

# Binge Drinking's Effects on the Developing Brain—Animal Models

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Adolescence typically is a time of experimentation, including alcohol use and, particularly, binge drinking. Because the brain is still developing during adolescence, such exposure could have long-lasting effects. Animal models and adolescent intermittent ethanol exposure (AIE) paradigms have been used to help elucidate the consequences of adolescent binge drinking. These studies have identified cognitive deficits, particularly in challenging cognitive tasks, and behavioral alterations such as greater risk preferences, impulsivity, and disinhibition. AIE also is associated with changes in affect when the animals reach adulthood, including increased social anxiety and, sometimes, general anxiety. Animal models have demonstrated that AIE can result in retention of certain alcohol-related adolescent phenotypes (i.e., reduced sensitivity to alcohol's aversive effects and increased sensitivity to alcohol's rewarding effects) into adulthood, which may motivate continued elevated alcohol use. The detrimental effects of adolescent alcohol exposure extend to a diversity of lasting alterations in the brain, including reduced neurogenesis, increased proinflammatory responses, changes in gene expression through epigenetic mechanisms, and alterations in the activities of various neurotransmitter systems. Further exploration of these mechanisms in animal models and humans may lead to improved prevention and intervention efforts.

**Key words:** Adolescence; alcohol exposure; alcohol use disorder; animal studies; binge drinking; brain development

Adolescence typically is a time of experimentation and emulation of adult behaviors, and many adolescents initiate alcohol and other drug (AOD) use during this developmental period. Brain development continues during adolescence, which could render the adolescent brain particularly vulnerable to alcohol's effects. Consequently, adolescent alcohol exposure could result in long-lasting changes in neuropsychological function and increased risk of developing alcohol use disorder (AUD). To better understand and minimize these risks, it is crucial to comprehensively study alcohol's impact on the adolescent brain. Such studies in humans face a number of challenges, however. For example, ethical constraints prevent the administration of alcohol to underage youth.

Moreover, in human adolescents it is difficult to discern whether observed correlations between alcohol use and the behavioral or neuropsychological measures under investigation reflect causes or consequences of alcohol use or are purely coincidental. Finally, despite significant progress in noninvasive imaging technologies, the complexity of the human brain and technical limitations of brain analyses hamper researchers' abilities to fully investigate how alcohol influences adolescent brain structure and function.

Animal models using laboratory animals such as mice and rats can help circumvent some of these problems. However, their use also is associated with certain limitations. Most importantly, no currently available animal model can fully represent complex

human behaviors such as alcohol use and addiction. Certain factors that influence adolescent neurobehavioral function and AOD misuse are not amenable to analysis using animal models, including variables such as verbal ability and language, and influences such as self-esteem, culture, media, or even parenting styles. Despite these limitations, much of what is currently known about the intricacies of brain development, neural substrates of AOD use and misuse, and adolescent responses to AODs has been obtained using animal models. This article summarizes some of the characteristics of animal models for studying alcohol's effects on the adolescent brain and reviews the findings of studies using those models that have shed light on functional and structural alterations

associated with adolescent alcohol use, the alcohol-induced persistence of adolescent phenotypes into adulthood, and the impact of adolescent alcohol use on later alcohol consumption.

## Characteristics of Animal Models

The potential usefulness and validity of animal models, especially for complex behaviors such as alcohol misuse and its consequences, depend primarily on the specific research questions being asked. The validity of such models can be assessed on three levels:<sup>1</sup>

- Face validity assesses whether the phenomenon under study in the model resembles the targeted human behavior in terms of its behavioral, cognitive, and physiological features. However, it is important to realize that even if certain behaviors or other effects appear similar across species, they may not share the same underlying mechanisms.
- The measure of construct validity focuses on the relevance of the phenomenon under investigation in the animal model to the concept being modeled. Investigators seek to determine how similar the animal model is to the biological foundation and neural underpinnings of the human behavior being modeled. This concept also considers the impact of moderators, such as previous experiences or the environment.
- The concept of predictive validity reflects how effectively the animal model predicts experimental findings or treatment outcomes in humans.

