Adolescent Opioid Abuse: Role of Glial and Neuroimmune Mechanisms

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Abbreviations

CNS, Central nervous system
CPP, Conditioned place preference
GFAP, Glial fibrillary acidic protein
GPCRs, G-protein coupled receptors
Iba1, Ionized calcium binding adaptor molecule 1
MAPKs, Mitogen-activated protein kinases
MORs, μ opioid receptors
MyD88, myeloid differentiation primary response 88
NAc, Nucleus accumbens
NF-κB, Nuclear factor-κB
NMUPO, Nonmedical use of prescription opioids
TLRs, Toll-like receptors
TLR4, Toll-like receptor 4
Abstract

Opioids are widely prescribed for pain management, and prescription opioid misuse in adolescents has become a major epidemic in the United States and worldwide. Emerging data indicate that adolescence represents a critical period of brain development, and exposure to opioids during adolescence may increase the risk of addiction in adulthood. There is growing evidence that disruptions in brain glial function may be implicated in numerous chronic neuropathologies. Evidence suggests that glial mechanisms have an important role in the development and maintenance of opioid abuse and the risk for addiction. This review will describe glial and neuroimmune mechanisms involved in opioid use disorders during adolescence, which may increase substance use disorder liability later in life. Moreover, this review will identify some important neuro-glial targets, involved in opioid abuse and addiction, to develop future preventions and treatment strategies.

Keywords: Opioid Addiction, Adolescence, Glia, Opioid Use Disorders, Drug Addiction, Microglia, Astrocyte, Neuroinflammation, Neuroimmune Mechanism
1. INTRODUCTION

Prescription opioid misuse and opioid use disorders (OUDs) represent a public health concern in the United States and worldwide (e.g., McCabe et al., 2019; Peacock et al., 2018). Evidence suggests an association between medical use of prescription opioids and subsequent nonmedical use of prescription opioids (NMUPO) during adolescence and young adulthood (McCabe et al., 2016, 2019, 2020; Miech et al., 2015). The prevalence rates of substance use disorders (SUDs) are significantly high among adolescents who report medical use of prescription opioids and history of NMUPO based on epidemiological data (McCabe et al., 2016, 2020). However, the neurobiological mechanisms of opioid abuse during adolescence remain unclear.

Adolescence in humans is a critical stage of brain maturation and behavioral development (e.g., Klinker et al., 2019; Lacagnina et al., 2017; Salamanzadeh et al., 2020; Schepis et al., 2008). Emerging evidence indicates that this unique developmental phase is highly sensitive to numerous environmental influences, including opioid abuse (Bava et al., 2013; Bell et al., 2013; Schwarz et al., 2011, Schwarz & Bilbo 2013). A growing body of evidence suggests that adolescent opioid exposure contributes to long-lasting changes in brain development, which subsequently compromises adult behavior and increases the vulnerability to addiction and cognitive dysfunction (Hanson et al., 2011; Kuhn et al., 2013; Moss et al., 2014; Rutherford et al., 2010; Vestal-Laborde et al., 2014). Thus, the trajectory and maturation of the central nervous system (CNS) development during adolescence increases its vulnerability to the effects of opioids. Simply put, exposure to licit and illicit drugs during adolescence
disrupts CNS maturation, which leads to long-term behavioral dysfunction (Guerri & Pascual, 2019; Winters et al., 2012). Adolescent opioid use also induces behavioral despair after sustained abstinence in animal models, suggesting potential comorbidity between depression and opiate addiction (Lutz et al., 2013), although it is difficult to disentangle anhedonia due to opioid withdrawal from the former. Previous data have also shown that adolescent morphine treatment significantly facilitates the development of tolerance to its analgesic effects and increases withdrawal signs in rodent models (Salmanzadeh et al., 2017). This suggests that neuroadaptation in the CNS may continue into adulthood. However, morphine treatment during adolescence had parameter-specific effects in adulthood, such that it did not affect morphine-induced onset or peak response of lateral paragigantocellular neurons (Salmanzadeh et al., 2018). Studies also evaluated the effect of opioid drug administration on impulsive-like behavior. Results from behavioral tests, related to reaction-time, 24 h and 25 days after administration of morphine to adolescent rats found a profound effect on motor impulsive behavior later in adulthood (Moazen et al., 2018). Regarding pharmacological effects of chronic morphine administration during adolescence, this treatment increased pain responsiveness, in the formalin test, in adult rats compared to controls (Ghasemi et al., 2018). Adolescent oxycodone exposure resulted in increased sensitivity to the rewarding effects of opioids in adult mice (Sanchez et al., 2016). Taken together, the neurobehavioral evidence suggests that opioid exposure during adolescence can induce a variety of effects different from those observed following exposure in adulthood (Spear, 2000, 2015). Therefore, adolescent opioid exposure can adversely influence neural, behavioral, and cognitive functioning long after this early-life exposure. While the
role of neuronal mechanisms is somewhat known, growing evidence suggests that disruptions in brain glial function are implicated in chronic neuroadaptations. This review will describe the glial and neuroimmune mechanisms involved in opioid use and/or abuse during adolescence that have been shown to increase drug abuse liability later in life. Moreover, this review will explore important neuro-glial targets involved in opioid abuse and addiction-related disorders.

