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Glial mechanisms underlying substance use disorders

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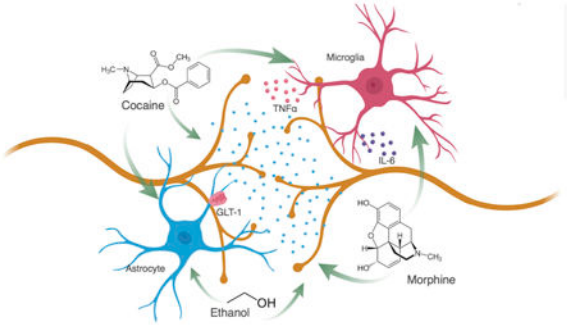
Graphical Abstract

Drugs of abuse have direct actions on both microglia and astrocytes and promote a reactive glial state where pro-inflammatory cytokines, such as IL-6 and TNFα, are released into the synaptic environment. Glia modulate neuronal activity through a variety of receptors including the astrocyte specific glutamate transporter, GLT-1. These drug-induced changes to glia alter neuron-glia interactions, circuit functions and ultimately drug-associated behaviors, and provide a promising therapeutic target for substance use disorders.



Keywords

microglia; astrocytes; addiction; opiates; psychostimulants

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Author Contributions

KL, SC, and FL performed literature searches and wrote sections of the manuscript. KL created the figures and SC and KL created the tables. KL, SC, and FL critically reviewed the manuscript. FL obtained funding. All authors approved the final version of the manuscript.

Conflict of Interest

KL, SC, and FL have no competing interests to declare.

Data Accessibility

All original figures are available upon reasonable request from corresponding authors.

Introduction

Addiction is a chronic relapsing disorder, characterized by the compulsion to seek and take drugs despite negative consequences. It is extremely damaging and costly, accounting for $740 billion annually in the United States resulting from healthcare, crime, and lost work productivity (Birnbaum *et al.*, 2011; CDC 2017; Xu *et al.*, 2014). There is also an immeasurable cost in the loss of life and immense individual suffering. In order to find effective therapies for addiction, we must identify effective targets for treatment by understanding the complex mechanisms that are fundamental to this impenetrable disorder. Critical, well-defined changes to the brain that underlie these behavioral stereotypes have been identified (Koob & Volkow, 2010). Although evidence has implicated both neurons and glia in mechanisms that contribute to these addictive behaviors, the largest body of work has focused on understanding neuronal mechanisms of addiction.

Extensive investigations of the pathogenesis of drug abuse have revealed the critical role of two dopamine-producing nuclei in the ventral midbrain, the ventral tegmental area (VTA) and the substantia nigra (SN). These two regions provide dopaminergic innervation to the forebrain, including prefrontal cortex (PFC), amygdala, hippocampus (HPC), dorsal striatum, and nucleus accumbens (NAc). Of these, the VTA to NAc pathway is noteworthy because it is consistently implicated in drug use across species, including humans (Volkow *et al.,* 2017). More specifically, this circuit is critical for motivated instrumental behaviors, such as self-administration and conditioned place preference (CPP) in both mice and rats. Fast-scan cyclic voltammetry studies have shown that natural rewards and drugs of abuse increase the amplitude of phasic dopamine release in the NAc (Phillips *et al.*, 2003; Vander Weele *et al.*, 2014). Although dopamine is a key neurobiological substrate that encodes rewarding properties of drugs of abuse, recent findings have shown other mechanisms that critically influence the reinforcing properties of drugs, including other neurotransmitters, transcription factors, and other substances released from neurons and glia alike. Whereas there is an expansive body of work on neural mechanisms underlying addiction (Hyman *et al.*, 2006; Kauer & Malenka, 2007; Kalivas, 2009), successful therapeutic targets remain elusive. Progress in understanding glial biology has revealed these cells to be potential key regulators in drug abuse. Glia were once viewed as merely immune response cells with no active role in cognitive function. Although microglia and astrocytes are master regulators of the central immune system, mounting evidence demonstrates that they influence processes beyond inflammation and immune surveillance. These cells actively regulate synaptic glutamate spillover, activity-dependent synaptogenesis and elimination, neuronal morphology, neurotransmitter synthesis, synaptic connectivity, and neural circuit function (Guerra-Gomes *et al.*, 2017; York *et al.*, 2017; Scofield & Kalivas, 2014). These functions of glial cells are significantly altered by exposure to abused drugs, and such changes likely contribute to the behavioral outcomes associated with substance abuse (Miguel-Hidalgo, 2009; Coller & Hutchinson, 2012).

