity [148] and potentiates the action of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) at the type-A GABA ($GABA_A$) receptor subunit [115, 162]. Additional contributions to the acute inhibitory effect of alcohol include activation of α1 glycine receptor [128]. Work has also demonstrated the importance of endogenous adenosine and its receptors in mediating behavioral effects of acute alcohol exposure and withdrawal [33, 70; reviewed in 21], in a sex dependent manner, as discussed below [22,23]. Further, acute alcohol inhibits function of glutamatergic N-methyl-D-aspartate (NMDA) and influences synaptic clustering of these receptors [89,104,109,146]. More recent work has suggested an acute effect of alcohol on alpha amino- 3-hydyroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors [101] and metabotropic glutamate receptors (mGluR) function or expression, possibly via effects on scaffolding proteins, such as Homer and PSD-95, that regulate both NMDA receptor and mGluR plasticity, or direct inhibitory effects on group I mGluR receptors [57, 107,142]. Acute alcohol exposure potentiates serotonin type-3 ($5\text{-}HT_3$) receptor function [90; reviewed in 95, 145], inhibits or stimulates 5'-triphosphate-gated purinergic receptors in a subunit-dependent manner, and also decreases agonist binding affinity [34,35,82]. Alcohol enhances

acetylcholine-evoked currents from nicotinic acetylcholine receptors (nAchR) at high alcohol concentrations [4,32], an effect that likely is associated with the high rate of co-dependence on alcohol and nicotine [94]. With regard to ion channels, alcohol produces numerous acute effects that promote inhibitory signaling via inhibition of calcium (Ca^{2+}) influx through voltage-operated calcium channels [58, reviewed in 85, 127]; effects on several K+ channels, including the small conductance calcium-activated K+ type 2 channel and inward rectifying K+ channels [7,102], as well as, multiple other ligand-gated and non-gated ion channels, reviewed elegantly in 45,88.

Chronic alcohol exposure produces changes reflective of neuronal adaptation to restore homeostasis of excitatory and inhibitory neurotransmission, some of which may occur in a sex-dependent manner and which may impact withdrawal-induced neurotoxicity. Specifically, sex differences in neuroadaptation of glutamatergic, GABAergic and adenosinergic receptor systems have been observed with use of preclinical models. It is evident that chronic exposure produces an upregulation of NMDA receptor density and/or function. Specifically, western blot analysis has demonstrated an increase in the protein expression of the NMDA receptor subunits GluN1, GluN2A, and GluN2B [28,57,71]. An increase in the GluN2B mRNA content (but not GluN1 of GluN2A mRNA) has also been observed after both continuous chronic [66, 136] and chronic intermittent alcohol exposure [136]. Further, changes in synaptic content and/or sensitivity of NMDA receptors may result from changes in trafficking of subunits or alternative splicing [24,30,146]. With regard to sex differences, there is evidence of sex differences in adaptation or plasticity of NMDA receptor subunits that may impact the susceptibility to alcohol withdrawal-associated excitotoxicity. Alele and Devaud [5] demonstrated that after three days of withdrawal from a chronic alcohol exposure regimen, female rats demonstrated greater levels of glutamate transporters GLT-1 and EEAC1, possibly suggesting differences in glutamate clearance from the synapse, though the implications of these findings for neurotoxicity have not been evaluated. Further, Devaud and Alele [40] reported persisting increases in GluN1, GluN2A and GluN2B subunit density in hippocampus of male rats following 9 days of alcohol intake via a liquid diet. Though females showed greater density of GluN2B subunit in cerebral cortex after this regimen, the hippocampal changes in GluN subunit were correlated with greater seizure susceptibility in male rodents during early withdrawal [42], particularly when considered in kind with evidence of sex-dependent changes in $GABA_A$ receptor subunits following alcohol exposure, discussed below. As noted above, changes reported above have not been linked to overt neuronal injury and may well be independent of injury, rather reflecting susceptibility to withdrawal seizures. As noted by Devaud and Alele [40] these sex differences in subunit changes observed after alcohol exposure are highly complex and do not clearly correlate with predicted behavioral responses to excitatory amino acid modulators during withdrawal. Rather, such data provide for a critically important foundation to a literature that continues to develop.