2. GLIAL CELLS AND BRAIN DEVELOPMENT

Glial cells are widely distributed throughout the CNS and are broadly classified based on morphological and functional characteristics (Bachtell et al., 2017; Lacagnina et al., 2017; Klinker et al., 2019; Rahman & Alzarea, 2019). The primary type of glial cells in the CNS include astrocytes, microglia, oligodendrocytes, and ependymal cells. Astrocytes are known to provide numerous structural and metabolic support functions for neurons and regulate the extracellular environment by clearing neurotransmitters, buffering ion concentrations, and releasing signaling molecules (Sofroniew & Vinters, 2010). In addition, astrocytes also play an essential role in immune cell trafficking and activation in the CNS (c.f., Rahman & Alzarea, 2019). Microglia are part of the innate immune system known as resident macrophages that maintain immunological functions in the CNS (c.f., Rahman & Alzarea, 2019). Functionally, microglia are immunocompetent cells that detect pathogens and engage in phagocytosis, lysosomal degradation, and secretion of various pro- and anti-inflammatory substances (c.f., Graeber, 2010). Oligodendrocytes are glial cells that wrap around neuronal axons, forming a myelin sheath that insulates the nerve fiber from the extracellular fluid and
allows for more efficient propagation of axonal signals (Nave, 2010). Ependymal cells line the spinal cord and brain ventricles, including the blood-brain barrier (BBB), where these cells produce, secrete, and circulate cerebrospinal fluid. Collectively, glial cells are important and are underappreciated targets, or regulators, of CNS function (Bachtell et al., 2017; Lacagnina et al., 2017; Klinker et al., 2019; Menassa & Gomez-Nicola, 2018; Rahman & Alzarea, 2019, Reemst et al., 2016).

The past few decades have witnessed a greater interest in the importance of glia in CNS development, plasticity and disease, including SUDs (Klinker et al., 2019; Lacagnina et al., 2017; Zuchero & Barrres, 2015). For instance, although the study of brain development has largely focused on neuronal development in the past, neuronal development coincides with the development of neuroglia, mediating their role in CNS development (Menassa & Gomez-Nicola, 2018; Reemst et al., 2016). Quantification methods indicate that at least 50% of all cells in the human brain are glia, with considerable variations and differences between studies and different brain regions, as well as differences in neuro-glial ratios across development (Azevedo et al., 2009; Lyck et al., 2009). For example, the glia to neuron ratio of the cerebral cortex is approximately 3.76, whereas it is only 0.23 in the cerebellum (Azevedo et al., 2009). Additionally, the finding that the human brain is made up of at least 50% glial cells has also been observed in primates and rodents, indicating a critical role for glia in numerous brain functions across multiple species (c.f., Reemst et al., 2016). For example, radial gill cells direct microglial migration, after which microglia control radial glial differentiation. On the other hand, astrocytes affect microglia maturation, which can
affect radial glial cells and neuronal functionality. Thus, as a highly interactive/integrated network, the developing CNS is strongly dependent on appropriate and coordinated signaling between neurons and glia, which affects adolescent brain function (c.f., Menassa & Gomez-Nicola, 2018; Reemst et al, 2016). Although microglia are the resident macrophages of the brain, recent research demonstrates that they have functions in addition to biological defense (Wu et al., 2015). Similarly, astrocytes have functions beyond their supportive role to also influence neurocircuit development (Kim et al., 2017). Both microglia and astrocytes exhibit significant changes in morphology, gene expression, and function following drug exposure, as seen by increasing evidence that microglia and astrocytes can perpetuate the detrimental effects of drugs of abuse on the brain (Klinker et al., 2019; Lacagnina et al., 2017; Melbourne et al., 2019). Thus, drug exposure during the critical period of adolescence may alter the interactive functions of glial cells with neurons well into adulthood.

