Impact of neuroimmune activation induced by alcohol or drug abuse on adolescent brain development

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**Abstract**

Evidence obtained in recent decades has demonstrated that the brain still matures in adolescence. Changes in neural connectivity occur in different regions, including cortical and subcortical structures, which undergo modifications in white and gray matter densities. These alterations concomitantly occur in some neurotransmitter systems and hormone secretion, which markedly influence the refinement of certain brain areas and neural circuits. The immaturity of the adolescent brain makes it more vulnerable to the effects of alcohol and drug abuse, whose use can trigger long-term behavioral dysfunction. This article reviews the action of alcohol and drug abuse (cannabis, cocaine, opioids, amphetamines, anabolic androgenic steroids) in the adolescent brain, and their impact on both cognition and behavioral dysfunction, including predisposition to drug abuse in later life. It also discusses recent evidence that indicates the role of the neuroimmune system response and neuroinflammation as mechanisms that participate in many actions of ethanol and drug abuse in adolescence, including the neurotoxicity and alterations in neurocircuitry that contribute to the dysfunctional behaviors associated with addiction. The new data suggest the therapeutic potential of anti-inflammatory targets to prevent the long-term consequences of drug abuse in adolescence.

1. Introduction

Adolescence is a developmental stage of brain maturation in which important structural and functional changes in synaptic plasticity and neural connectivity occur in different regions, including the cortical and subcortical structures, which undergo modifications in white and gray matter densities (Gogtay et al., 2004; Sowell et al., 2001). These changes concurrently occur with modifications in some neurotransmitter systems and hormone secretion, which markedly influence the refinement of certain brain areas and neural circuits (Schulz and Sisk, 2016; Vigil et al., 2011).

The immaturity of the adolescent brain makes it more vulnerable to the effects of abuse of alcohol and other drugs (Winters et al., 2012), and exposure to these substances in adolescence can cause dysfunctions in brain regions that undergo maturation, which lead to long-term behavioral and cognitive dysfunctions (see Pascual et al., 2018). Likewise, the non uniform maturation pattern, in which the limbic region (involved in emotion, motivation and reward behaviors) develops faster than the cerebral cortex region (reasoning), contributes to increased risk taking and novelty seeking among young teenagers (Dahl, 2004; Steinberg, 2004), which promotes alcohol and drug abuse. In fact the prefrontal cortex that is responsible for executive functions (Badre et al., 2010) is the last brain region to complete its maturation. Several studies clearly demonstrate that binge ethanol drinking in adolescence alters brain plasticity, and causes structural and functional changes in the prefrontal cortex that result in cognitive and behavioral deficits (Montesinos et al., 2015). Another major consequence is that alcohol or drug use initiation at an early age is an important predictor of developing a later substance use disorder (Aiken et al., 2018; Silins et al., 2018).

The mechanisms involved in the actions of ethanol and abuse of other drugs in the adolescent brain are uncertain, but evidence obtained over the last decade indicates the role of the neuroimmune system response in the neuropathological and behavioral consequences associated with binge ethanol drinking or drug abuse in adolescence (Brown et al., 2018; Crews et al., 2016; Montesinos et al., 2015, 2016b).

As adolescence is a critical stage of brain maturation and behavioral development, and one that is highly sensitive to environmental influences, including alcohol and drug abuse, we review the clinical and experimental evidence which demonstrates that exposure to alcohol...
and abuse of other drugs in adolescence impacts the adolescent brain maturation and causes long-term behavioral and cognitive dysfunctions. We also discuss the potential pathological bases and recent evidence that indicate the participation of the neuroimmune system response in glial cells, the influence of the innate immune toll-like receptors (TLRs) in neuroinflammation, and long-term behavioral dysfunctions associated with alcohol and drug abuse in adolescence.

2. The neuroimmune system

Although the central nervous system (CNS) has been considered an immune-privileged site, recent studies indicate that immune surveillance occurs under physiological and pathological conditions (Shrestha et al., 2013). However, the immune responses generated in the CNS differ from those that occur in the periphery because the blood brain barrier and tissue-resident glial cells confer nervous cells protection from potentially harmful immune cell-mediated damage. Recent studies have also shown that the peripheral immune system and the CNS can communicate using common molecular signaling cues (Prinz and Priller, 2017).

