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Mechanisms involved in the neurotoxic, cognitive, and neurobehavioral effects of alcohol consumption during adolescence

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Abstract

Studies over the last decade demonstrate that adolescence is a brain maturation period from childhood to adulthood. Plastic and dynamic processes drive adolescent brain development, creating flexibility that allows the brain to refine itself, specialize, and sharpen its functions for specific demands. Maturing connections enable increased communication among brain regions, allowing greater integration and complexity. Compelling evidence has shown that the developing brain is vulnerable to the damaging effects of ethanol. It is possible to infer, therefore, that alcohol exposure during the critical adolescent developmental stages could disrupt the brain plasticity and maturation processes, resulting in behavioral and cognitive deficits. Recent neuroimaging studies have provided evidence of the impact of human adolescent drinking in brain structure and functions. Findings in experimental animals have also given new insight into the potential mechanisms of the toxic effects of ethanol on both adolescent brain maturation and the short- and long-term cognitive consequences of adolescent drinking.

Adolescence is also characterized by the rapid maturation of brain systems mediating reward and by changes in the secretion of stress-related hormones, events that might participate in the increasing in anxiety and the initiation pattern of alcohol and drug consumption. Studies in human adolescents demonstrate that drinking at early ages can enhance the likelihood of developing alcohol-related problems. Experimental evidence suggests that early exposure to alcohol sensitizes the neurocircuitry of addiction and affects chromatin remodeling, events that could induce abnormal plasticity in reward–related learning processes that contribute to adolescents' vulnerability to drug addiction.

In this article, we review the potential mechanisms by which ethanol impacts brain development and lead to brain impairments and cognitive and behavioral dysfunctions as well as the neurobiological and neurochemical processes underlying the adolescent-specific vulnerability to drug addiction.

Keywords: Adolescence; Brain maturation; Binge alcohol drinking; Brain damage; Neurobehavioral dysfunctions; Mesocorticolimbic system

Introduction

Alcohol is one of the first drugs of choice among young people and adolescents, and heavy binge-drinking is becoming increasingly frequent in high school students in different countries. The proportion of high school students consuming alcohol in the United States and the rate of heavy drinking in the last 10 years are very high (Donovan, 2004). Reports from the European School Survey Project on Alcohol and Other Drugs (Hibell et al., 2007; Kuntsche et al., 2004) carried out in 35 European countries, indicate that young people today drink more and with a clearer focus on drunkenness than earlier generations. In south Europe or Mediterranean countries, such as Italy or Spain, there has also been a substantial rise in concern about youth drinking in recent years. One clear trend is a shift to binge-drinking as a natural habit, which is associated with drunkenness, especially among teenagers, in all the ''wine cultures'' with moderate alcohol consumption (Lopez-Frias et al., 2001; Peretti-Watel et al., 2006; Tur et al., 2003).

A substantial body of evidence in human and experimental animals has demonstrated the vulnerability of the central nervous system to the effects of ethanol and that exposure to ethanol during brain ontogeny can cause irreversible abnormalities in the brain structure and functions (Guerri et al., 2009). Recent studies with magnetic resonance imaging (MRI) have clearly shown that the brain continues to develop throughout adolescence and into adulthood and that the brain undergoes important structural and functional changes in synaptic plasticity and neural connectivity during the juvenile and adolescence periods (Giedd, 2004, 2008). These changes concomitantly occur

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with modifications in certain neurotransmitter systems and hormone secretion, which markedly influence the refinement of certain brain areas and neural circuits. In addition, dynamic processes allow the brain to specialize and sharpen its functions for the specific demands of its environment. By considering the enormous plastic changes occurring in the teenage/adolescent brain maturation stages and the vulnerability of the developing brain to the damaging effects of ethanol, one might expect that alcohol consumption in the juvenile and adolescence stages would have a significant impact on the adult brain functions (Paus et al., 2008).

Recent studies have shown the harmful consequences of alcohol abuse during adolescence. These studies demonstrate that adolescent drinking can induce brain structure abnormalities, deficits in memory, and poor academic performance (Brown and Tapert, 2004; Lopez-Frias et al., 2001; Medina et al., 2008; Nagel et al., 2005; Zeigler et al., 2005). The neurotoxic effects of ethanol have also been confirmed in experimental animals, providing further evidence of the vulnerability of the juvenile brain to the effects of ethanol and the long-term cognitive consequences (e.g., learning and memory processes) of binge-drinking during adolescence (Crews et al., 2000; Pascual et al., 2007).

At the behavioral level, adolescence is characterized by increased sensation-seeking, risk-taking behaviors, low levels of harm avoidance, impulsivity, and anxiety (Blakemore, 2008). These features are associated with changes in the secretion of gonadal steroids and stress-related hormones (Ceccarelli et al., 2007; Witt, 2007), which might explain the initiation pattern of alcohol and drug consumption. Likewise, the relatively late development of the prefrontal cortex (PFC) circuits involved in judgment and inhibitory control may underlie the propensity of adolescents to impulsivity and to ignore the negative consequences of their behavior, both of which could increase the risk of substance abuse.