Assessment of the validity of animal models of adolescent alcohol consumption and its consequences is an ongoing, iterative process, as research in these areas escalates in both human adolescents and laboratory animals. The current research supports cautious optimism in the use of such models. For example, findings have shown signs of consilience between human

adolescents and rodent models of adolescence when comparable assessment measures of alcohol sensitivity and consequences were used.<sup>2</sup>

## Animal Models of Alcohol Use and Its Consequences

One of the main factors for using rodent animal models for alcohol research is that these animals voluntarily self-administer AODs when given free access. For example, rodents often orally self-administer substantial amounts of alcohol, particularly if they are offered beer or sweetened beverages. Laboratory animals and humans exhibit similar behavioral and cognitive responses to acute AOD administration. Laboratory animals effectively model a broad diversity of alcohol effects seen in humans, ranging from euphoria and social stimulation at low alcohol levels to intoxication, motor impairment, sedation, and memory impairment at higher doses.<sup>3</sup> In addition, animals that are chronically exposed to AODs can develop physical dependence, characterized by dysphoria and physical signs of withdrawal (e.g., tremor, anxiety, insomnia, and even seizures) when access is terminated.<sup>4</sup> Such physical dependence can be accompanied by a tendency for relapse, particularly after re-exposure to the drug or exposure to stressors or drug-related cues. Experiments that used a conditioned place preference approach demonstrated that laboratory animals, even without physical dependence, can develop a preference for contextual cues associated with drug use.

Not only are the behavioral consequences of alcohol exposure often similar in humans and in animal models, but the neural substrates underlying these effects also exhibit across-species similarities. Numerous studies have identified sufficient similarities in brain structure and function between rodents and humans to support the validity of animal models in assessing the consequences of alcohol use on the brain. For instance, consider the prefrontal cortex (PFC), a brain re-

gion that comprises a notably greater proportion of the total brain matter in humans and other primates than in rodents. In humans, the PFC is thought to play a central role in executive functions, such as working memory, temporal processing, planning, flexibility, and decision-making, which influence behaviors such as drug self-administration and dependence. Comparative studies have indicated that rats also engage in these behaviors, and that the PFC is critical for mediating these processes in rodents, nonhuman primates, and humans.<sup>5,6</sup> In rats and humans, the PFC can be divided into subregions that are associated with similar cognitive functions across species.<sup>5</sup> Experimental animal models have been used successfully to reproduce features of neuropathological and neurochemical changes observed in humans who had neurodegenerative and psychiatric disorders that affected their cognitive function.<sup>7</sup>

Extensive studies also have established the relevance of animal models for investigating drug use behaviors and the consequences. For instance, brain reward systems using the neurotransmitter dopamine, including dopamine projection regions of the nucleus accumbens (NAc), amygdala, and PFC, are critically involved in drug self-administration and dependence in humans and animal models.<sup>8-10</sup> In addition, in humans and laboratory animals, specific brain structures and neurochemical systems are critical for different aspects of alcohol use and misuse (e.g., producing dependence or mediating craving and relapse).<sup>11</sup>

However, differences exist between the rodent and the human and non-human primate brains that should be considered when translating findings from animal studies to the neurological substrates and consequences of alcohol use in humans. For example, electrophysiological studies have suggested that the medial PFC in the rat brain combines elements (i.e., the anterior cingulate cortex and the dorsolateral PFC) that are separated in the primate brain.<sup>12</sup>

## **Animal Models of Adolescence**

Adolescence—that is, the transition from dependence on parents to the independence of adulthood—is not unique to humans and is, to some extent, experienced by all mammals. Similar biological changes, including alterations in the brain, are seen across a variety of mammalian species during adolescence.<sup>13–15</sup> Adolescence-associated neural alterations include regionally specific reductions in the number of synaptic connections between neurons and declines in the relative volume of certain cortical and subcortical areas.<sup>14</sup> Speed of information flow across distant brain regions increases,<sup>14</sup> as does the reactivity of some subcortical brain regions, including the NAc and amygdala.<sup>13,15</sup>