In the CNS, glial cells, microglia and astroglia are the principal cells that participate in the immune response, which not only serve as supportive and nutritive roles for neurons by contributing in remodeling circuit connectivity and plasticity, but also defend the CNS from stress and pathogenic insults by transiently up-regulating inflammatory processes (Tian et al., 2012). Among these cells, microglia, non parenchymal macrophages in the brain or mononuclear phagocytes, are capable of responding to pathogens and other insults. During stress stimuli or pathological events, these cells are activated, and their morphology changes and transforms into an ameboid phenotype, which is functionally similar to macrophages (Davis et al., 1994; Streit, 2000). Activated microglia produce and release a variety of factors, including cytokines, prostanooids, free radicals and pro-inflammatory mediators, which make these cells a good marker of neuroinflammation (Kettenmann et al., 2011). Other glial cells, astrocytes, are also capable of responding to a neuropathologic process by triggering the production of free radicals (Sofofroniew and Vinters, 2010).

Both microglial and astroglial cells express innate immune receptors, TLRs and cytoplasmic NOD-like immune receptors (NLRs) (Alfonso-Loeches et al., 2016, 2014; Blanco et al., 2008, 2005). These receptors detect and react not only to pathogens (PAMPs, pathogen-associated molecular patterns), but also to stress conditions, and to cell damage (DAMPs or damage-associated molecular patterns) (Montesinos et al., 2016b). Activation of TLRs triggers signaling pathways, such as the activation of transcription factor NF-κB, which culminates with the production of cytokines and inflammatory mediators (Montesinos et al., 2016b) (see Fig. 1). Recent studies demonstrate the participation of these receptors in neuroinflammation and in the long-term behavioral and neuropathological dysfunctions induced by binge ethanol drinking (Montesinos et al., 2016b) and drug abuse in adolescence (Hutchinson et al., 2012; Zhu et al., 2018).

2.1. Toll-like receptors and the innate immunity

Among the innate immune receptors, TLRs are the first identified and best characterized evolutionarily conserved family of receptors, which were discovered in the Drosophila melanogaster as a major defense against microbial infection (Medzhitov et al., 1997). Ten human and twelve murine TLRs have been characterized, although the most studied receptor is TLR4, which recognizes endotoxin lipopolysaccharide (LPS), a molecule found in the external membrane of gram-negative bacteria. Whereas receptors TLR1, TLR2, TLR4, TLR5, and TLR6 are located on the cell surface, TLR3, TLR7, TLR8 and TLR9 are located within the endosome/lysosome membrane (Nishiya and DeFranco, 2004). The activation of TLRs by PAMPs or DAMPs (Table 1) triggers different signaling pathways, including nuclear factor-κB (NF-κB) and mitogen-activated protein kinases (MAPKs), which lead to the production of inflammatory mediators, cytokines, chemokines and reactive oxygen species (ROS), and allow the intracellular pathogen to be killed.

Although glial cells, astroglia and microglia are the main immune cells that express several TLRs (e.g., TLR4) (Okun et al., 2011), neurons can also express TLR4, although the signaling pathways associated with the TLR4 response differ between both cell types (Leow-Dyke et al., 2012; Okun et al., 2011). Under physiological conditions, the release of cytokines by the glial TLR4 response can modulate neuronal functions by regulating synaptic and neural plasticity. Nevertheless, pathophysiological levels of pro-inflammatory cytokines can impair synaptic plasticity, and might underlie cognitive disturbances, memory dysfunctions and mood disorders (Khairova et al., 2009). Similarly, while NF-κB activation is associated with immune function regulation, in neurons the constitutive activation of this transcriptional factor is related with synaptic plasticity and memory processes (Snow et al., 2014), while NF-κB over-activation can cause neurotoxicity (Shih et al., 2015).

It is important to note that glial cells and neurons can be activated by the cytokines released from either the brain or peripheral blood. Additionally, certain compounds, like ethanol, can induce the release of LPS from the gut to the bloodstream (Mandrekar and Szabo, 2009) by activating TLR4 in liver Kupffer cells and peripheral monocytes, which can trigger the production of cytokines that can cross the BBB via diffusion or active transport, or even by BBB alterations (Rubio-Araiz et al., 2017). Indeed several studies have shown that the brain integrates neuro-immune communication and that the brain function is altered in diseases associated with peripheral immune dysregulation and inflammation, as occurs in some neurobehavioral dysfunctions associated with systemic neuroimmune activation (e.g., autoimmune diseases, liver failure, sepsis) (Pavlov et al., 2018).

3. Alcohol

Alcohol is the substance most normally used by adolescents. The common pattern followed by young teenagers and young adults is binge alcohol consumption. World Health Organization data (WHO, 2014) show that approximately 16% of the worldwide prevalence corresponds to drinkers aged 15 and upward. This pattern of alcohol consumption is defined as five drinks or more on one same occasion in the past 30 days, with 43% for males and 38% for females.