In this review, we argue that glia influence the cellular, molecular, and synaptic changes that occur in neurons following drug exposure, and that these neural–glial interactions influence drug-associated behaviors. Here we focus on microglia and astrocytes, rather than oligodendrocytes, endothial, or other glial cells, due to the large body of evidence

implicating microglia and astrocytes in addiction. We will evaluate the role of glia in addiction-like brain states and behaviors. First, we will discuss how abused drugs directly affect glia. We will then explore neural-glia communications, and how this affects circuit-glia interactions. Finally, we will review how these molecular, cellular, and circuit changes ultimately produce changes in drug-associated behavior, and discuss the clinical evidence for glia as a therapeutic target for addiction.

Direct Effects of Drugs of Abuse on Microglia and Astrocytes

Microglia and astrocytes are immune competent cells that prevail throughout the central nervous system (CNS). 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). Astrocytes are abundant, bushy cells that provide metabolic support for neurons, control synaptic microenvironments, and influence circuit development (Kim *et al.*, 2017). Both microglia and astrocytes exhibit significant changes in morphology, gene expression, and function following drug exposure. Furthermore, there is increasing evidence that microglia and astrocytes can perpetuate the detrimental effects of drugs of abuse on the brain.

Identifying Reactive Glia

Microglia and astrocytes can be identified by a host of general markers that include IBA1, Cd11b, and CX3CR1 for microglia and GFAP, S100b, and GLT-1 for astrocytes (Sofroniew & Vinters, 2010; Kim *et al.*, 2017). In the studies reviewed here, the predominant marker for microglia is IBA1, and for astrocytes, GFAP. It is important to note that IBA1 does not differentiate between central and peripheral macrophages, and that GFAP is only expressed at detectable levels in certain brain regions. However, both GFAP and IBA1 are upregulated after biological insult or during *activation*. Microglia and astrocytes can become *reactive* in response to harmful stimuli, including drugs of abuse, leading to stereotyped patterns of alterations in glial morphology and transcriptome. Reactive microglia have an increase in soma size and a simultaneous shrinkage and thickening of processes (Ramirez *et al.*, 2017; Coller & Hutchinson, 2012; Lacagnina *et al.*, 2017). Reactive astrocyte morphology is more difficult to define, although reactive astrocytes do proliferate (Liddelow & Barres, 2017). Reactive microglia and astrocytes can release cytokines and chemokines that are barely detectable in normal conditions, but that are increased following insult (e.g., exposure to drugs of abuse) and critically alter glial and neural function. Although cytokines released by microglia and astrocytes are categorized as either pro- or anti-inflammatory, the microenvironment that a cytokine is released into can alter its properties (Cavaillon, 2001). Generally, cytokines from the tumor necrosis factor (TNF) family, gamma-interferon (IFN-γ) family, and most cytokines from the interleukin (IL) family are considered pro-inflammatory. In contrast, IL-10, IL-13, IFN-α, and TGF-β are considered anti-inflammatory (Cavaillon, 2001; Sofroniew, 2014). Glial cells also release various chemokines, which are chemotactic cytokines that move in response to a chemical stimulus. These are classified into two families: CXC, which attract neutrophils and lymphocytes, or CC, which attract monocytes and T cells (Cavaillon, 2001). However, recent research demonstrates that chemokines participate in many functions in addition to chemotaxis.