3. NEUROIMMUNE RESPONSE AND ADOLESCENT BRAIN DEVELOPMENT

The innate immune responses, including toll-like receptors (TLRs), of neuroglia mediate many actions of drugs-of-abuse on the brain, which contributes to drug addiction-related behaviors (c.f., Guerri & Pascual 2019). For example, activated microglia produce and release numerous pro-inflammatory cytokines and other mediators, such as prostanoids, as well as free radicals associated with neuroinflammation (Kettenmann et al., 2011). Furthermore, both microglia and astrocytes express innate immune receptors, such as TLRs and cytoplasmic NOD-like immune receptors (NLRs) (Alfonso-Loeches et al., 2016; Blanco et al., 2008). Stimulation of TLRs triggers signaling
pathways, such as the mammalian target of rapamycin (mTOR) and nuclear transcription factor (NF-κB) pathways, which causes the production of cytokines, interleukins, and other inflammatory mediators (Montesinos et al., 2016). These receptors participate in neuroinflammation and the long-term behavioral and neuropathological dysfunction induced by drug use and/or abuse during adolescence (Hutchinson et al., 2012; Zhu et al., 2018). Although astrocytes and microglia are the main immune cells that express several TLRs (Okun et al., 2011), neurons can also express TLR4. However, the signaling pathways associated with a TLR4 response differ between cell types (Leow-Dyke et al., 2012; Okun et al., 2011). Under normal conditions, the release of cytokines by glial cells modulates neuronal function by regulating synaptic and neural plasticity. Nevertheless, pro-inflammatory cytokines can impair synaptic plasticity and may underlie cognitive disturbances, memory dysfunction, as well as mood disorders (Khairova et al., 2009). Here we discuss evidence indicating the participation of the neuroimmune response of glial cells, the influence of the innate immune TLRs in neuroinflammation, and long-term behavioral dysfunction associated with opioid use and/or abuse in adolescence.

4. OPIOID EXPOSURE AND GLIAL ACTIVATION

Accumulating evidence indicates that opioids stimulate glial cells via different opioid receptors (Fig. 1) expressed on the cell membrane (c.f., Zhang et al., 2020). The opioid receptors include μ, δ, and κ subtypes and these receptor subtypes are known heterotrimeric Gi/o protein-coupled receptors (GPCRs; Corbett et al., 2006; Waldhoer et al., 2004). Furthermore, μ opioid receptors (MORs) are the primary receptor subtype
mediating the rewarding and reinforcing effects of opioids. Previous studies have shown that these opioid receptors are expressed on both microglia (Chao et al., 1996; Dobrenis et al., 1995; Mika et al., 2014) and astrocytes (Gurwell et al., 1996; Meyer et al., 2017; Nam et al., 2018; Woo et al., 2018). Interestingly, activated glial opioid receptors produce mixed results (c.f., Zhang et al., 2020). For example, several studies have reported that stimulation of glial opioid receptors can reduce functional cellular activity (Chao et al., 1997; Hu et al., 2000). Furthermore, previous studies have shown that activation of opioid receptors can stimulate immune system (Muscoli et al., 2010). A recent study also found that activating astrocytic MORs induced glutamate release from astrocytes in the hippocampus (Nam et al., 2019). This resulted in a conditioned place preference (CPP), demonstrating a stimulatory role of Gi/o protein-mediated signaling by MORs and other GPCRs (Nam et al., 2019). It is possible that unique downstream cell signaling pathways are coupled with opioid receptors under distinct pathophysiological conditions, which may contribute to this inconsistency (Al-Hasani & Bruchas, 2011). Whether opioid glial activation and subsequent internal cell signaling pathways across development are similar, remain to be investigated.