Human and experimental studies demonstrate that binge ethanol drinking may affect the prefrontal and mesolimbic regions, which are involved in important adolescent ontogenetic changes (Gogtay et al., 2004). Thus the structural changes associated with cognitive impairment and anxiety-like behavior, and related with long-term drug abuse, have been observed in adolescents who have reported high alcohol consumption (see rev. Spear, 2016). Notably, gender differences in ethanol-induced brain structural alterations have been noted since female adolescent binge drinkers display greater cortical thinning than male adolescents. These effects are associated with worse visuospatial memory and the inhibition of attention performance in females than in males (Squeglia et al., 2012, 2009).

Although the molecular mechanisms of ethanol actions in the human adolescent brain are poorly understood, data about glial cells in culture and animal studies suggest the critical role of innate immune receptors TLRs in the neuroinflammation, brain damage and behavioral dysfunction induced by ethanol in adolescence (see below).
Fig. 1. Schematic of alcohol-induced neuroinflammation through the activation of immune receptor TLR4. Ethanol or PAMPs (e.g., LPS) or DAMPs (e.g., HMGB1) activate/s TLR4 by its recruitment with adaptor proteins CD14 and MD-2 in lipid rafts. This activation leads to the recruitment of the complex MyD88, IRAK and TRIF to trigger rapid downstream signaling pathways (e.g., IRAK/Traf-6 and MAPKs) and transcription nuclear factors (NF-xB and AP-1), which terminate in the generation of cytokines (e.g., IL-1β, IL-6, IL-8, TNF-α, etc.), chemokines (e.g., MCP-1) and inflammatory mediators (e.g., iNOS, COX-2), which lead to neuroinflammation. The expression of these inflammatory compounds, along with other stimuli such as alarmins (e.g., IL-33, HMGB1), can amplify the neuroinflammatory response. MyD88: myeloid differentiation primary response gene 88; IRAK: interleukin-1 receptor-associated kinase; MAPK: mitogen-activated protein kinase; AP-1: activator protein 1.

| Drug                          | Mechanisms                                                                 | References                                                                 |
|-------------------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Alcohol                       | Neuroinflammation                                                           | Pascual et al. (2018), Crews et al. (2016)                                  |
|                               | TLRs response                                                               |                                                                            |
|                               | Epigenetic changes                                                          |                                                                            |
|                               | Neurotransmitter system                                                     |                                                                            |
| Cannabis                      | Cannabinoid receptors signaling                                             | Chadwick et al. (2013), Kendall and Yudowski (2016), Zamberletti et al. (2015) |
| Opioids                       | Neuroimmune response                                                        | Al-Hassani and Bruchas (2011), Hutchinson et al. (2012), Cahill and Taylor (2017) |
|                               | Neuroimmune response                                                        |                                                                            |
| Cocaine                       | VTA dopaminergic system                                                     | Wong et al. (2013), Murisch et al. (2010)                                  |
|                               | Neuroimmune response                                                        | Northcutt et al. (2015)                                                    |
| Amphetamines                  | Free radicals/oxidative stress                                              | Parrott et al. (2004), Frau et al. (2013), Fernandes et al. (2016)          |
|                               | Neuroimmune response                                                        |                                                                            |
|                               | Ionotopic purinoceptor P2 × 7                                                |                                                                            |
| Anabolic androgenic steroids  | Neurotransmitter systems dysfunction                                         | Bertruzzi et al. (2018), Marshall-Gradinaruk et al. (2009), Riezzo et al. (2014) |
|                               | Immune response                                                             |                                                                            |
3.1. Molecular mechanisms of ethanol-induced neuroinflammation and the TLR4 response

3.1.1. Ethanol, glial cells and the TLR4 signaling response

The involvement of the ethanol-induced activation of the innate immune response has been shown in the early study by (Valles et al., 2004), which demonstrates that ethanol consumption triggers the induction of inflammatory mediators within the brain, and causes the induction of cytokines in astroglial cells in culture. Further studies done with cortical cultures of microglia and astrocytes demonstrate that ethanol, such as endotoxin lipopolysaccharide or LPS, is able to activate glial cells by inducing the TLR4 signaling response, triggering its translocation to lipid raft microdomains and promoting the recruitment of signaling molecules (phosphorylate kinases) in these microdomains by stimulating the downstream signaling response (NF-κB, AP-1), along with the release of cytokines and inflammatory mediators (e.g., IL-1β, TNF-α, COX-2, iNOS), in the medium of microglia and astrocytes in culture (Blanco et al., 2008, 2005; Fernandez-Lazarbe et al., 2009) (Fig. 1). By using either siRNA or cells from TLR4-deficient or knockout (TLR4-KO) mice, blocking the TLR4 response abolishes ethanol-induced cytokine and inflammatory mediators (Alfonso-Loeches et al., 2010; Fernandez-Lazarbe et al., 2009). In summary, in vitro studies conducted in glial cells demonstrate that ethanol is capable of activating the TLR4 signaling response, which leads to cytokine and chemokine production.