Changes in $GABA_A$ receptors upon chronic alcohol exposure show effects dependent on subunit peptide composition, and evidence of sex differences in responses to alcohol does exist. Chronic alcohol treatment upregulates some β polypeptide subunits of the $GABA_A$ receptor at some times points of intake or withdrawal, but also downregulates expression of the α_1, α_2, and α_5 subunits in rat cerebral cortex in a time-dependent manner [40, 92]. Devaud and Alele [40] reported evidence of increased β_2/β_3 density in cerebral cortex of female, but not male, rats after 9 days of alcohol intake via liquid diet and extended withdrawal. These subunits are thought be important in providing intra-membrane stability of $GABA_A$ receptors [reviewed in 50]. They interpreted their findings to suggest that this might reflect an increase in membrane-bound receptors, thus, providing greater basal inhibitory tone following alcohol exposure. However, their findings did not correlate readily with $GABA_A$ receptor function in females during alcohol withdrawal [discussed in 41].

Further, Devaud and Alele [40] have demonstrated an intriguing pattern of sex differences in regional changes in α4 subunit content following prolonged alcohol exposure. Male rats exposed to alcohol for 9 days via a liquid diet had markedly greater levels of α4 subunit of the $GABA_A$ receptor in both hippocampus and cortex, as compared to females. Notably, these elevations were maintained well into alcohol withdrawal and were postulated to be associated with reduced current at synaptic $GABA_A$ receptors, possibly contributing to the greater sensitivity of males to withdrawal seizure. Though this is still a possibility, more recent work has suggested that a lack of consensus exists with regard to effects of alcohol-induced changes in α4 and other $GABA_A$ subunit levels on development of alcohol dependence and/or withdrawal [reviewed in 16,17]. However, there are clear sex differences in behavioral responses during alcohol withdrawal that could theoretically be linked to differences in GABAergic signaling. For example, Reilly et al. [121] demonstrated that male rats show greater acoustic startle responses than females during withdrawal. Also, Koirala et al. [75] reported clear differences between male and female rats regarding tolerance to the motor incoordinating effects of acute alcohol administration during acute and protracted alcohol withdrawal. These findings provide clear evidence of diverse sex differences in neuroadaptation to prolonged alcohol intake, though the exact molecular underpinnings of these differences are unclear. Also, as noted above, there is no clear relationship established in the literature between withdrawal behavioral symptoms, including seizure, and overt neurotoxicity in either sex. Thus, there is a need to more thoroughly investigate links that may (or may not) exist between behavioral abnormalities observed during withdrawal, including seizure and neurotoxicity, particularly with regard to multiple withdrawals.

Alcohol Withdrawal Effects on the Brain: A Unique Role for NMDA Receptors in Promoting Neurotoxicity

Neuronal adaptation in function, affinity, and/or density of NMDA receptors, GABA receptors, Ca^{2+} channels or myriad other membrane or cytosolic proteins produced by chronic alcohol exposure has been long implicated to play a causal role in alcohol withdrawal syndrome (reviewed in 54,63, 67, 114]. That is, chronic alcohol exposure is postulated to induce changes in synaptic plasticity that initiate neuronal hyperexcitability during withdrawal [eg. 60]. Further, withdrawal from long-term in vivo alcohol exposure (13 months), as compared to alcohol exposure and no withdrawal, has been shown to result in significantly greater hippocampal cell death in the pyramidal cell layers [91]. Chronic alcohol exposure also decreases the rate of glutamate transport which causes increased levels of extracellular glutamate during alcohol withdrawal. Melendez et al. [97] observed increased extracellular glutamate concentration in the nucleus accumbens at 24 hours of alcohol withdrawal following 7 day chronic alcohol exposure in rodents, suggesting possible direct effects on anatomical regions associated with alcohol craving. Behavioral data collected in rodents supports NMDA receptor-mediated neuronal hyperexcitability, as it has been demonstrated that alcohol-dependent rats administered both NMDA (given intracerebroventricularly or intraperitoneally (IP)) and kainic acid (given IP) were more susceptible to seizure activity at 9 and 24 hours of alcohol withdrawal [129], than were non-dependent rats. Additionally, handling-induced seizures, audiogenic seizure severity, and overall seizure occurrence can be attenuated dose-dependently by the uncompetitive NMDA receptors antagonists MK-801 [100] and MRZ 2/579 [12]. In hippocampal explants, we have shown that NMDA receptor MK-801 exposure blocks the neuronal cytosolic Ca^{2+} accumulation and neurotoxicity produced by 24 hours of alcohol withdrawal in CA1 region pyramidal cells (eg. 57,103,113]. Similarly, Nagy and colleagues [105] demonstrated the efficacy of selective GluN2B antagonists in reducing neurotoxic effects of alcohol withdrawal in primary cortical neurons. Perhaps most importantly, the uncompetitive NMDA receptor antagonist memantine has demonstrated efficacy in reducing the severity of alcohol withdrawal in humans, in a manner similar to that of diazepam [77]. It remains to be

determined if sex differences in response to this and/or other pharmacological agents during withdrawal exist.