Additional studies have demonstrated that administration of opioid drugs increases the expression of glial fibrillary acidic protein (GFAP), an activation marker for astrocytes, and ionized calcium binding adaptor molecule 1 (Iba1), an activation marker for microglia, in animal models (Beitner-Johnson et al., 1993; García-Pérez et al., 2014). Furthermore, morphine administration altered glial signaling facilitating the release of
various cytokines and chemokines (Schwarz et al., 2011; Zhang et al., 2017). These findings suggest a strong association between opioids and neuroinflammatory signaling.

There is increasing evidence that TLR4 is a potential target where opioids (Fig. 1) can cause an innate immune response (Zhang et al., 2020). For example, opioid administration stimulates mitogen-activated protein kinase (MAPK) signaling downstream of TLR4, including p38, JNK, and ERK (Wang et al., 2012; Zhang et al., 2012). Interestingly, genetic deletion of TLR4 blocks this effect, suggesting a critical role for the TLR4 in opioid effects. Additionally, opioid-induced TLR4 stimulation promotes the production of pro-inflammatory signals from glial cells, which can be prevented by glial activation inhibitors or selective TLR4 antagonists (Eidson & Murphy, 2013; Liang et al., 2016). It is noteworthy to mention that morphine metabolites with no opioid receptor activity can bind and activate TLR4 (Due et al., 2012). This finding suggests that opioid-induced central immune signaling does not necessarily require activity at classical opioid receptors and their downstream signaling. Overall, these studies indicate that adolescence is a sensitive period for opioid-induced changes in the TLR4 signaling pathway as well as microglial function of microglia in MCL reward circuitry (c.f., Guerri & Pascual, 2019). This alteration in microglial function enhances the risk for adverse consequences later in life, since opioid exposure induces long-term changes in the neurocircuitry mediating OUDs (Schwarz & Bilbo, 2013). Given the role of the innate immune system in neuropsychiatric disorders, it is possible that adolescent opioid abuse may influence these disorders later in life through a complex mechanism, which will require further investigation (c.f., Guerri & Pascual, 2019).
5. OPIOID EXPOSURE AND GLIAL NEUROIMMUNE SIGNALING

As previously described, microglia and astrocytes are the primary cells in the CNS mediating an immune response; which, at least in part, protects the CNS from the effects of stress and pathogens by upregulating inflammatory signaling (c.f., Guerri & Pascual, 2019). Several studies indicate that the brain integrates neuronal and immune communication/signaling and that peripheral immune dysregulation and inflammation can alter brain function as well (c.f., Guerri & Pascual, 2019; Pavlov et al., 2018). Pharmacological manipulation of TLR4 inhibits opioid-induced dopamine release in the nucleus accumbens (NAc) as well as its CPP and self-administration (Chen et al., 2017; Hutchinson et al., 2012). The latter authors reported that the rewarding effects of opioids are mediated, at least in part, via a TLR4-triggered intracellular pathway. Similarly, intra-NAc injection of a p38 inhibitor was found to prevent the acquisition and maintenance of a morphine-induced CPP (Zhang et al., 2012). Furthermore, deletion of TLR4 prevented the phosphorylation of p38 and JNK, downstream signaling of TLR4-associated MyD88, a molecular inflammatory pathway (Hutchinson et al., 2012). A more recent study reported that selectively blocking microglial MyD88 activity did not change the acquisition of opioid reward but did prolong the extinction of this reward memory in preclinical models (Rivera et al., 2019). These results suggest that microglial TLR4-MyD88 activity (Fig. 1) plays an important role in opioid-induced rewarding effects and reinforcement behavior (Jacobsen et al., 2014).