3.1.2. Role of the TLR4 response in the neuroinflammation induced by binge-ethanol treatment in adolescence

In vivo studies confirm the stimulatory role of ethanol in TLR4 signaling by demonstrating that binge alcohol administration to adolescent rodents induces astrogliosis and microgliosis, increases cytokines and inflammatory mediators, and causes neuroinflammation and brain damage (Montesinos et al., 2015, 2016b; Pascual et al., 2007; Vetreno and Cresws, 2015). These effects are dependent of TLR4 signaling because the elimination of TLR4 in mice (TLR4-KO) abolishes binge-like ethanol treatment-related neuroinflammation and brain injury (Montesinos et al., 2015, 2016b).

Studies using binge-like ethanol drinking in adolescent animals have provided further evidence that high ethanol doses in adolescence induce the release of both proinflammatory compounds (e.g., TNF-α, IL-1β, MCP-1, MIP-1α) and DAMPs molecules, such as HMGB1, and that these effects are mediated by the activation of different TLRs since the up-regulation of TLR2, TLR3 and TLR4 is noted (Pascual et al., 2014; Vetreno and Cresws, 2012). However, the role of TLR4 in neuroinflammation has been clearly demonstrated in the study of Montesinos et al. (2015). This study shows that high ethanol doses in adolescent mice trigger the release and expression of inflammatory mediators (e.g., iNOS, COX-2) (Pascual et al., 2007), as well as cytokines and chemokines (e.g., TNF-α, IL-1β, MCP-1, MIP-1α, IL-17 A), in the prefrontal cortex of mice (Montesinos et al., 2015) (Fig. 2), but these effects of ethanol are not observed in TLR4-deficient mice. In line with these findings, the use of anti-inflammatory compounds has been found to prevent ethanol-induced neuroinflammation and neural damage. For instance, indomethacin inhibits the cyclooxygenase enzyme (Pascual et al., 2007), ethane-β-sulfam reduces cytokine release, reactive nitrogen species and neuronal loss (Stefanini et al., 2014), while oleyl-ethanolamide prevents neuroimmune TLR4/NF-κB danger sensing in the rat frontal cortex (Anton et al., 2017).

New findings in adolescent humans and mice (Pascual et al., 2017) show that acute alcohol intoxication induces higher levels of plasma cytokines and chemokines in human adolescent females than in adolescent males with comparable blood alcohol levels. Interestingly, increased levels of cytokines in female drinkers have been associated with both TLR4 mRNA blood levels than in males. These gender differences have also been corroborated in ethanol-treated adolescent mice, in which the levels of cytokines/chemokines in both plasma (e.g., IL-17 A, MCP-1, and MIP-1α) and the brain (e.g., IL-1β, IL-17 A, MCP-1, MIP-1α and fractalkine) were significantly higher in females than in males. This finding suggests that some cytokines or chemokines may be considered peripheral biomarkers of ethanol-induced neuroinflammation and brain damage. However, the same ethanol treatment has no effect on female and male TLR4 deficient mice, which supports the role of the TLR4 response in the neuroinflammatory effects of ethanol (Pascual et al., 2017).

Another mechanism involved in the actions of ethanol on the adolescent brain is the participation of epigenetic changes, such as changes in histone methylation and acetylation, which can affect gene transcription. Thus adolescent binge ethanol drinking either modulates the epizymes involved in H3K9 dimethylation in the amygdala in adulthood (Kyzar et al., 2017) or reduces brain-derived neurotrophic factor (BDNF) expression by increasing histone deacetylasyles in the CA1, CA2, and CA3 regions of the hippocampus (Sakharkar et al., 2016), effects that could contribute to long-term behavioral abnormalities, including anxiety. Other studies demonstrate that binge-like ethanol treatment in adolescent mice induces epigenetic changes in the promoter region of Snap2 and fosb by increasing their expression in the medial prefrontal cortex of young adult animals (Montesinos et al., 2016b). These effects might be associated with long-term rewarding and anxiogenic-related behavioral impairments, and with increased alcohol preference.