Drugs of Abuse Promote Reactive Glia

Both microglia and astrocytes exhibit significant changes in morphology, gene expression, and function following drug exposure (Lacagnina *et al.*, 2017). Furthermore, increasing evidence demonstrates that microglia and astrocytes can perpetuate the detrimental effects of drugs of abuse on the brain. Many drugs of abuse increase expression of microglial and astrocytic markers, increase cytokine and chemokine release, and promote a pro-inflammatory glial phenotype (Table 1; Ramirez *et al.*, 2017; Coller & Hutchinson, 2012; Lacagnina *et al.*, 2017). Indeed, psychostimulants, such as cocaine and methamphetamine, promote a reactive glial phenotype (Coller & Hutchinson, 2012; Lacagnina *et al.*, 2017) and increase IBA1 and GFAP expression (Fattore *et al.*, 2002; Thomas *et al.*, 2004; Liao *et al.*, 2016). The cytokines Il-1β, TNFα, and IL-6, among others, are also increased by psychostimulant exposure (Sriram *et al.*, 2006; Northcutt *et al.*, 2015; Liao *et al.*, 2016). Opioids and alcohol also increase glial markers and pro-inflammatory cytokine release (Alfonso-Loeches *et al.*, 2010; Wang *et al.,* 2012**)**. In contrast, nicotine suppresses reactive microglia in the adult brain (Li *et al.*, 2016; Noda & Kobayashi, 2017), and blocks LPS-induced increases in microglial activation and expression of Toll-like receptor 4 (TLR4, see below; Kim *et al.*, 2014; Li *et al.*, 2016). The anti-inflammatory state produced by nicotine results from activation of the α7 nicotinic acetylcholine receptor (nAChR) (Shytle *et al.*, 2004; Noda & Kobayashi, 2017), possibly in a metabotropic signaling mode (King *et al.*, 2017). Astrocytes have also been shown to express α7 nAChRs (Shen & Yakel, 2012), which modulate synaptic responsiveness by controlling glial release of the N-methyl D-aspartate (NMDA) receptor co-agonist, D-serine (Papouin *et al.*, 2017). Although nicotine has anti-inflammatory effects in adults, we have recently shown that adolescent nicotine exposure increases IBA1 expression and induces a pro-inflammatory microglia state (Linker *et al.*, 2017). Although the direct mechanism for this increase is unknown, this finding suggests that adolescent microglia may have different sensitivities to drugs of abuse. Indeed, adolescent morphine exposure increases subsequent TLR4 expression in adulthood, whereas adult morphine exposure does not (Schwarz & Bilbo, 2013).

Timing is Critical for Drug-Induced Glial Activation

Although nearly all drugs of abuse activate glia, it is pertinent to note that the timing of drug exposure is a vital factor determining drug-induced changes in glial responding. For example, 1-day and 7-day exposure to cocaine increases GFAP, whereas 14-day cocaine exposure does not (Fattore *et al.*, 2002). In addition, withdrawal from cocaine after extended self-administration decreases GFAP and astrocytic volume (Scofield *et al.,* 2016b). A similar pattern is observed after alcohol exposure, with increased GFAP expression after 4–12 week alcohol access and decreased GFAP following extended (36 week) access (Franke, 1995). There are also regional differences in inflammatory responses.