Adolescence-associated changes in dopamine-terminal regions, such as the amygdala and NAc, are particularly important in the context of adolescent AOD use, because these regions are critically involved in processing and responding to rewarding, aversive, and emotionally arousing stimuli, including social stimuli. In adolescents, when compared with adults, these brain regions often react in an exaggerated way to motivational stimuli.<sup>13,15</sup> In contrast, maturation of cognitive control regions in the PFC and other frontal regions occurs gradually during adolescence.<sup>16</sup> This maturational dissociation is thought to contribute to adolescent-characteristic behaviors, such as increased risk-taking and exploratory drug use.<sup>17</sup>

Such developmental alterations have been observed in humans and in animal models and have been matched by analogous behavioral changes in various species. Adolescent rats, for instance, show more peer-directed interactions, novelty-seeking or risk-taking behaviors, and consummatory behavior; find social stimuli, novel stimuli, and pleasant tastes particularly reinforcing; and voluntarily consume two to three times more alcohol than adult rats.<sup>18–21</sup>

Despite such similarities, there are, of course, marked differences between humans and rodents in the duration of this developmental period. Adolescence is relatively brief in rodents and in other mammals with a short life span. Adolescence in rats has been estimated to last only about a month (i.e., postnatal day [P] 25 to P55), with early to mid-adolescence ending at about P42, and late adolescence occurring from P43 to P55.<sup>22</sup> The experimental designs used to study adolescent alcohol use and its consequences, such as analyses involving operant self-administration, must be adapted to this relatively short time period.

To ensure the face validity of models, experimental designs for modeling human alcohol use and its consequences in animals must consider human drinking patterns. For example, alcohol misuse among human adolescents typically takes the form of binge drinking on weekends rather than daily drinking. This human adolescent behavior can be modeled by intermittent alcohol exposure. However, alcohol misuse among adults often involves more regular drinking patterns, which may be better represented by more continuous exposure models.

Despite these constraints, judicious use of animal models can complement studies in human adolescents and address questions that are ethically or technically not amenable to study in humans. Studies using animal models have identified numerous functional alterations associated with adolescent alcohol use, as well as a variety of neural alterations.

## **Functional Alterations Associated With Adolescent Alcohol Exposure**

Studies of the lasting consequences of repeated alcohol exposure during adolescence in animal models have identified numerous functional alterations across domains, ranging from cognition and behavior, to affect, and

to later alcohol consumption. These studies typically use alcohol exposure levels that produce blood ethanol concentrations of .08% or more—the level required to meet the definition for binge drinking specified by the National Institute on Alcohol Abuse and Alcoholism<sup>23</sup> (see **Drinking Patterns and Their Definitions** in this issue). Blood ethanol concentrations in these studies often average .15% to .20%, which is well within the binge-drinking range observed in field studies of human adolescents.<sup>24</sup> Usually, each alcohol exposure during a rat's adolescence is followed by a short period of abstinence before the next exposure period, a design sometimes called adolescent intermittent ethanol exposure (AIE).

## **Cognitive and Behavioral Alterations**

Animal studies have helped identify a variety of cognitive deficits resulting from repeated adolescent alcohol exposure, particularly deficits in tasks that are thought to require hippocampal functioning.<sup>25</sup> Other identified deficits reflect aspects of executive functioning, where prefrontal cortical brain regions are thought to play a particularly important role.<sup>16</sup> Interestingly, the observed effects are highly specific. Learning of some less cognitively challenging tasks, such as passive avoidance or simple operant conditioning tasks, does not seem to be affected by adolescent alcohol exposure.<sup>26,27</sup> Alcohol-exposed animals sometimes exhibit deficits on more challenging tasks, such as conditional discrimination and object recognition tasks.<sup>28</sup> For adolescent animals exposed to ethanol, tasks that demand some degree of cognitive flexibility or self-control seem to be particularly vulnerable to performance impairment. These tasks include reversal learning,<sup>29</sup> extinction, and set-shifting tasks.<sup>30</sup> Adolescent alcohol exposure also is associated with a greater vulnerability to disruptions in spatial memory that are induced by ethanol challenge in adulthood.<sup>25</sup>