The neurotransmitters system has also been found to be involved in the effects of ethanol in adolescence. Among them, activation of both the mesolimbic dopamine system and the glutamate and gamma aminobutyric acid (GABA) systems has been shown in adolescent rodents with binge-like ethanol drinking. For instance, adolescent binge drinking in rats induces higher levels of basal extracellular dopamine in the nucleus accumbens shell and dopamine receptors D1 and D2 in adolescents compared with adults (Pascual et al., 2009). However, a similar treatment in adolescence reduces choline acetyltransferase (a cholinergic marker) in the basal forebrain, and tyrosine hydroxylase (a dopaminergic marker) in the prelimbic and infralimbic cortices, which suggests a reduction in the cholinergic and dopaminergic neurotransmitter systems (Boutros et al., 2016; Vetreno et al., 2014). Alterations to the dopaminergic system might be involved with the reward and reinforcement effects of abuse of alcohol and other drugs (Koob and Weiss, 1992; Robbins and Everitt, 2002), while alterations to the GABAergic system in the adolescent frontal cortex have been associated with impulsiveness and cognitive control (Silveri, 2014). Likewise, changes in the basal glutamate levels and glutamatergic NMDA receptors have also been observed in adolescent ethanol-treated animals (Pascual et al., 2009; Szumlinski et al., 2007; Ward et al., 2009), although developmental differences in the composition of NMDAR subunits in adolescent vs. adult animals may explain the enhanced vulnerability of the adolescent brain to ethanol dependence (Pian et al., 2010).

3.2. Neuropathological consequences of alcohol abuse in adolescence

Several neuropathological consequences have been observed in adolescents with binge ethanol drinking. For instance in adolescence, synapses and neural circuits are remodeled to acquire a normal cognitive function (Glantz et al., 2007), and changes in important specialized proteins in both pre- and postsynaptic terminals occur that are crucial for synaptic neurotransmission (Washbourne et al., 2002). Binge ethanol drinking in adolescence alters synaptic transmission, as demonstrated by down-regulating synaptic proteins (e.g., synapsin IIa, syntaxin 4, SNAP-25 and synaptotagmin). These effects have been associated with ultrastructural alterations in synapses, such as a reduction in both postsynaptic thickness and synaptic vesicle number, and an increase in convex synaptic curvature and synaptic cleft width, effects related with poor synaptic transmission efficacy (Kovalenko et al., 2006). The adolescent brain is also characterized by synaptic pruning with a reduction in dendritic spine connections. Recent findings reveal that binge ethanol treatment in adolescence may affect structural
Fig. 2. Potential mechanism of the abuse of ethanol or other drugs, which is able to induce brain damage by neuroinflammation through the activation of the TLR4 immune response in glial cells in the brain by causing long-term cognitive and behavioral impairments and predisposition to drug abuse. Figure modified from (Guerri and Pascual, 2010).

synaptic proteins (PSD-95 and SHANK3) by impairing synaptic pruning maturation, effects that might involve autophagy processes (Montesinos et al., 2018). The involvement of the immune response, such as the TLR4 pathway and the presence of cytokines, may modify synaptic transmission and plasticity (see rev., Vezzani and Viviani, 2015). Similarly, TLR4-deficient animals have shown no changes in the synaptic pruning connections and synaptic proteins between ethanol- and saline-treated TLR4-KO mice (Montesinos et al., 2018, 2015).

Another important neuropathological consequence of binge ethanol drinking in adolescence is white matter alterations. The structure of nerve myelinated fibers in the CNS is essential for correct information processing (Simons and Trotter, 2007), and several human and animal studies have demonstrated the involvement of myelin dysfunctions induced by binge ethanol drinking in adolescence. For instance, human neuroimaging studies show frontal myelin tract alterations (Bava et al., 2013) and reduced white matter integrity in the prefrontal cortex and superior longitudinal fasciculus (De Bellis et al., 2005; Elofson et al., 2013; Medina et al., 2008), effects that might be related with long-term memory, learning and executive dysfunctions. Studies into adolescent mice have also demonstrated that binge ethanol drinking induces ultrastructural myelin sheath disarrangements in the prefrontal cortex (e.g., inter-laminar splitting and irregular fiber shapes of myelin sheaths) and in the down-regulation of the expression of several myelination-related proteins (e.g., MBP, PLP, CNPase or NG2) (Montesinos et al., 2015) (Fig. 2), effects related with the TLR4 immune response since the use of TLR4-deficient mice protects against myelin alterations (Montesinos et al., 2015).