This article will briefly review the dynamic processes involved in brain maturation during adolescence and will discuss the potential mechanisms by which ethanol impacts brain development and leads to brain impairments and long-term cognitive and behavioral dysfunctions. Specifically, the mechanisms involved in ethanol-induced alterations in the brain structure and cognitive deficits, as well as the neurobiological and neurochemical processes by which early alcohol intake predisposes to the later alcohol abuse, will be addressed.

Structural and functional maturational changes of the brain during adolescence

The human brain undergoes changes in terms of its morphology, volume, composition, and function during brain development and maturation (Dekaban, 1978; Giedd et al., 1999). Important alterations in brain weight occur during fetal and neonatal development but changes in brain structure and functions in childhood and adolescence are more subtle than those in the first 4 years of life (Paus et al., 2001). These changes in functional maturation of the neural pathways connecting individual brain regions is an essential condition for the successful cognitive, motor, and sensory functions from infancy, through childhood and adolescence, and into adulthood.

Several in vivo studies using MRI (Gogtay et al., 2004; Paus, 2005; Paus et al., 2001; Sowell et al., 2002, 2003) have demonstrated that the cortical and subcortical components of the brain change dramatically during childhood and adolescence. Robust alterations are observed in secondary and tertiary expanses of the cerebral cortex, which not only encompass components of the temporal, parietal, and prefrontal cortices, but also the alterations in key subcortical structures within the medial temporal lobe, which include the amygdala, hippocampus, and brain structures with high densities of sex steroid receptors (Gogtay et al., 2004; Toga and Thompson, 2003; Sowell et al., 2004). Concomitantly, gray matter volumes decrease and follow inverted U-shaped developmental curves during childhood, whereas white matter volume changes tend to be more linear and less variant across regions (Gogtay et al., 2004; Toga and Thompson, 2003; Sowell et al., 2004).

At cellular level, changes in gray and white matter volumes appear to be associated with an overproduction of axons and synapses in early puberty and with rapid pruning in later adolescence (Giedd et al., 1999; Huttenlocher and de Courten, 1987; Sowell et al., 2004). These events are likely to reflect a restructuring or refinement of the connection between neurons and glial cells (Fields and Stevens-Graham, 2002). Specifically, three main processes occur during both the brain maturation stages and the transition from juvenileness/adolescence to adulthood (Lenroot and Giedd, 2006):

(1) *Proliferation*, in which a rapid growth of gray matter and the formation of new connections within the brain take place. For instance, the maximum size in the frontal- and parietal-lobe gray matter occurs at about 12 years in males and at 10.2–11 years in females, whereas the maximum size in other brain regions, such as the temporal-lobe gray matter, is not reached until 16.5 and 16.7 years for males and females, respectively (Giedd et al., 1999).
(2) *Pruning* or gray matter maturation. New synaptic connections are established, whereas others are eliminated or pruned back to reflect in part, a decrease in the gray matter volume (e.g., PFC) (Bourgeois and Rakic, 1993; Huttenlocher, 1984; Huttenlocher and Dabholkar, 1997; Huttenlocher and de Courten, 1987; Shaw et al., 2008; Tamnes et al., 2009). Pruning is greatly influenced by experience and this makes the adolescent brain extremely versatile and able to make changes depending on the demands of the environment (Blakemore, 2008).

(3) *Myelination*. Increased myelinated axons speed up the communication among neurons and make more stable connections (Perrin et al., 2009; Sowell et al., 2001). The progressive myelination of axons results in developmental increases in cortical white matter through adolescence and into adulthood serves to accelerate the information flow along axons, and is presumed to increase the overall speed of information processing within the brain (e.g., Sowell et al., 2003).

Among the brain regions showing marked ontogenic changes during adolescence is the PFC (for reviews, see Crews et al., 2007; Spear, 2007). The volume of PFC declines from adolescence to young adulthood (Sowell et al., 1999, 2001; van Eden et al., 1990), and this reduction is associated with both a refinement in the neural circuits and the connectivity. For instance, a loss of synapses, especially the excitatory glutamatergic inputs to the PFC, occurs during adolescence (Huttenlocher, 1984), whereas the dopamine (DA) and the serotonin (5-hydroxytryptamine) inputs to the PFC increase during adolescence and then decrease later in life (Kalsbeek et al., 1988; Rosenberg and Lewis, 1994). These remodeling changes in the neural circuits are associated with cognitive functional modifications and with the acquisition of executive functions (e.g., response inhibition, attention, working memory) (Casey et al., 2000; Paus, 2005). For example, level of intelligence is associated with the trajectory of cortical development, primarily in the frontal regions (e.g., the PFC) implicated in the maturation of intelligent activity. More intelligent children demonstrate a particularly plastic cortex with an initial accelerated and prolonged phase of cortical increase, which leads to equally vigorous cortical thinning by adolescence (Shaw et al., 2006). MRI studies also reveal that the hippocampus volume is significantly larger in older male adolescents than in younger male adolescents. As in other brain regions, less cerebral gray matter volume and significantly greater cerebral white matter volume are found in older adolescents compared with younger adolescents (Suzuki et al., 2005), changes that might reflect maturation of the memory functions.