Other studies have assessed the effects of AIE on risk-taking behavior, impulsivity, and disinhibition, all behavioral propensities that could promote experimentation with AODs. Such studies have demonstrated that animals with adolescent alcohol exposure exhibited greater risk preferences on a probability-discounting task.<sup>31,32</sup> AIE has been associated with increased impulsivity and greater disinhibition, as indicated by an increase in time spent in open or lighted test areas.<sup>30,32,34</sup>

### **Changes in Affect**

Animal studies also have demonstrated changes in measures of affect in adult animals that were exposed to alcohol as adolescents. For example, AIE animals exhibited depression-like signs, such as reduced consumption of a sugar solution or increased immobility in a swim test.<sup>35-37</sup> Similarly, alcohol exposure during early to mid-adolescence was associated with reliable increases in social anxiety in adulthood.<sup>38,39</sup> Interestingly, this effect seems to be sex-specific and is only observed in males. Other studies in male rats after AIE have detected increases in general anxiety, as indicated by decreased time on the open arms (relative to time on the closed arms) of an elevated plus maze.<sup>37,40,41</sup> However, increases in general anxiety have not always been observed.<sup>36,42</sup>

It is challenging to distinguish disinhibition and anxiety in animal studies. For example, although the elevated plus maze test was developed and validated as a test of anxiety, results from it are sometimes interpreted in terms of disinhibition. Increased time spent in an environment that animals perceive as more risky (i.e., the open arm of an elevated maze) could indicate either greater disinhibition, decreased anxiety, or some interaction of the two, with increases in disinhibition perhaps contributing to a suppression in anxiety.<sup>30,34</sup> In studies of adolescent alcohol exposure, AIE has been found to increase open-arm time in some

studies, suggesting greater disinhibition, but to decrease open-arm time in others, a pattern of findings consistent with a profile of increased anxiety. It is possible that adolescent alcohol exposure can be characterized by profiles of both increased anxiety and disinhibition. Competition between these propensities—depending, for example, on the perceived stressfulness of the situation or the animals' previous handling—may explain these reliable but opposing outcomes.<sup>43</sup>

### **Retention of Adolescent Phenotypes Into Adulthood**

One surprising long-lasting consequence of adolescent alcohol use observed repeatedly in AIE studies is the retention of adolescent phenotypes into adulthood. In rodent studies, adolescents have been shown to differ from adults in a variety of alcohol-related phenotypes. In instances where researchers could assess similar effects in human adolescents, the analyses uncovered comparable age-related differences.<sup>2</sup> For example, like their human counterparts, adolescent animals often voluntarily consume significantly more alcohol per drinking occasion than adults.<sup>18,44,45</sup> This elevated alcohol intake is particularly notable in male animals and mirrors intake by human adolescents.<sup>46</sup>

Adolescents often differ from adults in their sensitivity to alcohol's effects, with the direction of these differences dependent on the effect studied. Adolescents are less sensitive to many of alcohol's undesired effects, such as alcohol-induced motor impairment, sedation, aversion, and social impairment, which normally serve as cues to limit intake.<sup>47</sup> Adolescents are also less sensitive to acute withdrawal (i.e., hangover effects) after moderate to high alcohol consumption. In animal models, this effect has been reflected in reduced levels of withdrawal-associated anxiety.<sup>48,49</sup> In contrast to the attenuated sensitivity of adolescents to many of alcohol's undesired effects, adolescents

are often more sensitive to certain desired effects of alcohol, such as its rewarding and social facilitating effects.<sup>47</sup> Adolescents are also usually sensitive to the disruptive effects of acute alcohol intoxication on learning and memory.<sup>25</sup> Collectively, adolescent-associated attenuated sensitivity to aversive effects and increased sensitivity to desirable effects of alcohol could contribute to enhanced susceptibility to the initiation and escalation of alcohol use during adolescence,<sup>47</sup> with intoxication having pronounced disruptive effects on learning and memory.<sup>25</sup>