3.3. Long-term behavioral consequences

Human and animal studies have described the long-term behavioral impairments induced by binge alcohol drinking in adolescence. Poorer performance in many neurocognitive domains, including attention and information processing, memory, visuospatial functioning, language abilities and executive functioning (Jacobs and Tapert, 2013), have been described in adolescents with binge alcohol use. Stronger cognitive alterations of binge alcohol drinking have been observed in young females than in males (Scaife and Duka, 2009; Squeglia et al., 2009; Townshend and Duka, 2005). Some of these effects have also been observed in rodents exposed to binge ethanol drinking in adolescence, in which long-term cognitive impairments have been shown (Montesinos et al., 2015; Pascual et al., 2007; Schulteis et al., 2008; Vetreno et al., 2016), effects which might be related with synaptic and white matter alterations (Montesinos et al., 2018, 2015).

Another important consequence of alcohol consumption in adolescence is predisposition to alcohol drinking in later stages. Indeed NCANDA (National Consortium on Alcohol and Neuro Development in Adolescence Project) data show the potential risk factors that may contribute to early drinking in at-risk adolescents before they initiate heavy alcohol use (Sullivan et al., 2016). Experimental animal models confirm that binge ethanol drinking in adolescence increases voluntary ethanol drinking in adult rodents (e.g., Alaux-Cantin et al., 2013; Gass et al., 2014; Montesinos et al., 2016a; Pascual et al., 2009), effects that are stronger in female than in male animals (Strong et al., 2010).

Anxiety-like behavior induced by binge ethanol treatment in adolescence has been associated with epigenetic changes as the pharmacological administration of histone deacetylase inhibitors (e.g., trichostatin A, sodium butyrate) reverses or enhances behavioral effects. Trichostatin A is able to attenuate anxiety-like behavior and ethanol intake in adulthood by normalizing deficits in the histone H3 acetylation of Arc (activity-regulated cytoskeleton-associated protein) and BDNF genes (Pandey et al., 2015), while sodium butyrate enhances the ethanol-induced long-term acquisition, extinction and reinstatement effects of ethanol-treated adolescent rats by promoting the gene histone acetylation of cFos, Cdk5 and FosB in the prefrontal cortex (Pascual et al., 2012).
The role of ethanol-induced neuroinflammation and the TLR4 response has also been demonstrated in behavioral studies (Fig. 2). For instance, the administration of the COX-2 inhibitor, indomethacin, is able to restore the long-lasting neurobehavioral deficits induced by intermittent ethanol treatment in adolescent animals (Pascual et al., 2007). Likewise, the use of TLR4 receptor-deficient mice protects against long-term cognitive, anxiety-like behavioral impairments and ethanol intake in binge ethanol drinking adolescent mice (Montesinos et al., 2015, 2016b). In fact the administration of LPS, a TLR4 ligand, increases voluntary alcohol intake (Blednov et al., 2011). A correlation between ethanol-induced HMGB1/TLR expression in the prefrontal cortex and spatial learning deficits has also been shown in adolescent rats with intermittent ethanol treatment (Vetreno and Crews, 2012).

To summarize, human and animal studies clearly demonstrate that binge ethanol drinking in adolescence can induce important short- and long-term cognitive and behavioral dysfunctions. Evidence also shows that the innate immune system and the TLRs response are important targets of alcohol-induced neuroinflammation and behavioral dysfunctions.

4. Drug abuse

In addition to the effects of alcohol abuse in adolescent brain maturation, other drug abuse also impact the adolescent brain, although the role of the neuroimmune system activation in the behavioral dysfunction is less clear than in alcohol abuse. We will shortly describe some evidences supporting the role of the neuroimmune response in the actions of some drug abuse in adolescence.

4.1. Cannabis

Marijuana (cannabis) is one of the commonly used illicit substances in adolescence (Patrick et al., 2011), whose initiation in adolescence leads to heavy use in both late adolescence and young adulthood. Some consequences of cannabis use and abuse include failed education, persisting mental health problems and progression to other substance use (e.g., Coffey and Patton, 2016).