Subcortical regions also undergo considerable remodeling during adolescence (Gogtay et al., 2004; Sowell et al., 2004; Toga and Thompson, 2003), particularly interconnecting network of circuitry with the PFC, including the amygdala and other DA mesocorticolimbic terminal regions (for reviews, see Spear, 2002, 2007). Ontogenetic alterations in the pattern of DA production and utilization also occur in the mesocorticolimbic brain regions during adolescence. For instance, DA synthesis and turnover in the PFC are higher in early adolescence than in adulthood, whereas DA synthesis and/or turnover in nucleus accumbens (NAc) and striatum are lower in earlier adolescence than in its later stages (e.g., Andersen et al., 1997, Teicher et al., 1993). In the striatum and NAc of rats, the DA receptors are overproduced with subsequent pruning during the adolescent stage, suggesting a maturational remodeling of the reward pathways (Tarazi and Baldessarini, 2000; Teicher et al., 1995). These developmental events during adolescence may alter the relative balance of DA activity between PFC and striatal or mesolimbic terminal regions, resulting in grater predominance of DA activity in the PFC during early adolescence. Stressors seem to exacerbate the shift in DA balance toward mesocortical than mesolimbic/striatal DA activity during early adolescence (Spear, 2000). These remodeling changes in the neurocircuitry involving the DA projections to mesolimbic brain regions and the PFC could acquire a special functional significance for the adolescence, as this circuitry forms a part of the reward system modulating the typical behavior of adolescents (for reviews, see Spear, 2000, 2007) and the motivation to natural (e.g., food) and non-natural reward, such as alcohol and other drugs of abuse (Robinson and Berridge, 2003). Therefore, neurochemical immaturity of the mesocorticolimbic system might contribute to the initiation of alcohol intake and to the adolescent-specific vulnerability to drug addiction.

Studies in experimental animals demonstrated that the ontogenetic transition patterns as well as physiological and behavioral characteristics of adolescents are common among mammalian species (for reviews, see Spear, 2004, 2007). For instance, absolute PFC volume declines in the adolescent rat (van Eden et al., 1990), and synapse elimination has been observed during adolescence in the PFC of nonhuman primates (Rosenberg and Lewis, 1994). Maturation changes are also evident in the hippocampus of adolescent rodents (Dumas and Foster, 1998). Although the complexity of the human brain and behavior cannot be extrapolated to experimental animals, numerous similarities are found between human adolescents and adolescents of other species. Thus, it is reasonable to support the use of animal models to study the neurochemical and behavioral consequences of alcohol abuse during adolescence (see the following sections).

In summary, adolescence is a developmental stage in which brain undergoes remodeling and functional changes in synaptic plasticity and neuronal connectivity in different brain regions, including the PFC, the temporal cortex, the parietal cortex, and a number of subcortical structures, which undergo alterations in white- and gray-matter densities. In addition, the forebrain DA projection regions and reward circuitry system also undergo important remodeling alterations. These changes in the plasticity might confer the special vulnerability of the adolescent brain to both the deleterious effects of ethanol and to the acquisition of alcohol use disorders (AUD).

Neurotoxicological, neurological, and long-term behavioral consequences of young drinking

Human studies

Clinical and experimental studies demonstrate that alcohol affects adolescent and adult brain functions and

behaviors differently and that adolescents are more vulnerable to the deleterious effects that alcohol has on brain functions and behavior. Indeed, by using MEDLINE search for the neurological and cognitive effects of underage drinking (Zeigler et al., 2005) and self-administered questionnaire in conjunction with school performance (Lopez-Frias et al., 2001), these studies have reported that teenage drinking is associated with cognitive deficits with implications for learning and intellectual development as well as poor academic achievements. Neuropsychological testing has been done to show alterations in the visual–spatial functioning and in verbal and nonverbal information in youths with alcohol episodes (Brown et al., 2000) or in adolescents with AUD during abstinence (Brown and Tapert, 2004). However, memory problems are among the most common dysfunctions in adolescents with AUD and these effects have been associated with abnormalities in the brain's response to a spatial working memory task. By measuring the blood oxygen level–dependent (BOLD) response to a spatial working memory task, Tapert et al. (2004) showed a reduction of the brain BOLD response in the left precentral gyrus and the bilateral cerebellar areas, although a greater response was found in bilateral parietal cortices. These findings suggest that alcohol may modify some brain maturation processes that are critical for cognitive processes. According to this assumption, some studies have demonstrated structural abnormalities in the brain areas involved in memory and learning processes, such as the hippocampus and the PFC, of adolescents with AUD. Indeed, smaller hippocampal volumes have been shown in adolescents who began drinking at an earlier age when compared with those individuals who began later (De Bellis et al., 2000; Nagel et al., 2005) and that these effects are independent of other comorbid conduct disorders (Nagel et al., 2005). Neuroimaging studies have further shown PFC abnormalities, including white matter differences in adolescents with AUD (De Bellis et al., 2005; Medina et al., 2008).