Animals given repeated alcohol exposure during adolescence often retain adolescent-typical phenotypes into adulthood.<sup>50</sup> This persistence can be observed through baseline behavioral, cognitive, electrophysiological, and neuroanatomical assessments, as well as in the animals' responses to alcohol challenges in adulthood.<sup>51</sup> For example, animals exposed to alcohol during adolescence maintained an enhanced sensitivity to alcohol's rewarding and stimulatory effects into adulthood.<sup>38,52-54</sup> This persistent sensitivity could promote alcohol consumption in adulthood. In other studies, animals that experienced AIE retained their adolescent-typical insensitivities to alcohol's sedative, motor-impairing, and aversive effects, which could permit the maintenance of elevated alcohol drinking during adulthood.<sup>53,55-58</sup> Also, the decline in sensitivity to alcohol-induced deficits in spatial working memory that normally occurs between adolescence and adulthood did not occur in animals exposed to alcohol in adolescence.<sup>59</sup> As a result, adult animals exposed to AIE retain adolescent-like vulnerability to alcohol-induced memory impairments and show more memory disruption under the influence of alcohol than adults without a history of adolescent alcohol exposure.

Generally, retention of these adolescent phenotypes into adulthood is associated with alcohol exposure during adolescence; equivalent alcohol exposure during adulthood does not induce similar effects.<sup>55,58</sup> Moreover,

adolescent phenotypes are more pronounced if adolescent alcohol exposure is episodic, rather than continuous, reflecting typical adolescent binge-drinking consumption patterns.<sup>55</sup> An episodic exposure pattern can result in withdrawal episodes following each exposure, which could result in escalating withdrawal signs (e.g., increased anxiety-like behavior, lower seizure threshold, and more severe seizures), particularly in adolescents.<sup>60,61</sup>

Researchers are trying to uncover the neurobiological mechanisms that underlie the retention of adolescent phenotypes after adolescent alcohol exposure. One line of investigation has explored whether animals exposed to AIE retain into adulthood an immature balance of enhanced excitation to inhibition in the brain. Some analyses have assessed the role of gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the brain. Studies found that in the hippocampus, inhibitory effects of GABA responsible for baseline levels of tonic inhibition normally are attenuated in adolescents; however, after AIE, this attenuation is maintained into adulthood.<sup>50,62</sup> Ethanol potentiation of this tonic inhibition is more marked in adolescents than adults—an effect that is maintained into adulthood after AIE.<sup>50,62,63</sup> These adolescent-typical neurophysiological characteristics and their persistence into adulthood may contribute to alcohol's enhanced memory-impairing effects in adolescents and to long-lasting memory impairment seen in adulthood after AIE.<sup>51</sup> More work is needed to identify the overall prevalence of persistent adolescent-typical immaturities after adolescent alcohol exposure under various baseline and challenge conditions, and to further characterize the mechanisms underlying these persisting effects.

## Effects on Later Ethanol Consumption

Another potential long-term consequence of adolescent alcohol exposure

that may reflect the persistence of adolescent phenotypes is elevated alcohol consumption during adulthood. Findings are mixed as to whether adolescent alcohol exposure increases adult alcohol consumption. The hypothesis is supported by findings that alcohol-preferring rats given free access to ethanol in their home cages throughout adolescence acquired an operant self-administration task for alcohol in adulthood more quickly than animals that did not have access to alcohol during adolescence.<sup>64,65</sup> Moreover, these animals exhibited greater resistance to extinction of the operant task, more spontaneous recovery of self-administration, and elevated response levels during reacquisition of the operant task compared with animals with no history of alcohol exposure. Similar findings were obtained in mice. Animals that had voluntary access to alcohol throughout adolescence consumed more alcohol as adults than mice whose access to alcohol was delayed until adulthood.<sup>66</sup> Rats exposed to alcohol through intermittent intraperitoneal administration in early to mid-adolescence later exhibited increased alcohol consumption, an effect that was not apparent when alcohol exposure was delayed until late adolescence.<sup>41,67</sup>

The findings of increased adult consumption levels following adolescent exposure are not universal, however. In some studies, adolescent rats exposed to alcohol vapor, and mice or rats given free access to alcohol in their home cages did not exhibit increased alcohol consumption during adulthood.<sup>44,68,69</sup> Other researchers found that animals given free access during adolescence to alcohol through an operant task demonstrated no increased operant response during adulthood, although they did show increases in some alcohol-related responses.<sup>30,42</sup>

