

Commentary

Stress and Alcohol Priming of Brain Toll-Like Receptor Signaling in Alcohol Use Disorder

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Toll-like receptor signaling in brain is emerging as an important contributor to the pathology of stress and alcohol use disorders (AUDs). Toll-like receptor 4 (TLR4), a member of the interleukin-1 receptor/toll-like receptor (TLR) superfamily is expressed in brain. Microglia have basal expression, whereas neuronal and astrocyte expression increases the following priming by stress or alcohol. The endotoxin-LPS receptor discovered to be TLR4 in the late 20th century, more recently, has been found to respond to the endogenous agonist, high mobility group box 1 (HMGB1). Studies in rodents find ethanol exposure increases brain expression of both HMGB1 and TLR4 as well as other neuroimmune genes in association with increased ethanol drinking (Blednov *et al.*, 2012) and neurodegeneration (Coleman and Crews, 2018). Further, human studies find increases of TLR4 and HMGB1 in post-mortem brain of individuals with AUD that positively correlate with life-time alcohol consumption (see Fig. 1; Crews *et al.*, 2017).

Evidence suggests that ethanol priming of HMGB1-TLR4 signaling occurs through multiple mechanisms including direct ethanol induction in brain cells as well as brain responses to systemic immune signals. Excessive alcohol and stress can increase gut 'leakage' of endotoxin into the blood that increases circulating immune signals. These signals may both prime brain HMGB1-TLR4 signaling as well as increase craving for alcohol in individuals with AUD (Temko *et al.*, 2017; Leclercq *et al.*, 2018). Cycles of binge drinking and stress have been suggested to increase the risk for development of AUDs through sensitization or priming of synaptic circuitry increasing craving and risks for AUD. Alcohol- and stress-induced increases in TLR4 and HMGB1 may sensitize specific neurocircuits through autocrine and paracrine signaling across neurons and glia within a brain region. HMGB1-TLR4 signaling is emerging as a mechanism of innate immune memory, which in brain is plasticity related to experience that could be a mechanism of allosteric adaptation that is proposed to drive alcohol seeking (see Fig. 1). Innate immune signals in brain lead to persistent and progressive synaptic changes, most often enhancing excitability.

Seizures provide an example of the persistent and progressive changes due to increased HMGB1-TLR4 expression in hippocampus. Epilepsy and hippocampal seizures provide an example of

HMGB1-TLR4 synaptic plasticity. An initial seizure is known to cause a reduction in seizure threshold that increases risk of subsequent seizures and which persists and progresses with each additional seizure. Hippocampal seizures increase HMGB1-TLR4 expression that endures and lowers seizure threshold through excitation-induced neuronal release of HMGB1 triggering TLR4 glutamate release increasing excitability (Maroso *et al.*, 2010). Similarly, exposure to stress and alcohol causes modest changes in HMGB1 and TLR4 expressions that persist, and with repeated exposures progressively sensitize responses of neurocircuits.

HMGB1 and TLR4 expressions in post-mortem prefrontal cortex (PFC) of individuals with AUD are increased and correlated with life-time alcohol consumption, but moderate-drinking controls do not show a relationship with life-time alcohol consumption; perhaps a seizure-like threshold of binge drinking or stress are needed to induce plasticity (see Fig. 1). Individuals with AUD show a broad range of consumption that is considerably greater than control moderate drinkers. The progressive and persistent development of AUD is consistent with binge drinking- and stress-induced progressive and persistent increases in HMGB1-TLR4 signaling. However, there are multiple innate immune signaling molecules, many of which are increased by HMGB1-TLR4 signaling that could also contribute to the development of AUD. Further, cycles of moderate ethanol exposure have been found to prime anxiety-like behavior through this signaling (see below). How this signaling contributes to changes in synaptic circuitry that might contribute to AUD is poorly understood and needs further investigation. In this issue of *Alcohol and Alcoholism*, two articles explore TLR4 interactions, one rat study focused on amygdala GABA–corticotropin-releasing factor (CRF) responses to repeated stress and one human study on how TLR4 gene methylation impacts subjective responses to alcohol.

The study by Karolya *et al.* (2018) finding interactions between TLR4 methylation and alcohol subjective and physiological responses extends previous studies reporting hyper-methylation of the TLR4 receptor DNA in individuals with AUD. In this study over 200 subjects were divided into heavy drinkers, defined as greater than 14 drinks per week or light-moderate drinkers, and the level of TLR4 DNA CpG site methylation, a known regulator of gene

