Biological Psychiatry: Celebrating 50 Years

# Commentary

# Connecting the Dots: Adolescent Alcohol, Enhancer RNA, and Anxiety

Jennifer T. Wolstenholme and Michael F. Miles

The overwhelming drug of choice for abuse by adolescents is alcohol, and when they drink, it is often in intermittent binges, consuming more than four drinks in a few hours (1). Considerable epidemiological evidence documents that adolescent exposure to ethanol can increase the risk for drug dependence, affective disorders, or cognitive impairment in adulthood (2). Similar results from adolescent ethanol exposure have been found in animal models (3) and also occur with other drugs of abuse (4). Such findings document that adolescence is a critically sensitive developmental period whereby ethanol can evoke long-lasting changes in brain function, possibly due to alterations in ongoing synaptic pruning and maturation of myelinated fibers. However, the molecular mechanisms underlying how adolescent ethanol might evoke persistent alterations in brain function and behavior in adulthood remain unclear.

Epigenetic regulation of gene transcription represents an attractive potential mechanism driving long-term neuroadaptations following ethanol binges during adolescence. Histone modifications (i.e., acetylation and methylation) and DNA methylation positively and negatively regulate gene transcription and are possibly one of the main upstream regulators of ethanol-induced transcriptional reprogramming (5). These modifications work in concert and can dynamically change in response to external stimuli, such as exposure to drugs of abuse (3,6,7). Several research groups have made important strides toward understanding the roles of swift and dynamic histone acetylation and methylation changes in modulating the early molecular processes involved in the expression of ethanol consumption and anxiety-like behavior following ethanol binges during adolescence. For example, intermittent ethanol exposure leads to global increases in *Hdac2* and *Hdac4* expression, and decreases in histone H3 lysine 9 acetylation (H3K9ac) in the central nucleus of the amygdala are associated with decreased dendritic spine density in adulthood (7). Furthermore, systemic administration of histone deacetylase inhibitors attenuated anxiety-like behavior and increased ethanol consumption observed after adolescent ethanol exposure (3,7). Importantly, these epigenetic therapeutic effects were only seen with adolescent ethanol exposure, and adult-exposed behaviors were not affected by histone deacetylase inhibition. Unfortunately, many of these studies have focused on global epigenetic mechanisms within the brains of these ethanol-exposed adolescents, and the exact signaling targets whereby these epigenetic modifications act have not been previously identified.

In the current issue of *Biological Psychiatry*, Kyzar *et al.* (8) present data showing that adolescent intermittent ethanol (AIE) decreased the number of synapses and reduced the expression of genes involved in synaptic plasticity in the central and medial nuclei of the amygdala in adult rats. Focusing on the activity-regulated cytoskeletal-associated protein gene *Arc* as a potential target underlying reduced synapse levels in adult rats, this report outlines a series of mechanistic findings that connect initial epigenetic alterations in upstream regulatory sites of the *Arc* gene with subsequent changes in *Arc* expression and behavior. Most intriguingly, the investigators also implicated a novel mechanism involving enhancer RNA (eRNA) production in AIE-induced changes in adult anxiety and gene expression. Together, these findings identify a detailed series of epigenetic events evoked by AIE having important implications on long-term behavioral alterations in adulthood.

Earlier work by Pandey *et al.* (7) and Kyzar *et al.* (9) demonstrated that AIE induced decreases in *Bdnf* and *Arc* expression, altered histone modifications, and caused corresponding decreases in the dendritic spine density in amygdala of male adult rats, along with increased anxiety and ethanol intake. Therefore, the current report focused on mechanisms of *Arc* expression because this is a known downstream target of brain-derived neurotrophic factor and is an important modulator of synaptic plasticity. Importantly, *Arc* is known to be regulated by synaptic activity through interactions between an upstream synaptic activity response element (SARE) and the *Arc* promoter via a novel mechanism involving eRNA transcription from the SARE site and decoying of a transcription repressor, negative elongation factor, by binding to the *Arc* SARE eRNA. Recent studies have suggested that eRNA, possibly through multiple complex actions, may be involved in the regulation of transcriptional profiles associated with behavioral neurological diseases, such as autism and schizophrenia (10). However, no previous work has implicated eRNA expression in any behavioral responses to ethanol or other drugs of abuse in either adolescents or adult animals.