Cannabis contains psychoactive components (e.g., Δ9-tetrahydrocannabinol or THC) that might interfere with the brain's endogenous endocannabinoid system, which is involved in neurodevelopment. Therefore, THC and other related compounds could interfere with normal adolescent neurodevelopment, predisposing young people with cannabis use to motivational and psychotic disorders (Chadwick et al., 2013). Studies have shown that marijuana use/abuse in adolescence can impair brain maturation and neurodevelopmental trajectories by changing neurochemical communication and having a toxic effect on brain tissue. Cannabis use in adolescence impair white and gray matter structures (e.g., changes in myelin, axons and synapses), which affect healthy brain development from childhood to young adulthood, and lead to subtle cognitive functioning and success in daily functioning (Jacobs et al., 2015; Jacobs and Tapert, 2014). Further evidence from both human and experimental animals has demonstrated that adolescent cannabinoids persistently change the mesolimbic regions of the adult brain that sufficiently predict future self-administration behavior, a phenotype relevant to drug addiction vulnerability (e.g., Tomasiwicz et al., 2012). Although the neurobiological mechanisms underlying the effects of cannabis in adolescence remain largely unknown, cannabis mediates some of its effects on the central nervous system by interacting with CB1 and CB2 cannabinoid receptors (Zimmer et al., 1999). Indeed, CB2 receptor expression is associated with inflammation, it is primarily localized in CNS resident macrophages and in microglia, acting as a glial cell modulator (Kendall and Yudowski, 2016). Experimental studies also demonstrate the role of neuroinflammation in the prefrontal and evidence an association between microglial activation, along with cytokine production, and the development of long-term cognitive deficits induced by adolescent THC treatment (Zamberletti et al., 2015). Recent findings also demonstrate that cannabis use leads to the over-activation of a pro-hallucinogenic signaling pathway of 5-HT2AR (serotonin 2A receptor) through the regulation of Akt/mTOR (Ibarra-Lecue et al., 2018), effects that can explain the risk for adult psychosis in adolescent cannabis use (Arseneault et al., 2002).

4.2. Opioids

Opioids and their receptors have been classically used to treat pain and related disorders, and remain the most widely used analgesics in clinical terms. Opioid peptides and their receptors are expressed throughout the nociceptive neural circuitry, in addition to other critical regions of the CNS such as reward and emotion-related brain structures. To date, different receptors have been described [mu (µ), delta (δ), kappa (κ), opioid receptor like-1 (ORL1)], and their genes have been characterized at the cellular, molecular, and pharmacological levels (see rev., Al-Hasani and Bruchas, 2011).

Despite few studies indicating opioid use in adolescence, growing evidence suggests that medically and non-medically prescribed opioid exposure in adolescence, along with other risk factors, can increase the risk of substance use disorders in young adults (McCabe et al., 2016; Miech et al., 2015). Indeed when used recreationally, opioids produce a powerful, euphoric high and feeling of relaxation, but these positive effects are associated with the highly addictive action of drug abuse. Those adolescents whose abuse can also suffer negative consequences, such as having problems with peers, and suffering organ and brain alterations, including the neurological consequences of opioid addiction (NiDa, 2015). Strikingly, neuroinflammation has been involved in chronic opioid dysfunctions and addictive-like behaviors (Cahill and Taylor, 2017). Indeed the TLR4/MD2 recognition of opioids has been suggested to be involved in neuronal reinforcement mechanisms, which supports a role of central proinflammatory immune signaling and neuroinflammation in opioid and drug reward (Hutchinson et al., 2012).

4.3. Cocaine

Cocaine is one of the most widely abused drugs used also by adolescents, whose intake is associated with serious social, medical and economic problems (Tolou-Shams et al., 2010). Repeated cocaine use causes long-lasting changes and cellular/molecular alterations that lead to dysfunctions in neuroplasticity in the nucleus accumbens (Cooper et al., 2017). Different studies demonstrate the vulnerability of the adolescent brain to the effects of cocaine abuse. For instance, animal studies have shown that cocaine self-administration causes greater impairment in the orbitofrontal cortex-related learning task in adolescent than in adult rats (Harvey et al., 2009). Similarly, adolescent rats are more sensitive than adults to lower cocaine doses by showing a greater escalation of cocaine intake and marked activity of ventral tegmental area dopamine neurons, a feature known to be associated with increased self-administration behavior (Wong et al., 2013).