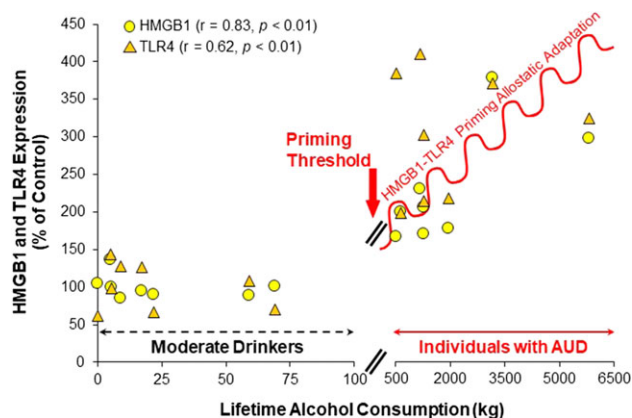


Fig. 1. HMGB1 and TLR4 expressions in post-mortem PFC of moderate drinkers and individuals with AUD. Shown are cellular expressions in orbital frontal cortex determined using immunohistochemistry and the life-time alcohol consumption in kilograms of absolute alcohol as determined by New South Wales Brain Research Center for moderate-drinking controls and individuals with AUD. Note: Individuals with AUD consume from 5 to 50 fold more alcohol than moderate drinkers. A priming threshold of about 100 kg life-time consumption represents less than one drink per day and controls show no induction of HMGB1 or TLR4. In contrast, individuals with AUD have progressive increases with increasing alcohol consumption. These findings are consistent with multiple cycles of binge drinking contributing to the increased HMGB1-TLR4 found in AUD that likely primes neurocircuitry contributing to subjective responses to alcohol and the accumulation of stress- and alcohol-induced withdrawal anxiety (adapted from Crews *et al.*, 2013).

expression, was assessed. Individuals were also assessed for subjective and physiological responses to i.v. alcohol exposure to blood alcohol levels up to 0.06 g/dl (i.e. a moderate, but not binge drinking blood alcohol concentration). Heavy drinkers had positive associations between TLR4 DNA methylation and subjective stimulation, tension and arousal. Light drinkers had the opposite response. These studies suggest a relationship between TLR4 methylation and subjective physiological responses to acute alcohol intoxication. Acute ethanol can release HMGB1 from neurons that can trigger TLR4 signaling impacting subjective responses to alcohol. However, preclinical studies find HMGB1-TLR priming occurs at blood alcohol concentrations (BACs) higher than those that can be easily studied in humans. Karoly *et al.* infused alcohol to 0.06 g/dl while determining subjective responses. One would expect subtle changes at BACs below the binge drinking level and they cleverly use regression analysis to disclose subtle differences in arousal and positive feelings with TLR4 methylation. TLR4 methylation and subjective responses to alcohol were found to be dependent upon regular drinking levels, with higher regular drinking being associated with arousal and more positive subjective responses to alcohol. Studies of individuals with AUD find increased DNA methylation of multiple genes, but this study focused on a single gene (i.e. TLR4) in non-alcoholic drinkers that is consistent with TLR4 signaling contributing to subjective responses to alcohol in all individuals.

Varodayan *et al.* (2018) assessed synaptic processes underlying the effects of CRF on the central nucleus of the amygdala (CeA) GABA_A-mediated spontaneous inhibitory post-synaptic currents (sIPSCs). Wild-type (WT) and TLR4-KO rats were used to test the hypothesis that TLR4 plays a role in CRF modulation of GABA in the CeA. No differences in baseline responses were found between

WT and TLR4-KO in baseline sIPSCs or CRF increased sIPSCs. Restraint stress did not cause a change in either WT or TLR4-KO. The lack of difference in responses is consistent with the idea that priming of HMGB1-TLR4 signaling by stress or alcohol is needed to observe the role of TLR4. Interestingly, although single or repeated stress did not alter sIPSCs in WT or TLR4-KO, it did change the CRF-induced increase in sIPSCs following repeated stress. CRF increases IPSCs, but repeated stress led to a loss of the CRF potentiation in the TLR4-KO animals. CRF in amygdala is primed by stress and alcohol. These findings are consistent with repeated stress priming HMGB1-TLR4 signaling that sustains CRF increased GABAergic sIPSCs. Breese and colleagues (2016) have reported that multiple cycles of moderate ethanol consumption and stress in rats increase amygdalar excitability and ethanol withdrawal anxiety-like behavior. HMGB1, other neuroimmune signaling molecules, and CRF increase with cycles of exposure that shows parallel increases in ethanol withdrawal anxiety-like behavior. They found that injection of TLR4 agonists, other immune signaling molecules, or CRF mimics a cycle of moderate ethanol exposure in the priming of anxiety consistent with immune-CRF signaling increasing anxiety-like behavior. Further, ethanol withdrawal anxiety is blocked by CRF and HMGB1 antagonists (Whitman *et al.*, 2013). This is consistent with the findings presented by Varodayan *et al.* that the TLR4-KO did not alter sIPSPs after a single stress, but did alter sIPSPs after multiple stress exposures. These studies suggest that TLR4-CRF interactions in amygdala require priming to observe a change. Controls show no effect since they are not primed by cycles. These studies further support the role of cycles of stress or alcohol contributing to increases in alcohol withdrawal-induced anxiety. Thus, the studies reported here extend the link between amygdala HMGB1-TLR4 signaling, CRF and GABA synaptic sIPSCs that likely contribute to withdrawal anxiety and possibly craving for alcohol.

The above mentioned two papers in this issue of *Alcohol and Alcoholism* on TLR4 receptors relating human subjective responses to alcohol and rat stress synaptic circuitry continue to support the role of HMGB1-TLR4 signaling in stress and alcohol-induced pathologies.

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