Further studies have indicated that TLR4’s effects are partially mediated by proinflammatory cytokine tumor necrosis factor alpha (TNFα) activity (Eidson et al.,
These authors also indicated that TNFα, an inflammatory pathway end-product, and the sequestration of soluble TNFα prevent TLR4-mediated opioid-induced neuroinflammation. Thus, TNFα may modulate opioid reward processing since it contributes to altered opioid sensitivity in humans (Reyes-Gibby et al., 2008). Additionally, Niwa et al. (2007) reported that TNFα disrupted a morphine-induced CPP. Interleukin-1β (IL-1β) is another important proinflammatory cytokine thought to mediate TLR4-induced alterations in neuronal plasticity (Guerri & Pascual, 2019). Like TNFα, IL-1β is also a product of TLR4-signaling since its production involves the activation of TLR4 with a concomitant increase in pro-IL-1β expression (Hutchinson et al., 2011). IL-1β has been found to play a role in morphine-induced reward-related behavior (Zhang et al., 2020). For example, single nucleotide polymorphism in the IL1B gene was found to be associated with an increased risk of opioid, and alcohol, dependence in humans (Liu et al., 2009). Previous studies revealed that opioid addiction shares similar characteristics with chronic pain, indicating possible common mechanisms and neuroadaptations between these two neuropathologies (Elman & Borsook, 2016). In addition, two polymorphisms of brain-derived neurotrophic factor (BDNF) were found to be associated with the age of onset in heroin-dependent patients, suggesting an important modulatory role for BDNF in opioid abuse and addiction (Jia et al., 2011; Meng et al., 2012). Taylor et al. (2016) found that chronic opioid use activates microglia and its release of BDNF in the ventral tegmental area (VTA). A previous study showed a single dose of BDNF into the VTA of naive animals recapitulated opioid-associated withdrawal behavior (Vargas-Perez et al., 2009). This supports a role for
microglial-derived BDNF in the regulation of opioid-induced rewarding effects and related behavior.

Besides the inflammatory activity discussed above, previous reports have indicated that opioid-induced neuroimmune activation via TLR4 contributes to deficits in glutamate turnover by inhibiting glutamate uptake (Eidson et al., 2017). In addition, chronic opioid exposure reduces the expression of both astrocytic glutamate transporters (GLT-1) as well as glutamate aspartate transporters, likely diminishing synaptic clearance of glutamate (Ozawa et al., 2001; Shen et al., 2014). Thus, restoring deficits in GLT-1 levels could prevent many chronic opioid-induced behavioral effects (c.f., Bachtell et al., 2017). Further studies indicate that administration of the GLT-1 activator, MS-153, attenuated opioid-induced CPP; whereas overexpression of GLT-1 in the NAc prevented an opioid-induced CPP (Fujio et al., 2005). Interestingly, several studies suggest that injection of the beta-lactam antibiotic, ceftriaxone, upregulates endogenous GLT-1 levels and may effectively reduce opioid-induced hyperthermia, tolerance to this effect, and reinstatement of heroin-seeking behaviors (Rawls et al., 2010; Shen et al., 2014). Further evidence has indicated that reducing opioid-induced increases in glutamate with chronic administration of the cysteine pro-drug, N-acetylcysteine, effectively reduces heroin-seeking behaviors (Zhou & Kalivas, 2008). Importantly, both ceftriaxone and N-acetylcysteine display anti-inflammatory properties making it difficult to separate their ability to suppress immune activation from their effects on restoring glutamate homeostasis (c.f., Bachtell et al., 2017). Taken together, these data indicate that opioid-induced neuroimmune signaling and disrupted glutamate regulation may be
important mechanisms that alter the behavioral effects of acute and/or chronic opioid administration. Therefore, given the critical role of glial neuroimmune mechanisms in opioid effects, it is clear that adolescent opioid use likely alters neuroimmune signaling controlling neural development (e.g., Brenhouse et al., 2016).