The role of the central immune activation and pro-inflammatory cytokines production seem also to participate in the neuropathological changes, behavioral dysfunctions and addiction induced by cocaine (Muriach et al., 2016; Northcutt et al., 2015; Zhu et al., 2018). For instance, cocaine self-administration increases the mRNA expression of the proinflammatory cytokine interleukin-1β receptor in the ventral tegmental area and reduces cocaine-primed drug seeking, which suggest that chronic cocaine produces inflammatory signaling that contributes to cocaine seeking (Muriach et al., 2010; Northcutt et al., 2015; Zhu et al., 2018). Interestingly, the blockade of TLR4 suppresses cocaine-induced extracellular dopamine in the nucleus accumbens and the pharmacological antagonism of interleukin-1 receptor in the ventral tegmental area reduces cocaine-primed drug seeking, which suggests that chronic cocaine produces inflammatory signaling, which
contributes to cocaine seeking. Interestingly, the blockade of TLR4 suppresses cocaine-induced extracellular dopamine in the nucleus accumbens (Northcutt et al., 2015) as well as cocaine rewarding effects (Ang et al., 2001; Crews et al., 2011). A recent study also shows that both TLR3 deficiency and an intra-nucleus accumbens injection of TLR3 inhibitors significantly attenuate cocaine-induced condition place preference, locomotor activity and self-administration in mice (Zhu et al., 2018). New data also reveal the participation of inflammation in cocaine-dependent individuals, and suggest that cytokines are novel biomarkers in addicted populations for treatment development (Fox et al., 2012). These results suggest that cocaine-induced neuroinflammation and immune activation in specific brain regions (cerebral cortex, ventral tegmental area and amygdala) of adolescents can contribute to not only the neuropathological changes associated with cocaine abuse (Buttner, 2012; Cadet et al., 2014), but also to cocaine addiction.

4.4. Amphetamines and MDMA

Amphetamines and analog derivative 3,4-methylenedioxymethamphetamine (MDMA or ecstasy) are the psychostimulant drugs usually consumed by adolescents and young adults (Schulz, 2011). MDMA causes the rapid release of serotonin and the inhibition of its re-uptake, which also affect some other neurotransmitters, such as dopamine and noradrenaline (Baumann et al., 2007), and increase the production of free radicals and oxidative stress (Parrott et al., 2004). In adolescence, MDMA consumption causes several long-lasting behavioral impairments in mood and cognitive functions (Llorente-Berral et al., 2013).

The most consistent effect of MDMA exposure on rats is serotoninergic deficit in various forebrain regions, including the striatum, hippocampus and cortex (Battaglia et al., 1991; Piper, 2007), although MDMA also induces neurotoxicity and cell death (Schmedt, 2003). Recent studies evidence that MDMA neurotoxicity is linked with neuroinflammation-associated astroglialosis (Frau et al., 2013; Johnson et al., 2002) and microglia activation (Connor et al., 2005; Lopes-Rodriguez et al., 2014; Monks et al., 2004). Some studies have shown that microgliosis is an early response to methamphetamine abuse, and this response is maintained in the long-term after abstinence (Sekine et al., 2008). The activation of microglia by methamphetamine temporally precedes neuronal damage and is dose-dependent (LaVoie et al., 2004; Thomas et al., 2004). Ionotropic purinoceptor P2 × 7 (P2 × 7R) seems to play a role in methamphetamine-induced microglial activation responses (Fernandes et al., 2016).

In short, human and experimental studies support the role of microglial activation, neuroinflammation and neural death in the neurotoxicity and behavioral dysfunctions associated with the consumption of methamphetamines.

4.5. Anabolic androgenic steroids

Anabolic androgenic steroids (AAS) are synthetic androgens used both clinically and illicitly, but their use has increased dramatically among adolescent males (Lumia and McGinnis, 2010) given their muscle building properties (Buckley et al., 1988). AAS use is associated with mood and anxiety disturbances, and with increased aggressiveness, and psychotic and behavior disorders (Piacentino et al., 2015). Experimental studies have revealed that AAS steroids disrupt physiological function in specific brain areas (e.g., amygdala-fugal pathway), affecting several neurotransmitters pathways (e.g., serotonergic, dopaminergic, glutamatergic, GABAergic) (Bertozzi et al., 2018), events that might be associated anxiety disorders as well as dysfunction of the reward system (Piacentino et al., 2015; Zotti et al., 2014). Likewise, AAS abuse impairs the immune system (Marshall-Gradiskik et al., 2009) by increasing the levels of pro-inflammatory cytokine and TNF-α mediated apoptosis (Riezzo et al., 2014).

5. Conclusions

Evidence demonstrates that alcohol or drug abuse in adolescence can alter normal physiological processes, which leads to long-lasting cognitive and behavioral dysfunction, including predisposition to substance use disorders. Recent studies support a role of the innate immune response and TLRs in glial cells in many actions of alcohol and drug abuse in the brain, including neural damage cognitive dysfunctions and alterations to neurocircuits that contribute to drug addiction-related behaviors. Targeting the immune response, such as using anti-inflammatory compounds (Pascual et al., 2007; Vetreno et al., 2018), exercise (Pascual et al., 2007; Vetreno et al., 2018) or inhibiting microglial activation (e.g., minocycline) (Agrawal et al., 2011; Wang et al., 2018), are a potential therapeutic strategy to treat the neuroinflammation and alcohol drinking associated with alcohol and drug abuse in adolescence.

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