Notably, gender differences have been observed in alcohol–induced brain damage. Thus, although PFC abnormalities have been noted in both young males and females with AUD, female subjects show smaller volumes than their male counterparts who, in turn, present larger volumes compared with same-gender controls (Medina et al., 2008). These findings correlate with functional neuroimaging studies that report that male subjects with AUD present increased frontal activation, whereas female drinkers display limited frontal activation in response to a spatial working memory. Ethanol also induces greater impairments in brain functioning (measured by BOLD) and in gray matter volume in females than in males, thus suggesting that female adolescents are more vulnerable than males to adverse alcohol effects, and may be at an increased risk of behavioral deficits (Caldwell et al., 2005). These results confirm the well-established notion that females are more vulnerable than men to alcohol–related organ damage (Spear, 2002). Female alcoholics also appear to be more susceptible to frontal lobe gray matter reduction than male counterparts (Schweinsburg et al., 2003). Although the mechanisms involved in gender differences remain elusive, several factors could influence brain–related ethanol effects in female adolescents, including gender–specific brain development (e.g., Giedd et al., 1999; Lenroot and Giedd, 2006), differential gene expression linked to enhanced alcohol-related neurotoxicity in females (Hashimoto and Wiren, 2008), increased blood alcohol concentration among females despite similar drinking patterns, and differences in alcohol metabolisms (Baraona et al., 2001). Finally, hormonal and receptor differences in response to alcohol may also play an important role in these gender differences. Hormonal receptor levels are associated with gender differences in brain functioning during spatial tasks (Williams and Meck, 1991) and also with alcohol-induced changes in hormone distributions (Emanuele et al., 2001; Kim et al., 2003), which could partially account for the gender differences noted in alcohol–related neural abnormalities.

In summary, studies into human adolescents demonstrate that heavy drinking during juvenile/adolescent stages could impair brain structure and cognitive functions and suggest that alterations in neuronal reorganization and connectivity might affect brain maturation. Although genetic factors might influence the effects of ethanol on brain damage and cognitive dysfunctions (Rose et al., 2004), animal research has confirmed the sensitivity of the adolescent brain to the harmful effects of ethanol on brain maturation.

Animal studies and mechanisms involved in ethanol–induced adolescent brain damage

Studies into experimental animal models have also provided evidence of the vulnerability of the adolescent brain to the toxic effects of ethanol (Carpenter-Hyland and Chandler, 2007; Chandler, 2003; Crews et al., 2000; Pascual et al., 2007; Spear, 2000). Compared with adults, alcohol–exposed adolescent animals are more likely to exhibit cognitive deficits, including learning and memory dysfunctions (Markwiese et al., 1998; White and Swartzwelder, 2005), although some findings suggest that adults are more sensitive than adolescents to ethanol-induced disruption of performance in a nonstressful spatial memory task (Rajendran and Spear, 2004). Notably, some of the cognitive effects, such as learning impairments, induced by repeated ethanol treatment in adolescent rats continue into adulthood (Pascual et al., 2007; Sircar and Sircar, 2005). However, adolescent rats are less vulnerable than adults to a number of acute effects of ethanol, including ethanol-induced sedation (Silveri and Spear, 1998) and motor impairments (Little et al., 1996; White et al., 2002), effects that might enable adolescents to drink larger amounts of alcohol without noticing its intoxicating effects.

Although the behavioral and neurobiological mechanisms for the ontogenetic differences in alcohol sensitivity

remain unclear, it has been shown that adolescents are more sensitive than adults to not only the memory impairing effects of alcohol, but also to the impact of alcohol on the brain function underlying memory formation. Thus, using hippocampal slices taken from 30-day-old adolescent and 90-day-old adult rats, Swartzwelder et al. (1995a, 1995b) have shown that ethanol greater attenuates NMDA–mediated synaptic activity in the immature than in the mature hippocampus and that ethanol more potently inhibits the induction of long-term potentiation (LTP) in immature versus mature animals. This developmental sensitivity of the NMDA currents to alcohol was also observed in both the hippocampus and pyramidal cells in the posterior cingulate cortex (Li et al., 2002). According to these in vitro data, adolescent rats reveal a greater impairment than adults in the acquisition of a spatial memory task after acute ethanol exposure (Markwiese et al., 1998). These results suggest that ethanol disrupts memory and memory–related brain functions (e.g., LTP) more powerfully in adolescent animals than in adults, effects that could contribute to the greater vulnerability of adolescents to ethanol–induced memory impairments and the reduction of synaptic plasticity.

Regarding the toxic effects of ethanol on the adolescent brain, some studies have demonstrated that young rats exposed to heavy binge-like episodes of ethanol display greater damage in the frontal-anterior cortical regions than adults, including the olfactory frontal cortex, the anterior perirhinal, and the piriform cortex (Crews et al., 2000). Likewise, intermittent ethanol administration to juvenile/adolescent rats (from postnatal days 30 to 45) increases neural death in both the hippocampus and the PFC and that these animals present behavioral and cognitive deficits at the end of ethanol treatments and at the adult stage (Pascual et al., 2007). These results suggest that intermittent ethanol intoxication during adolescence induces brain damage, impairing the normal processes of brain maturation and plasticity, and causing long-lasting behavioral consequences. Notably, ethanol–induced brain damage seems to be greater in female adolescent rat than in their male counterparts and brain injury is greater in adolescent animals than in adult animals (Pascual and Guerri, unpublished results), which corroborate the human studies on gender differences in alcohol–induced brain damage.