Several variables may influence whether adolescent alcohol exposure increases adult alcohol consumption, which may explain the diverse findings. These variables include the sex of the animals, genetic background

(i.e., the strain of rats or mice used), amount and mode of adolescent alcohol exposure, and assessment method of adult alcohol intake.<sup>43</sup> Also, when adolescent rats were given either a sweetened alcohol solution or the sweetened solution without alcohol, both groups later increased intake only of the solution they were exposed to during adolescence, not the alternate solution.<sup>70</sup> This suggests that increased alcohol intake during adulthood after consuming alcohol during adolescence may reflect increased acceptability of a familiar solution, rather than alcohol-specific effects. Although the existing data suggest that in some cases adolescent alcohol exposure can lead to increased consumption during adulthood, researchers still need to further clarify the circumstances in which these intake-enhancing effects emerge.

## Neural Alterations

Alcohol exposure during adolescence has detrimental and potentially long-lasting effects not only on cognition, affect, and behavior, including future alcohol consumption, but also on the structure and function of the brain. Particularly pronounced effects include reductions in the formation of new brain cells (i.e., neurogenesis), long-lasting neuroinflammation, changes in gene expression through epigenetic mechanisms, and alterations in the activities of neurotransmitter systems in several vulnerable brain regions.

### Neurogenesis and Cell Death

Adolescence is associated with a variety of neuroanatomical changes, including enhanced neurogenesis in some brain regions (e.g., the hippocampus).<sup>71</sup> Reductions in the numbers of neurons and in the connections between neurons (a process known as pruning) may occur in other regions of the brain (e.g., the PFC).<sup>72</sup> One of the most consistent neurological findings associated with adolescent alcohol ex-

posure is a reduction in neurogenesis and a region-specific increase in cell death and cell damage in the brain. The regions most commonly affected include the frontal cortex, hippocampus, amygdala, NAc, and cerebellum—regions that also undergo significant developmental changes during adolescence.<sup>71,73-75</sup> The adolescent brain seems to be particularly vulnerable to the effects of alcohol exposure because similar disruptions were not observed after equivalent exposure in adulthood.<sup>73</sup> The effects of binge-like exposure during adulthood occurred in different regions of the brain and were less pronounced than the effects of exposure during adolescence.<sup>74</sup>

Adolescent alcohol exposure affects not only the overall number of brain cells in specific brain regions but also their connections with each other. Recent studies investigated the effects of AIE on the structure and function of synapses in the hippocampus, a brain region associated with learning and memory.<sup>76</sup> The analyses found that AIE resulted in a greater proportion of immature relative to mature dendritic spines (specialized sites on neurons that receive and amplify input from signal-emitting neurons) in the brains of AIE animals compared with those of nonexposed adult animals. Animals with AIE also manifested more robust long-term potentiation as adults when they were compared with nonexposed animals, a pattern of neurophysiological activation similar to the pattern normally seen in adolescents. Long-term potentiation is the strengthening of synaptic connections when the synapses are repeatedly activated. Although this process is necessary for learning, greater than normal long-term potentiation has been linked to memory deficits and other learning-related behavioral changes.<sup>76</sup>

### **Neuroinflammation**

Adolescent alcohol exposure has been shown to induce long-term increases in expression of several neu-

roimmune genes that encode proinflammatory signaling molecules.<sup>77</sup> Adolescent exposure also has been shown to activate Toll-like receptor 4 (TLR4), a receptor in the innate immune system that plays a central role in initiating innate immune responses throughout the body.<sup>77</sup> Ethanol-induced TLR4 activation triggers the expression of various transcription factors that, in turn, promote the expression of proinflammatory cytokines and other mediators of inflammation. In the short term, such proinflammatory responses may be adaptive. However, when these responses are maintained over longer periods, the result is long-lasting neuroinflammation.