Drugs of Abuse Act Directly on Toll-like Receptor 4

The mechanisms by which drugs of abuse activate glia vary. For some drugs of abuse this can occur through direct actions on glial receptors. Microglia and astrocytes express a wide array of receptors that may or may not be glial-specific. These include cytokine and chemokine receptors, numerous neurotransmitter receptors (including dopamine receptors),

neuropeptide receptors, interferon receptors, Toll-like receptors, and many others (Pocock & Kettenmann, 2007; Sofroniew, 2014). Microglia and astrocytes also have cell-type specific receptor expression, notably the fracktalkine (CXC3CL1) receptor for microglia and glutamate transporter 1 (GLT-1) for astrocytes (Scofield & Kalivas, 2014; Ransohoff & El Khoury, 2015). Both of these signaling proteins are critical for glial modulation of synapses Toll-like receptors (TLRs), are an important receptor family expressed by both microglia and astrocytes, and they are the most extensively studied immune receptors for biological defense and drug response (Lacagnina *et al.*, 2017). TLR activation promotes an adaptor protein, myeloid differentiation primary response gene 88 (MyD88), to assist translocation of the transcription factor Nf-κβ to the nucleus, which then induces the production of Il-1β, TNFα, IL-6, IL-10, and other cytokines and chemokines (Lee & Kim, 2007; Trotta *et al.*, 2014). These cytokines are released into the extracellular milieu and bind to receptors expressed in microglia, astrocytes, and neurons, causing changes to intracellular signaling and cell function.

The Toll-like receptor 4 (TLR4), in particular, is a critical interface for direct and indirect effects of many drugs of abuse on glia (Figure 1). Extensive studies have demonstrated that opioids can act directly on TLR4 as an agonist at a non-classical binding site (Watkins *et al.*, 2009; Coller & Hutchinson, 2012). Opioids bind to the MD-2 binding pocket for lipopolysaccharide (LPS; TLR4 agonist and classical immune activator) to induce TLR4 oligomerization, which triggers downstream signaling to Nf-κβ and the production of pro-inflammatory cytokines (Figure 1; Wang *et al.*, 2012). Furthermore, the (+) isomers of the opioid receptor antagonists, naloxone and naltrexone, act as direct TLR4 antagonists (Wang *et al.*, 2016). Interestingly, Northcutt et al., (2015) demonstrated that cocaine interacts with the same TLR4 binding domain as (+)-naloxone, which blocks cocaine-induced increases in cytokine release (Figure 1). Alcohol also induces a TLR4-dependent release of pro-inflammatory cytokines (Alfonso-Loeches *et al.*, 2010). Knockdown of TLR4 genetically, or with an siRNA, blocks alcohol-induced increases in microglial (Cd11b) and astrocytic (GFAP) pro-inflammatory markers (Alfonso-Loeches *et al.*, 2010). The mechanism by which alcohol enhances activation of TLR4 is unclear, but one possibility is through modulation of lipid rafts in astrocyte membranes, and endocytosis of active TLR4 receptors (Figure 1; Blanco *et al.*, 2008; Pascual-Lucas *et al.*, 2014). Even with these complexities, the above studies demonstrate that most drugs of abuse can produce profound pro-inflammatory states in both microglia and astrocytes, and that TLR4 receptors are a central regulator of their downstream inflammatory signaling.

Distinct Alterations in Neuron-Glial interactions Following Drug Exposure

Cytokines and Chemokines Modulate Synaptic Plasticity and Neurotransmitter Synthesis—

As reviewed above, exposure to drugs of abuse increases microglial and astrocytic markers, largely promoting a pro-inflammatory state that triggers glial cells to release an arsenal of cytokines and chemokines, but how do these changes affect neurons? Glial-derived cytokines and chemokines can alter neurotransmitter synthesis and release, neuronal activity, and synaptic plasticity (Cui *et al.*, 2014). Increasing evidence has demonstrated that the pro-inflammatory cytokine, TNFα, regulates synaptic activity via AMPA receptor trafficking and astrocyte glutamate release (Santello & Volterra, 2012).