The underlying mechanisms of ethanol–induced brain damage during adolescence are poorly understood, although two mechanisms have been proposed. The first suggests that intermittent ethanol drinking might induce brief episodes of withdrawal from alcohol and might induce excitotoxic neuronal damage. Ethanol withdrawal has been shown to increase aberrant synaptic activation of NMDA receptors (Hendricson et al., 2007), and also produce a marked cellular injury, as demonstrated in CA1 pyramidal neurons from hippocampal explants (Prendergast et al., 2004; Self et al., 2005). This process can result in either discrete excitotoxic neuronal damage or a more severe and complicated alcohol withdrawal syndrome, including withdrawal seizures (Becker et al., 1997). However, De Bellis et al. (2005) reported that of the 14 adolescents with AUD who presented PFC reduction, only two individuals displayed withdrawal symptoms. In addition, excitotoxicity appears to result in enhanced glutamatergic transmission by upregulating the NMDA receptors after chronic ethanol consumption, and a reduction in the levels and function of the NMDA receptors has been observed in animals exposed to ethanol during adolescence (Pascual et al., 2009; Swartzwelder et al., 1995a).

The second mechanism indicates that ethanol induces adolescent brain damage by mechanisms involved in neuroinflammation. Alcohol intake has been demonstrated to possibly induce inflammatory mediators in the brain by activating glial cells, and by stimulating intracellular signaling pathways that trigger the induction of proinflammatory cytokines (IL-1β, TNF-α), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and neural cell death (Blanco et al., 2005; Valles et al., 2004). A recent study demonstrated that intermittent ethanol administration to adolescent rats upregulated the COX-2 and iNOS levels, and increased cell death in the neocortex, hippocampus, and cerebellum (Pascual et al., 2007). It is noteworthy that an increase in brain inflammatory mediators concomitantly occurs with short- and long-term cognitive deficits in animals treated with ethanol during the adolescence stage (Pascual et al., 2007), thus suggesting an association between brain damage and long-term cognitive effects. Likewise, a single dose of ethanol during middle adolescence has been shown to decrease neural progenitors proliferation and neural survival in young adult animals (Crews et al., 2006), suggesting that the adolescent binge-drinking may induce long-term effects on the neurogenesis associated with either learning or other behaviors undergoing maturation during brain adolescent development.

Concerning the potential treatments for the damaging effects of ethanol on the adolescent brain, Pascual et al. (2007) demonstrated that the administration of a nonsteroidal anti-inflammatory compound, indomethacin (an inhibitor of COX-2 activity) (Kluska et al., 2005; Shibata et al., 2003), before administering ethanol to adolescent rats abolished the induction of the COX-2 and iNOS expressions and cell death, and prevented ethanol–induced behavioral deficits (Pascual et al., 2007). Remarkably, elevated levels of COX-2 and iNOS have also been observed during excitotoxicity, ischemia, and neural injury (Heales et al., 1999; O'Banion, 1999; Yamada et al., 1999), whereas COX-2 inhibitors have been shown to prevent neuronal loss and to ameliorate brain injury caused by excitotoxicity (Iadecola et al., 2001), ischemic brain injury, brain inflammation, and neurodegeneration (Minghetti, 2004; Willard et al., 2000). COX-2 inhibitors also improve the behavioral and cognitive functions associated with neurodegeneration (Minghetti, 2004). These findings suggest that inflammation could play a prominent role in the

pathophysiological mechanisms of both ethanol–induced brain damage and neurobehavioral deficits in adolescent animals.

Regarding the molecular mechanism by which ethanol induces neuroinflammation, it has been shown that ethanol, by stimulating the innate immune response and, specifically the Toll-like receptor 4 (TLR4) and interleukin-1 receptor I (IL-1RI) receptor (Blanco and Guerri, 2007; Blanco et al., 2005; Fernandez-Lizarbe et al., 2008), activates the signaling pathways and transcription factors (nuclear factor-kappa B [NF-κB] and activator protein-1 [AP-1]), which can lead to the induction of cytokines and inflammatory mediators in the brain, causing neural dysfunction and death (for a review, see Blanco and Guerri, 2007 and Fig. 1).

In summary, human and animal studies have demonstrated the vulnerability of the adolescent brain to the harmful effects of ethanol. The results of these studies show that heavy drinking during adolescence can induce neural damage, particularly in the PFC and the hippocampus, as well as cognitive deficits. These data suggest that alcohol, by inducing neural death and damaging specific areas, alters neural developmental plasticity and impairs brain maturation, thus leading to lifelong behavioral impairments (for a review, see White and Swartzwelder, 2005). Furthermore, dysfunctions in the PFC also occur in alcoholics and have been associated with impaired cognitive functions and compulsive behavior, events that predispose to alcohol abuse (Duka et al., 2003; Moselhy et al., 2001). Therefore, ethanol–induced PFC impairments in adolescents might not only underlie the cognitive dysfunctions, but also predispose to alcohol abuse and dependence (Crews et al., 2007).