In the brain, ethanol-induced activation of TLR4 and its subsequent actions can contribute to brain damage associated with excessive alcohol exposure.<sup>77</sup> For example, in animal studies, activation of TLR4 using a bacterial compound (i.e., lipopolysaccharide) induced a long-lasting reduction in neurogenesis similar to that observed after AIE.<sup>71</sup> In mice that did not produce TLR4, adolescent alcohol exposure did not result in the characteristic inflammatory, cognitive, and behavioral consequences usually associated with this exposure.<sup>40,77</sup>

The role of TLR4 and neuroinflammation in the functional and neural consequences of adolescent alcohol exposure is supported by findings that treatment with an anti-inflammatory compound (i.e., indomethacin) prevented the typical cell death and behavioral deficits seen after AIE.<sup>28</sup> These observations suggest that anti-inflammatory agents may represent a new class of pharmacotherapeutic interventions for preventing, ameliorating, or even reversing some of the long-term consequences of adolescent alcohol exposure.

### **Epigenetic Mechanisms**

Adolescent alcohol exposure also influences gene expression by modifying epigenetic regulatory mechanisms.

Adolescent animals exposed to alcohol show alterations in histone acetylation, which, in turn, influences DNA methylation and the level of gene expression.<sup>41,78,79</sup> Such epigenetic alterations have been identified in the amygdala, NAc, and PFC, which are brain structures involved in memory processing, decision-making, and emotional reactions. For example, rats with AIE exhibited persistent increases in histone deacetylation and reductions in histone acetylation in the amygdala,<sup>41</sup> resulting in reduced expression of certain genes (e.g., brain-derived neurotrophic factor [BDNF]). When the alcohol-induced deacetylation was prevented by treatment with a histone deacetylase inhibitor, histone acetylation levels in the amygdala normalized, and the transcription of BDNF was restored.<sup>41</sup> The effects of AIE on histone acetylation levels also may contribute to observed behavioral and neural effects of AIE. Treatment with the deacetylase inhibitor attenuated anxiety-like behaviors, reversed the increase in alcohol intake during adulthood, and normalized the decline in neurogenesis usually exhibited by AIE animals.<sup>41,80</sup>

### **Neurotransmitter Systems**

Alcohol exerts its dose-dependent and region-specific effects largely through direct or indirect interactions with the major neurotransmitter and neuromodulatory systems in the brain, including the GABA system discussed earlier, as well as the dopamine, serotonin, glutamate, acetylcholine, and endocannabinoid systems.<sup>81</sup> However, there is specificity in these effects, and not all systems and brain regions are equally vulnerable. Many of these alcohol-sensitive neurotransmitter and neuromodulatory systems and affected brain regions undergo developmental transformations during adolescence, and they may be especially vulnerable to alcohol-induced perturbations during development. Indeed, AIE has been shown to be associated with alterations in several of these systems, including:

- **Changes in the activity of the dopamine system in the NAc.**

Several studies have reported enhanced dopamine function in neurons projecting to the NAc, a pivotal component of the brain's reward system, following AIE. These neurons exhibited increased dopamine-mediated neurotransmission under normal conditions and after an alcohol challenge.<sup>78,82,83</sup> The neurons also exhibited higher basal extracellular dopamine levels.<sup>78,84</sup> Given the critical role that dopamine plays in facilitating reward-related motivation and behaviors, these findings suggest that AIE may enhance the rewarding experiences associated with alcohol, which could promote further alcohol ingestion.

- **Changes in the activity of the glutamate system.** Glutamate is the primary excitatory neurotransmitter in the brain and acts via several types of receptors, including the N-methyl-D-aspartate (NMDA) receptor. AIE has been reported to increase NMDA receptor binding in the frontal cortex, as well as the expression of one subunit of this receptor (i.e., the NR2B subunit).<sup>85</sup>

Other research has reported a decrease in the subunit's phosphorylation.<sup>78</sup> Altered NMDA functioning in the PFC has been suggested to disrupt functioning of that brain region and to contribute to the impulsive behavior and the lack of control over drinking that is characteristic of individuals with AUD.<sup>78</sup>

- **Changes in the acetylcholine system in the basal forebrain.**

One reliable consequence of AIE observed in rodent studies is a long-lasting decrease in the basal forebrain of the number of neurons that exhibit activity of the choline acetyltransferase enzyme, which is required for synthesis of the neurotransmitter acetylcholine. This effect is seen following adolescent, but not adult, alcohol exposure.<sup>29,31,36,86</sup> These findings suggest

that adolescent alcohol exposure impairs the normal cholinergic neurotransmission in the basal forebrain that is crucial for ensuring cortical plasticity and learning. Hence, AIE-induced deficits in the cholinergic system may contribute to future cognitive deficits.