Through a multilayered analysis at the level of messenger RNA (mRNA) or protein expression, and both transcription activator and repressor DNA-binding protein analysis, the current report by Kyzar *et al.* (8) showed striking parallel molecular responses in the central and medial nuclei of the amygdala but not the basolateral amygdala. These involved decreased expression for *Arc*, *Arc* SARE eRNA, and the *Kdm6b* demethylase, and accompanying increased anxiety-like behavior in adult rats that were exposed to AIE. Lysine demethylase (KDM6B) is known to remove repressive methylation on H3K27me2/3, thus activating expression of synaptic activity–related genes such as *Arc*. The net expected outcome of AIE-induced decreases in KDM6B and *Arc* SARE eRNA expression were documented by chromatin immunoprecipitation analyses, showing increased H3K27me3 and negative elongation factor E occupancy at the *Arc* promoter,

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https://doi.org/10.1016/j.biopsych.2019.04.004
ISSN: 0006-3223

along with expected coordinate decreases in occupancy by KDM6B and the activating histone mark, H3K27ac.

The difficulty in interpretation of such complex bimodal changes in mRNA/protein expression or chromatin binding by multiple factors regards showing causality for such events rather than merely identifying correlated responses. Importantly, this report by Kyzar *et al.* (8) used three different approaches to infer causality in AIE between chromatin alterations: *Arc* SARE eRNA, *Arc* expression, and the behavioral response of increased anxiety in adult animals. An initial detailed correlation analysis used the ability of acute ethanol exposure to decrease anxiety, counteracting the response to AIE. Therefore, AIE-exposed adult animals treated with acute ethanol saw coordinate normalization of anxiety behavior, *Arc* mRNA, *Arc* SARE eRNA, Kdm6b mRNA, ARC protein and KDM6B protein, and chromatin immunoprecipitation profiles for all DNA binding proteins studied. Although still only a correlation analysis, the highly coordinated molecular and behavioral response across so many interacting factors is striking.

However, the reversal of AIE responses by acute ethanol raises other questions. The duration of such reversal responses in adult animals with acute ethanol was not documented by Kyzar *et al.* (8). Presumably, the alterations in *Bdnf*/*Arc* expression in the amygdala after AIE are generating increased anxiety and thus driving risk for increased ethanol consumption. Indeed, animal model studies have found increased consumption after AIE (7). If ethanol consumption reversed such alterations for a prolonged period, then the disease would be self-limiting in terms of the anxiety component contribution. Therefore, future studies are needed to determine how long these alterations in amygdala *Arc* expression persist in adulthood, particularly after re-exposure to ethanol.

A more definitive and elegant causal argument was made by Pandey *et al.* (7): direct downregulation of either KDM6B or *Arc* SARE eRNA in ethanol-naïve animals using amygdala infusion of small interfering RNA or antisense locked nucleic acid, respectively. In both cases, the treatments phenocopied the effect of AIE exposure in terms of behavioral responses, gene expression, and chromatin occupancy. Such studies provide the first direct linkage of alternations in eRNA expression with ethanol behavior.

This detailed analysis by Kyzar *et al.* (8) provides highly complex and novel insight into mechanisms whereby AIE might produce altered risk for excessive ethanol consumption or affective disorders in adulthood, at least in male rats. Future studies are needed to identify whether the same events are operative in females and with other drugs of abuse. The implication of *Arc* SARE eRNA as a mechanism in epigenetic regulation of gene expression after AIE is both highly novel and potentially significant for understanding molecular mechanisms of ethanol-induced plasticity as a whole. *Kdm6b* expression might be the driving force for *Arc* SARE eRNA expression changes, but exactly how AIE might alter *Kdm6b* expression is not definitively known. Alterations in the epigenetic landscape of the *Arc* SARE and promoter sites might initiate changes in eRNA expression that drive subsequent changes in *Arc* expression, but specific eRNA expression itself might provide a more selective molecular target for future intervention strategies than efforts to alter global chromatin modifications, such as histone acetylation. The potential ability to selectively reverse an epigenetically driven alteration in expression of genes key to disrupted behavioral plasticity might provide a novel long-term intervention for alcohol use disorder.

## Acknowledgments and Disclosures

This work was supported by National Institute on Alcohol Abuse and Alcoholism Grant Nos. AA026347 (to JTW) and AA022537 (to JTW and MFM).

The authors report no biomedical financial interests or potential conflicts of interest.

## Article Information

From the Departments of Pharmacology and Toxicology (JTW, MFM) and Neurology (MFM) and the Virginia Commonwealth University Alcohol Research Center (JTW, MFM), Virginia Commonwealth University, Richmond, Virginia.

Address correspondence to Michael F. Miles, M.D., Ph.D., Scientific Director, Virginia Commonwealth University Alcohol Research Center, Departments of Pharmacology and Toxicology and Neurology, Virginia Commonwealth University, Box 980613, Richmond, VA 23298; E-mail: Michael.Miles@vcuhealth.org.

Received Apr 2, 2019; accepted Apr 3, 2019.

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