Neurochemical mechanisms involved in the vulnerability of adolescents to alcohol abuse and dependence

Another important long-lasting consequence of alcohol use during adolescence is the greater risk of developing alcohol dependence in adulthood. Indeed in both prospective and retrospective human studies, an early onset of alcohol use typically emerges as a reliable predictor of a later problematic use and dependence on alcohol and other drugs (DeWit et al., 2000; Grant and Dawson, 1997; Hawkins et al., 1997; Labouvie et al., 1997). For instance, survey data indicate that the rate of lifetime alcohol dependence was 40% when individuals started drinking at 14 years and younger, whereas only 10% of individuals started drinking at 21 years and younger (Grant and Dawson, 1997). Furthermore, the age of the first use of alcohol and other illicit drugs was similar across six different populations (~11–14 years), and also showed a fundamental uniformity of onset patterns per age which contrasted with wide variations in lifetime prevalence across different countries (Vega et al., 2002). Some studies suggest the existence of certain predispositions, disorders, or genetic background in certain adolescents, for substance abuse disorders (e.g., adolescents with AUD). A strong correlation has also been found between family history and the risk of initiating drinking under the age of 15 (Dawson, 2000). Behavioral under control is measurable very early in life and is a predictor of both earlier alcohol use and elevated risk for later AUD (Caspi et al., 1996; Wilens and Biederman, 2006; Zucker and Wong, 2005). Besides, hostility significantly predicts risk taking which, in turn, predicts substance abuse (Ohannessian and Hesselbrock, 2008). These findings suggest that some risk factors (e.g., specific genetic background, environmental, and familiar) could predispose certain adolescents to the initiation of alcohol abuse and AUD later in life.

Another possibility that may concomitantly occur with genetic predisposition, is that early exposure to alcohol could sensitize the brain regions and/or developmental processes involved in drug addiction, causing long-term effects on neurobehavioral functions and increasing propensity to later abuse. The *rewarding power* of abused drugs is classically ascribed to mesolimbic dopaminergic projections. The mesocorticolimbic DA pathway compromises the ventral tegmental area, the NAc, and the associated limbic structures. This pathway participates in the reward and reinforced effects of drugs of abuse, including alcohol (Koob and Weiss, 1992; Pierce and Kumaresan, 2006; Robbins and Everitt, 2002) and substantial evidences demonstrate that the limbic system and the mesocorticolimbic DA pathway undergo important remodeling during adolescence (as previously mentioned).

Studies using animal models have begun to explore the impact of the mesocorticolimbic DA pathway response on adolescent and adult animals and have shown that although ethanol elicits a prolonged DA response in the NAc septi of both adolescent and adult animals pretreated with intermittent doses of ethanol, the DA levels tend to be higher in adolescent (160%) than in adult rats (Pascual et al., 2009). A prolonged increase in the extracellular DA levels has also been observed in alcohol-preferring rats exposed to ethanol from postnatal day 30 to 60 when compared with saline-treated animals (Sahr et al., 2004). However, another important difference between young and adult animals is the higher extracellular accumbal DA baseline levels in adolescent alcohol-treated animals when compared with ethanol-treated adult rats (Pascual et al., 2009). Notably, elevated levels of extracellular DA noted in ethanol-exposed adolescents continue into adulthood (Badanich et al., 2007). These findings suggest that changes in extracellular DA during adolescence could both sensitize the dopaminergic system and mediate the increased likelihood of engaging in drug use initiation during adolescence, and might even mediate the long-term consequences of alcohol abuse. Indeed, binge-like ethanol treatment during the juvenile/adolescent period increases ethanol intake in adulthood rats, and these animals display high accumbal extracellular DA levels (Pascual et al., 2009). Similarly, alcohol drinking during periadolescence by alcohol-preferring rats that present high accumbal extracellular DA levels (McBride et al., 2005), also increases the

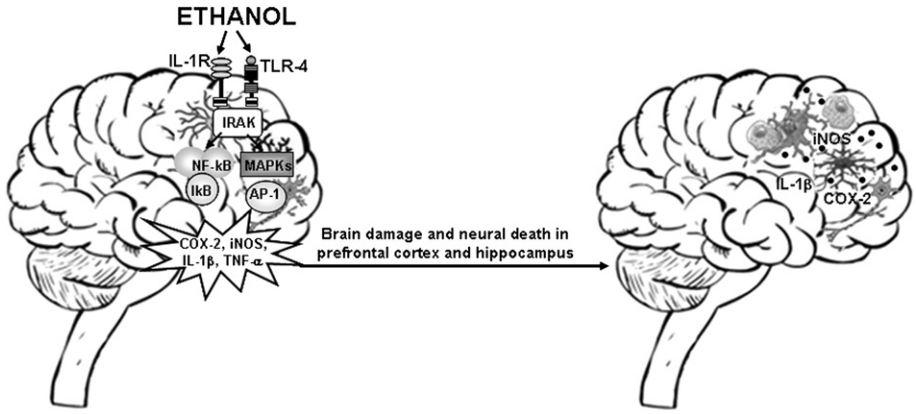

Fig. 1. Potential mechanism of ethanol-induced brain damage by neuroinflammation. Ethanol activates the TLR4 and IL-1RI pathways in glial cells in the brain, leading to inflammatory responses with the production of cytokines and inflammatory mediators and neural damage.

acquisition of ethanol-self administration in adulthood (Bell et al., 2006). Studies conducted in C57BL/6J and BAL mice (Blizard et al., 2004; Ho et al., 1989) have also confirmed that postweaning two-bottle choice exposure slightly, but significantly, increases ethanol consumption and preference in adulthood. Conversely, forced ethanol exposure during adolescence in rats does not enhance the reinforcing properties of ethanol in adult animals (Slawecki and Betancourt, 2002; Vetter et al., 2007). Multiple factors (e.g., differences between species, genetic factors, alcohol administration paradigm, age of initiation, or housing conditions) may explain the variations in findings across different studies.