Evidence of sex differences in the neuroadaptation of NMDA and $GABA_A$ receptors to alcohol, discussed above, may correlate most closely with sex differences in seizure susceptibility. However, some evidence does exist suggesting that differences in NMDA receptor subunit function impact alcohol withdrawal associated excitotoxicity differently in the sexes. For example, we have observed significant sex differences in hippocampal subregion sensitivity to the GluN2 subunit modulating polyamine spermidine, suggesting that female hippocampus is more susceptible to withdrawal excitability and neurotoxicity in hippocampal explants. This suggests a discord between sex differences in sensitivity to withdrawal seizure and neurotoxicity. GluN2 subunits confer sensitivity to the endogenous polyamines putrescine, spermine, and spermidine which function to allosterically promote channel opening [154]. These polyamines are small, aliphatic molecules that affect multiple membrane proteins in mediating cell proliferation, immune responses, function of excitatory amino acid receptors and response to injury, both in the CNS and peripheral tissues (for review, 114,154]. While no sex difference existed in basal alcohol withdrawal hippocampal neurotoxicity, it was found that application of exogenous spermidine produced a significant increase in hippocampal CA3 pyramidal cell death that was markedly elevated in explants obtained from neonatal female rats [113]. Further, it was recently demonstrated that this unique sensitivity of the female hippocampus to neurotoxic effects of a polyamine during alcohol withdrawal was dependent on some degree of tissue maturation [9], as it was not observed in hippocampi obtained from early neonatal animals. Importantly, this suggests a hormone-independent sex difference rendering portions of the female hippocampus more sensitive to excitatory effects of GluN2 subunit modulators. It will be of importance to examine the extent to which these phenomena, observed with use of preclinical models, are observed in tissues of human alcohol-dependent individuals. Lastly, Butler et al. [22,23] reported that hippocampal slice cultures derived from neonatal female rats, and in particular the pyramidal cell layer of the CA1 region, demonstrated more neurotoxicity during alcohol withdrawal when exposed to the adenosine A1 receptor selective antagonist 8-Cyclopentyl-1,3-dipropylxanthine or the non-selective A1 antagonist caffeine. Perhaps most importantly, it was demonstrated that this sex-specific form of neurotoxicity was prevented by co-exposure to the competitive NMDA receptor antagonist D,L,-2-amino-5-phosphovalerate. These findings demonstrate a greater sensitivity of female hippocampi produced by relief of tonic A1 inhibition (via relief from Gi or G_0 modulation, reviewed in 21) during alcohol withdrawal and downstream activation or supersensitivity of NMDA receptors to endogenous ligand binding. These findings, considered with those demonstrating specific sex differences in GluN2 subunit sensitivity during alcohol withdrawal discussed above may provide for a putative molecular target to be exploited in moderating alcohol withdrawal, in a sex-specific manner.

Sex Differences in Hormonal Responses and Alcohol Withdrawal

A comprehensive discussion of sex differences in neuroadaptation to alcohol requires discussion of evidence that the hypothalamic-pituitary-adrenal (HPA) axis and possibly brain steroid synthesizing pathways may be differently altered by prolonged alcohol exposure (reviewed in 44) and that these changes may influence withdrawal seizure susceptibility and possibly, neurotoxicity. Alcohol itself induces synthesis of neuroactive steroids that modulate $GABA_A$ activity both as a result of HPA axis activation [150] and due to brain steroidogenesis [130], effects that contribute to the behavioral depressant effects of alcohol, including anticonvulsant effects. Others have reported that combined adrenalectomy and gonadectomy increased the severity of handling-induced convulsions (HICs) during acute alcohol withdrawal in male C57BL/6J (B6) and DBA/2J (D2) mice, as

well as female D2 mice [51]. In withdrawal seizure prone and resistance mice, this same surgical procedure exacerbated HICs during withdrawal from administration of a single alcohol dose in females, but not males [141]. Findings consistent with this suggestion demonstrated that the anti-convulsant effects of the neuroactive steroid pregnanalone were more prominent in female rats than in males [6]. Gorin-Meyer et al. [53] provided evidence that female rodents are more sensitive to the withdrawal-promoting effects of neuroactive steroid loss (via use of the 5 alpha reductase inhibitor finasteride), suggesting a sex-specific role for neuroactive steroids in moderating withdrawal severity in females. One interpretation of these studies presented by Finn and colleagues is that the surgical procedure resulted in reduced levels of neuroactive steroids known to moderate $GABA_A$ receptor function, though as noted above, brain steroidogenesis may also influence the severity of withdrawal.