TNFα decreases inhibitory synaptic strength by promoting endocytosis of $GABA_A$ receptors through p38 MAPK (Pribiag & Stellwagen, 2013), and also enhances excitatory synaptic efficacy by increasing AMPA receptors (GluR1) at the cell surface (Beattie *et al.*, 2002; Stellwagen *et al.*, 2005). Furthermore, TNFα is required for astrocyte glutamate release (Santello *et al.*, 2011). This glutamatergic change is critical as an imbalance in glutamate synaptic plasticity is a hallmark of addiction (Kalivas, 2009; Scofield & Kalivas, 2014; Scofield *et al.*, 2016a). IL-1β increases excitatory transmission through an increase in surface expression of GluR1, but IL-6 and IL-10 do not produce these receptor changes (Stellwagen *et al.*, 2005). However, IL-6 may downregulate NMDA receptor function or expression (Ali *et al.*, 2000; Pizzi *et al.*, 2004). Increasing evidence has demonstrated that TNFα regulates synaptic activity via AMPA receptor trafficking and astrocyte glutamate release. These cytokine-derived changes to glutamate and GABA transmission may be critical to understanding addiction-like states, as these neurotransmitter systems are significantly altered after repeated drug exposure. Lewitus et al. (2016) demonstrated that semi-chronic cocaine exposure increased TNFα levels which resulted in a significant reduction in AMPA/NMDA receptor ratios in D1-containing medium spiny neurons. This alteration in glutamate receptor signaling is evident 24 hours after repeated cocaine exposure (Kourrich *et al.*, 2007; Lewitus *et al.*, 2016). Furthermore, disruption of cocaine signaling at TLR4, and prevention of subsequent TNFα production, blocks cocaine-induced dopamine release (Northcutt *et al.*, 2015).

Chemokines are also key glial-derived neuronal modulators. CXC motif chemokine 12 (CXCL12) increases dopamine neuron action potential frequency, and switches the firing pattern of depolarized dopamine neurons from tonic to burst firing (Skrzydelski *et al.*, 2007). Chemokine ligand 2 (CCL2) also increases striatal dopamine release (Guyon *et al.*, 2009). The receptor for CCL2, chemokine receptor 2 (CCR2), has distinct expression throughout the brain, with high levels in the cortex, striatum, and basolateral amygdala (BLA) – regions involved in reward and addiction (Banisadr *et al.*, 2002). Importantly, CCR2 knockout mice also exhibit attenuated cocaine-induced immediate early gene activation in the striatum (Trocello *et al.*, 2011). These preliminary studies suggest that CCL2, its receptor CCR2, and CXCL12 may contribute to the rewarding effects of drugs by their modulation of striatal dopaminergic firing.

Cytokines have also been shown to stimulate intra-neural signaling pathways that alter the synthesis of neurotransmitters essential for reward and addiction-like behaviors (Cui *et al.*, 2014). Dopamine is synthesized by a series of chemical reactions where phenylalanine is converted to tyrosine by phenylalanine hydroxylase (PAH) and tyrosine is converted into the precursor of dopamine, L-DOPA, by tyrosine hydroxylase (TH). Tetrahydrobiopterin (BH4) is an essential co-factor for both enzymes, and BH4 production is stimulated by pro-inflammatory cytokine release, suggesting that inflammatory cytokines may promote dopamine synthesis via BH4 (Cui *et al.*, 2014; Figure 2). However, inflammatory cytokines also increase reactive oxygen species that ultimately decrease the oxidation-labile BH4 and, in turn, decrease TH production (Neurauter *et al.*, 2008). Many studies in the depression field have demonstrated that a chronic excess of inflammatory cytokines, associated with sickness and mood disorders, ultimately causes a decrease in dopamine levels (Neurauter *et al.*, 2008; Felger & Miller, 2012). This mechanism is not well understood in the context of

addiction, but recent evidence suggests drug-induced changes in cytokines may have different effects on BH4-controlled TH. TLR4 pro-inflammatory signaling has been shown to *increase* TH production in VTA dopaminergic neurons, which contrasts with cytokine-induced decreases in TH observed in depression (Aurelian *et al.*, 2016). This discrepancy in the impact on TH may reflect differences in the duration or concentration of cytokines. Indeed, acute cytokine exposure increases dopamine whereas extended exposure decreases dopamine and TH levels (Dunn, 2006). Clearly this research area is understudied and should be further investigated in relation to how drug-induced increases in cytokines alter neurotransmitter production.