Stressors of adolescence have been suggested to contribute to the high sensitization of the mesocorticolimbic DA system and to the initiation of ethanol during adolescence (for a review, see Spear, 2002) because stressors can selectively activate the mesocorticolimbic DA projections (Robbins and Everitt, 2002; Wise, 1994). In fact, stress increases the drinking onset in young male and female rats (Fullgrabe et al., 2007; Siegmund et al., 2005). Nevertheless, some studies have shown no association between early alcohol consumption and stress (Tambour et al., 2008) or novelty seeking, anxiety, and stress hormone levels in adolescent rats (Schramm-Sapyta et al., 2008), suggesting that not only stressors mediate the high alcohol consumption and the relapse-like behavior in adolescent animals.

Developmental changes also occur with glutamate NMDA receptors (NMDAR), which peak in early adolescence and decline significantly thereafter (Guilarte and McGlothan, 1998; Insel et al., 1990). Such synaptic pruning could contribute to the loss of excitatory glutamate input to NAc (Frantz and Van Hartesveldt, 1999b), and also to a reduction in accumbal NMDAR (Frantz and Van Hartesveldt, 1999a) during adolescent brain maturation. These ontogenetic changes in glutamate and DA, and also in their receptors, could certainly contribute to neurochemical remodeling in adolescents, especially in the limbic brain regions, and might underlie the higher ethanol consumption noted in adolescents compared with adult rats.

Experimental studies have demonstrated that intermittent ethanol treatment during adolescence, in addition to sensitizing the mesocorticolimbic DA system, also induces alterations in the dopaminergic and glutamatergic neurotransmissions, events that might affect the remodeling and functions of adolescent brains. Thus, intermittent binge-like ethanol exposure has been shown to decrease the levels of both DRD2 and NMDAR-2B phosphorylation in several brain areas, including the PFC and the hippocampus of adolescent animals. A similar ethanol treatment has been seen to have no significant effects on the levels of these proteins in the adult brain (Pascual et al., 2009). Notably, reductions in the levels of DRD2 have been reported in ethanol-preferring rats (Kanes et al., 1993; McBride et al., 1993), whereas the overexpression of DRD2 has been seen to reduce both alcohol preference and intake (Thanos et al., 2001, 2004, 2005), suggesting that these receptors are involved in alcohol abuse. The above findings also suggest that the developmental expressions in DRD2 and NMDAR-2B phosphorylation are sensitive to the ethanol-inhibition during adolescence, contributing to the greater sensitivity of young animals to ethanol-induced inhibition of LTP.

Glutamatergic transmission is vital for the control of the ventral tegmental area and is also critical for weighing the novelty and importance of a stimulus, an essential output of this brain region. Alcohol exposure during adolescence enhances the synaptic localization of the NMDA receptors and leads to the increased size of the dendritic spines (Carpenter-Hyland and Chandler, 2007). This increase in size may represent a structural-based mechanism that supports the formation and stabilization of maladapted synaptic connections that, in a sense, "fix" addictive behavior in adolescent and young adult brains (Carpenter-Hyland and Chandler, 2007).

Finally, the ethanol-induced inhibition of the NMDA receptor activity could attenuate the NMDA-mediated transmission in the PFC to not only lead to dysfunctions in the PFC, but also to impulsive behavior and lack of control over drinking, which characterize alcoholism (Weitlauf and

Woodward, 2008). As PFC reductions and abnormalities occur in human adolescents with AUD (Medina et al., 2008), inhibition of NMDAR-2B activity might contribute to loss of control and alcohol addiction.

In summary, adolescence and early adulthood appear to represent a period of significantly greater vulnerability to addictive drugs. Refinements in the synaptic functions and neuronal architecture in the adolescent brain are thought to represent learning–based adaptive processes that are important for developing an adult-like cognitive phenotype. However, this period of heightened neuroplasticity also confers a greater vulnerability to addictive drug actions. Sensitization of the mesocorticolimbic DA pathway, along with changes in the glutamatergic and dopaminergic neurotransmission, might mediate the vulnerability of adolescents to the long-term consequences of alcohol addiction (Fig. 2).

Potential role of epigenetic modifications in the long-term effects of early alcohol consumption

Changes in the gene expression in the brain reward regions are thought to contribute to the pathogenesis and persistence of drug addiction. Recent studies suggest that drugs of abuse and related environmental stimuli, such as drug-associated cues or stress, converge on the genome to alter specific gene programs. Specifically, the epigenetic mechanisms that alter the chromatin structure in specific gene promoters can lead to potent and often long-lasting changes in the gene expression, which contribute to neurobehavioral alterations or addictive-like behavior (Renthal and Nestler, 2008).