There also exists intriguing evidence of sex differences in corticosterone signaling following prolonged alcohol intake. However, the issue of corticosterone elevations in either sex during alcohol exposure and/or withdrawal is highly contentious as conflicting findings have been reported. It has been known for several decades that alcohol administration acutely elevates corticosterone secretion [47], resulting from activation of the HPA axis [80,119]. However, the consequences of this phenomenon are still not fully understood. In fetal alcohol exposed rodents, highly complex and sometimes contradictory alterations in HPA axis activation are observed in male and female offspring, including differences in the timing and magnitude of HPA axis activation, as well as, hormonal responses to qualitatively different types of stressors [reviewed in 161]. Alele and Devaud [6] reported that adult outbred male but not female rats had markedly elevated plasma corticosterone levels during withdrawal from 14 days of alcohol (6 %) intake that were correlated with enhanced seizure susceptibility following pentylenetetrazol injection. Similar findings were previously reported by Janis et al. [69]. We have recently reported 4-5 fold increases in plasma corticosterone during acute withdrawal from a 4-day alcohol binge regimen [135]. In contrast, Silva and Medeira [137] reported no differences in plasma corticosterone levels among control rats; those consuming 20% alcohol solution for 6 months; or those undergoing alcohol withdrawal. Many studies exist which demonstrate either increased corticosteroid levels during withdrawal or blunted HPA responses reflected in reduced corticosteroid levels or levels similar to those of controls, in both preclinical and clinical studies [eg. 1,123, reviewed in 114]. Adinoff and colleagues, in particular, provide compelling evidence of possible sex differences in HPA axis function during withdrawal/abstinence. They reported that in a small sample of abstinent alcohol-dependent females, no alterations in adrenocorticotropic hormone or cortisol were observed. They earlier reported increased salivary cortisol levels in both alcohol-dependent males who were either intoxicated or in acute withdrawal [3]. Thus, while there remains a lack of uniformity regarding data describing effects of alcohol intoxication and withdrawal on HPA axis function in either sex, evidence does exist that such sex differences may exist, with moderating variables (eg. duration of drinking history, comorbidity, rodent strain, age) yet to be fully characterized. With regard to neurotoxicity, it is known that corticosteroids promote expression and/or trafficking of NMDA receptors in a glucocorticoid receptor (GR)-dependent manner [reviewed in 114] and that this influence promotes alcohol withdrawal hippocampal neurotoxicity *in vitro* [103]. Sousa et al. [138] demonstrated a link between chronic stress or corticosterone administration and microstructural hippocampal deficits, as well as, impaired spatial working learning and memory. However, the limited evidence that does exist does not suggest that this is a sex-dependent effect, at least in the hippocampal formation. This understudied area shows significant promise given recent evidence that mifepristone, which is in part an antagonist at GRs, reduced the severity of alcohol withdrawal in male rats [135]; reduced voluntary intake of alcohol in rodents under limited access [74]; attenuated binge alcohol-induced neurodegeneration in rodents [29]; and

reduced compulsive responding and escalated alcohol intake subsequent to chronic, intermittent alcohol vapor exposure in male rodents [152].

Conclusions

Findings from both preclinical and clinical studies suggest the possibility that profound sex differences may exist regarding neuroadaptation of amino acid signaling systems and the HPA axis to chronic alcohol intake and that these differences may influence the topography of alcohol dependence in both sexes; the severity of alcohol withdrawal; and possibly, withdrawal neurotoxicity. It is clear that significant contradiction or at least a lack of characterization of many moderating variables exists. There is a need to clarify the presence or absence of sex differences with regard to progression to dependence and alcohol-related neurodegeneration. Further, the role that hormonal status has in influencing neuroadaptation to alcohol and withdrawal is unclear, owing in part to the disruptive effects of prolonged alcohol intake on steroidogenesis and cyclicity [eg. 5]. As a critical outcome, expanding our knowledge in these, and other relevant, areas has the potential to significantly impact our predictions about the topography of alcohol dependence in males and females; assessment of risk related to dependence; and to guide the development of novel biologically-based approaches to treat alcohol dependence and related organ pathologies.

Acknowledgments

The authors acknowledge the support of AA013388, AA013561, AA001274, and AA018588. We also acknowledge the many achievements of Daniel W. Hommer, M.D.

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