Astrocytic GLT-1 is Important for Glutamate Signaling—Astrocytes also modulate synaptic function by tightly controlling synaptic glutamate levels through the astrocyte-specific glutamate transporter GLT-1, which is responsible for ~90% of synaptic glutamate clearance (Rao *et al.*, 2015). GLT-1 is decreased following cocaine, nicotine, and heroin exposure, demonstrating a consistent decrease across drug groups (Smith *et al.*, 2015). Drug-induced downregulation of GLT-1 is associated with glutamate synaptic spillover to activate extrasynaptic NMDARs (Shen *et al.*, 2014), which is critical for drug-induced plasticity and reinstatement.

Microglial and Astrocytic Creation and Elimination of Synapses—Glia also modulate synapse elimination and formation. Specifically, microglia prune synapses via phagocytosis of dendritic spines (Nimmerjahn *et al.*, 2005; Wake *et al.*, 2009, Wu *et al.*, 2015) in an activity-dependent manner (Tremblay *et al.*, 2010; Paolicelli *et al.*, 2011). Several mechanisms have been elucidated for this pruning, most notably the classical complement cascade and fracktalkine-CX3CR1 signaling (Stevens *et al.*, 2007; Paolicelli *et al.*, 2011). Foundational studies *in vitro* have also demonstrated that astrocytes are key for promoting synapse formation, since cultured neurons develop very few synapses in their absence (Clark *et al.*, 2013). Astrocytes secrete thrombospondins (TSP1 and TSP2) and the matricellular protein, hevin (SC1), to promote synaptic formation (Christopherson *et al.*, 2005; Kucukdereli *et al.*, 2011). Although astrocytes are classically known to promote synapse formation while microglia prune synapses, both cell types contribute to synaptic formation and elimination. Microglia can promote synaptic formation through the anti-inflammatory cytokine IL-10, and astrocytes can promote synaptic elimination through SPARC (Kucukdereli *et al.*, 2011; Miyamoto *et al.*, 2015).

Drugs of Abuse Influence Glial Modulation of Synaptic Plasticity—We highlight the significant role that glia play in synaptic modulation due to the long-lasting effects of drugs of abuse on synaptic density, structure, and function (Lüscher & Malenka, 2011; Gipson *et al.*, 2014). There is minimal, but promising, evidence that glia are also involved in drug-induced changes to synaptic plasticity. For example, alcohol-induced depression of long-term potentiation (LTP) is blocked in the absence of glial-derived chemokine CCL2 (Bray *et al.*, 2013). Adolescent intermittent alcohol exposure also increases thrombospondin-2 and PSD95 protein levels, suggesting that alcohol may increase synapse count through secreted astrocyte chemokines (Risher *et al.*, 2015). These effects are not limited to alcohol, as cocaine self-administration and extinction also decrease astrocyte-

synaptic contacts (Scofield *et al.*, 2016b), and morphine decreases microglial NAc levels of fracktalkine, a chemokine involved in microglial synaptic pruning (Schwarz *et al.*, 2013). Taken together, these studies demonstrate that different drug exposure paradigms can increase or decrease synaptic count or contacts through glial-derived factors. Although these few studies are encouraging, this field remains largely unexplored.

Drugs of Abuse Critically Alter Circuit-Glial Network Interactions

Some brain circuits and regions are particularly sensitive to inflammation, and heterogeneous glial populations respond to neural and foreign stimuli differently. This diverse and elaborate circuit-glia intercommunication remains poorly understood, but is an important component of brain function in health and disease. Diversity of microglia and astrocytes throughout the brain is well understood (Jessen *et al.*, 1984; Reynolds & Herschkowitz, 1987; Lawson *et al.*, 1990). However, the extent to which these cells differ functionally and in smaller, discrete brain regions is a more recent discovery. Understanding this heterogeneity is crucial to understanding the role of glia in drug use, as drug exposure has region-specific effects.