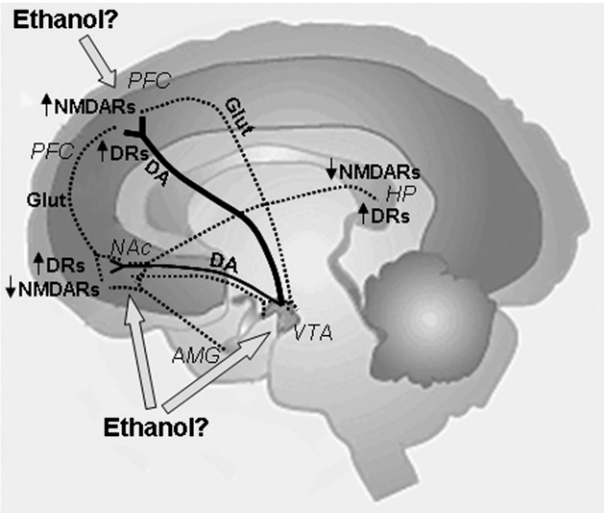

Fig. 2. Schematic representation of the dopaminergic and glutamatergic pathways in the adolescent rat brain. During adolescence, the DA projections into the PFC increase (thicker line), and there are changes in the expression of DA and NMDA receptors. The possible sites of ethanol action on the mesocorticolimbic dopaminergic circuitry during adolescence are indicated. PFC, prefrontal cortex; NAc, nucleus accumbens; AMG, amygdala; HP, hippocampus; DRs, dopamine receptors; NMDARs, NMDA receptors; DA, dopamine; glut, glutamate.

A common form of epigenetic modification involves the addition of molecules to the DNA structure. Thus, posttranslational modifications in the chromatin, such as acetylation, phosphorylation, and methylation of histones on DNA, positively or negatively modulate the transcriptional activity of the underlying genes without causing major alterations to the genetic structure (Grewal and Moazed, 2003). Indeed, enzymes that induce histone acetylation, such as histone acetyltransferases, cause nucleosome relaxation and promote the gene expression, whereas enzymes that remove acetyl groups from the histone, such as the histone deacetylases (HDACs), pack the DNA into a more condensed chromatin, block the access to transcriptional activators, and lower the gene expression (Hsieh and Gage, 2005). These associations exist in brain in vivo studies in response to drugs of abuse. Recent studies have demonstrated that HDAC inhibitors improve the plasticity associated with memory formation which, in turn, is associated with both drug abuse (McClung and Nestler, 2008) and the formation of long-term contextual fear memory (Levenson et al., 2004).

Other conditions, such as changes in dopaminergic and glutamatergic inputs, have also been shown to induce chromatin remodeling by histone modifications (Li et al., 2004; Schroeder et al., 2008), and both events have been associated with drug-related behavioral sensitization and reward (Fischer et al., 2007; Kumar et al., 2005; Li et al., 2004; McClung and Nestler, 2008; Schroeder et al., 2008). Consistently with these findings, a recent study has demonstrated that intermittent ethanol treatment during adolescence not only alters dopaminergic and glutamatergic neurotransmissions, but also induces changes in the acetylation of histones H3 and H4 in the frontal cortex, NAc, and the striatum (Pascual et al., 2009). These results suggest that ethanol-induced alterations in the dopaminergic and glutamatergic systems during adolescence may trigger histone modifications, and that these events might participate in the long-term behavioral alterations induced by early alcohol consumption. In accordance with this hypothesis, a recent study has demonstrated that cocaine administration to adolescent rats reduces histone H3 methylation and causes long-term behavioral consequences (Black et al., 2006).

Although the association between alcohol and epigenetic mechanisms is poorly understood, a novel role has been recently demonstrated for amygdaloid chromatin remodeling in the process of alcohol addiction, suggesting that HDAC inhibitors may be potential therapeutic agents in treating alcohol withdrawal symptoms (Pandey et al., 2008). Therefore, because addiction is more persistent when drug use begins at an early age, it is possible that alcohol and other illicit drugs could induce chromatin remodeling and abnormal plasticity in reward–related learning processes during brain maturation, events that might contribute to the long-lasting consequences of alcohol abuse and to adolescents' vulnerability to drug addiction.

Numerous unanswered questions pertaining to this action mechanism of ethanol, or of other illicit drugs, must

be elucidated to discover which genes are modulated by acetylation or methylation and which genes participate in the long-lasting neurobehavioral alterations induced by ethanol consumption during adolescence.

Conclusions

- Adolescence is a critical stage of development in which the brain undergoes neuromaturation and reorganization characterized by changes in neurotransmission, plasticity, and synaptic remodeling. The results of human and animal research suggest that alcohol exposure during adolescence adversely affects brain development and maturation, causing brain damage, structural alterations, and cognitive deficits.
- Neurochemical immaturity and the heightened neuroplasticity in the limbic brain regions might confer a greater sensitivity of adolescence to addictive drug actions. Sensitization of the mesocorticolimbic DA pathway, along with changes in the glutamatergic and dopaminergic neurotransmission as well as chromatin remodeling might mediate the vulnerability of adolescents to the long-term consequences of alcohol addiction.
- Although prevention and alcohol control policies are important for reducing the devasting consequences of young drinking, a better knowledge of the molecular events underlying the effects of ethanol on the teenage brain could also lead to the development of therapeutic strategies to treat disorders associated with adolescent alcohol use.

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