Repeated alcohol use during adolescence induces specific alterations in a variety of neural systems that play critical roles in neural, cognitive, and behavioral function. It is possible that some of these neural alterations reflect positive adaptations to AIE to mitigate long-term consequences of the alcohol exposure. Yet, these potential compensations do not appear to be sufficient, given the growing list of long-term consequences of AIE on later neurocognitive and behavioral function.

## Conclusions and Future Directions

Adolescence is characterized by social and emotional development and often is accompanied by experimentation with AODs. Brain development continues during adolescence, and, increasingly, adolescence is being viewed as a period of enhanced brain plasticity and experience-related brain sculpting. Many adolescent experiences (e.g., education, sports, and positive social interactions) provide beneficial long-term sculpting. Other influences, such as repeated exposure to alcohol, can be detrimental and have long-term effects on neural functioning, cognition, and behavior, including enhanced AOD consumption, that persist into adulthood.

Studies conducted primarily using rodent models of adolescence have shown that propensity for the initiation and escalation of alcohol use during adolescence may be promoted by adolescents' greater sensitivity to the socially facilitating and rewarding effects of alcohol, combined with a reduced sensitivity to other effects (e.g., social and motor impairment, and sed-

ative and aversive effects) that likely serve as cues to terminate intake. Animal studies have shown that repeated exposure to alcohol during adolescence, especially AIE that mirrors binge-drinking patterns observed in human adolescents, induces specific patterns of sustained neurobehavioral alterations that may promote further drinking. Particularly worrisome are reports that adolescent alcohol exposure may lead to the retention of adolescent phenotypes—including adolescent-typical responses to alcohol—into adulthood. Other cognitive, behavioral, and affective consequences have been reported after AIE, including impaired performance of executive functions, memory impairment, reduced cognitive flexibility, greater risk preference and disinhibition, and elevated social (and sometimes general) anxiety. In many cases these effects are specific to adolescent alcohol exposure and are not evident after equivalent alcohol exposure during adulthood.

Animal studies also have identified lasting neural alterations induced by AIE that may contribute to behavioral and cognitive changes. These changes include reduced neurogenesis, increased neuroinflammation, epigenetic alterations, and alterations in numerous neurotransmitter systems, including glutamate, GABA, the balance between these excitatory and inhibitory systems, dopamine, and the basal forebrain cholinergic system. When different age groups were compared, the consequences typically were more pronounced after adolescent alcohol exposure than after equivalent adult exposure. Likely anatomical targets for these long-term effects include the hippocampus, amygdala, NAc, and PFC. These neural systems underlie the developmental shifts in sensitivity to drug rewards and drug aversion that normally occur during adolescence and adulthood. These systems are also involved in neurodevelopmental processes related to socioemotional

functioning and advanced aspects of cognitive functioning.

Despite the progress achieved using animal models for understanding the consequences of adolescent alcohol exposure and, particularly, the intermittent, binge-like exposures characteristic of this age, many questions remain. For example, additional research is needed to elucidate how AIE affects the neural mechanisms underlying the enhanced reward and attenuated aversive sensitivities that are normally seen during adolescence and are maintained into adulthood after AIE, as well as how these mechanisms contribute to later alcohol consumption. It also will be crucial to determine if lasting functional consequences of adolescent alcohol exposure can be prevented, attenuated, or reversed by blocking alcohol-induced neural alterations. Similarly, researchers need to further elucidate the persistence of adolescent phenotypes into adulthood that has been reported after adolescent alcohol exposure. The breadth and limitations of this adolescent-like persistence across different functional domains, its stability over time, and whether it can be reversed or modified all need to be examined. It is undoubtedly useful and necessary to use animal models to study contributors to and consequences of adolescent-typical behaviors such as alcohol consumption. Nonetheless, the findings are only useful if they prove valid, applicable to predicting the effects of adolescent alcohol exposure in humans, and ultimately relevant to prevention and treatment.

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