6. GLIAL TARGETS, OPIOID ABUSE, AND THERAPEUTIC SIGNIFICANCE

Given the above, it is clear that glial activity significantly influences the abuse potential of opioids. Therefore, glial cells are potential targets to develop drugs that prevent or treat opioid abuse. As of now, the following agents have been investigated, which target glial pathways to reverse glial activation and associated neuroinflammatory and/or other neuroimmune mechanisms.

For example, peroxisome proliferator-activated receptor-γ (PPARγ) is a nuclear receptor expressed by both microglia and astrocytes (Warden et al., 2016; Zhang et al., 2020). Several preclinical studies indicate that pioglitazone, a PPARγ agonist, effectively suppresses opioid reward-related behavior in animal models (de Guglielmo et al., 2015, 2017). PPARγ may represent a novel therapeutic strategy for treating opioid addiction, as the activation of this nuclear receptor significantly suppresses the production of pro-inflammatory cytokines from microglia and astrocytes (Kielian & Drew, 2003). Consistent with this, a recent study reported that pioglitazone has pharmacological efficacy in altering the addiction liability of oxycodone in opioid users (Jones et al., 2016). A small molecule Ibudilast, a glial modulator and non-selective phosphodiesterase (PDE) inhibitor, reduces neurobiological markers associated with opioid-induced pro-inflammatory responses, while it attenuates both antagonist-
precipitated and abstinence-induced morphine withdrawal in animal models (Hutchinson et al., 2009). More recently, ibudilast was found to reduce opioid-related withdrawal symptoms (Cooper et al., 2016). These findings show the potential clinical utility of glial modulators for treating opioid withdrawal in humans. Minocycline, a tetracycline antibiotic, inhibits the activation of microglia. In preclinical studies, minocycline prevented the development of tolerance to opioid analgesia and opioid-induced hyperalgesia (Arout et al., 2019). This exploratory investigation sought to determine if minocycline changes pain threshold and tolerance in individuals with OUDs maintained on an opioid agonist treatment (Arout et al., 2019). These studies indicate minocycline is a potential therapeutic for OUDs and other SUDs. Further preclinical and clinical investigations will provide additional insights into its potential therapeutic utility.

7. CONCLUSIONS

In this review, we have explored evidence that opioid abuse during adolescence can modify brain functions and produce drug–specific neurobehavioral consequences. The preclinical and clinical data discussed above indicate that exposure to opioids during adolescence can cause long-lasting neurobiological, cognitive, affective, and behavioral dysfunctions. The net result is an impaired brain function that lasts into adulthood. Moreover, the development of multiple brain regions is particularly sensitive to adolescent opioid exposure. In addition, we have reviewed that neuroimmune signaling pathways mediate opioid-induced glial activation and associated cellular and molecular mechanisms. Furthermore, recent studies have revealed an important role for neuroimmune mechanisms, including glial activation, in many actions of opioids in the
brain. These actions include neural damage, cognitive dysfunction, and alterations to reward neurocircuitry. Therefore, targeting neuroimmune signaling, including glial activation is a viable therapeutic strategy for future prevention and treatment of OUDs.
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**Fig. 1** Opioids and putative glial mechanisms. Opioids activate microglia and astrocytes via opioid receptors, and the toll-like receptor (TLR4). Opioids are direct agonists at TLR4 and bind to a unique binding site in the myeloid differentiation factor 2 (MD-2) domain of the TLR4 receptor. TLR4 activation causes the translocation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) to the nucleus via the myeloid differentiation primary response 88 (MyD88) pathway and MyD88-independent pathways. The stimulation of TLR4 by opioids causes the activity of intracellular mitogen-activated protein kinases (MAPKs) that control the formation of microglial or astrocytic markers and pro-inflammatory cytokines. Opioid-induced glial activation also involves other receptors and signaling mechanisms. Protein P38 is associated with MAPK signaling. See text for detail. Astrocyte activation markers connexin 43 (CX43) and glutamate transporter-1 (GLT-1); Microglial activation markers such as cluster of differentiation molecule 11b (CD11b), Ionized calcium binding adaptor molecule 1 (Iba1).