Microglia and Astrocytes are Multifarious Cell Types—Mounting evidence shows that microglia are very diverse throughout the CNS, both constitutively and after insult. Systemic LPS, a TLR4 agonist, induces IL-1β and TNFα cytokine production in the HPC, PFC, and other cortical brain regions, with the highest induction in the striatum (Tyagi *et al.*, 2010; Noh *et al.*, 2014). An autoimmune paradigm also significantly increases IL-1α in the striatum as compared to the HPC and amygdala (Gentile *et al.*, 2015). Taken together, these studies demonstrate that the striatum may be particularly sensitive to inflammatory insults. Microglia have distinct cell body densities, morphologies, transcriptomes, and receptor expression in the subnuclei of the basal ganglia (NAc, VTA, SN; De Biase *et al.*, 2017). Heterogeneity within these nuclei suggests that each basal ganglia region possesses a unique population of microglia with a variety of prospective functions (De Biase *et al.*, 2017). Some of the most differentially expressed gene functional groups within these subnuclei are related to vesicle release and secretion, lysosome function, and reactive oxygen species homeostasis. Understanding these spatial differences in microglia is key to interpreting the regional changes in microglia following drug exposure, and how these region-specific changes to glia may modify reward circuitry.

Astrocytes are also a remarkably diverse cell type, which exhibit regionally distinct transcriptomes, electrophysiological properties, morphologies, calcium signaling, and synaptic proximity (Chai *et al.*, 2017). Astrocytes are sparse in regions with a high density of neuronal cell bodies, but are dense in regions occupied by dendrites and axons (Khakh & Sofroniew, 2015). As such, there are regional differences in astrocyte-to-neuron ratios throughout the brain. For example, there is a lower number of astrocytes per neuron in the striatum as compared to the HPC (Chai *et al.*, 2017). Although the functional outcomes of this differential distribution remain poorly understood, sparsely populated striatal astrocytes have larger calcium responses to excitatory and inhibitory designer receptors exclusively activated by designer drugs (DREADD) modulation as compared to the HPC (Chai *et al.*, 2017).

Different Drugs of Abuse Alter Glia in a Region-Specific Manner—Drugs of abuse produce varied regional changes to microglia. As discussed earlier, opioids activate TLR4 receptors and, therefore, promote the production of several pro-inflammatory (IL-1β, TNFα, IL-6) and anti-inflammatory (IL-10) cytokines through downstream activation of the glial transcription factor Nf-κβ (Figure 1). However, this cytokine induction is not homogeneously distributed throughout the brain (Table 2). Chronic morphine increases IL-1β in the HPC and dorsal periaqueductal grey (dPAG), while it increases IL-6 in the SN and IFN-γ in the NAc. FACS sorted tissue in the NAc has shown that morphine induces production of chemokines CCL4, CCL17, and chemokine receptor CCR4, as well as the anti-inflammatory cytokine IL-10, by microglia, but not astrocytes (Schwarz *et al.*, 2013). Opioids also induce reactive phenotypes in microglia and astrocytes broadly and differentially throughout the CNS. Following chronic morphine exposure, astrocytic GFAP is increased in the locus coeruleus, nucleus of the solitary tract, caudate putamen (CPu), NAc, dentate gyrus (DG), dPAG, SN, PFC, and other midbrain nuclei, but not the dorsal raphe nucleus (DRN) (Marie-Claire *et al.*, 2004; Alonso *et al.*, 2007; Hutchinson *et al.*, 2009). Morphine also promotes morphological changes in astrocytes in the NAc and lateral septal nucleus (Lazriev *et al.*, 2001). Furthermore, microglial IBA1 expression is increased by morphine in the VTA, SN, NAc, DG, and CPu, but not the mPFC or DRN (Hutchinson *et al.*, 2009).

Alcohol also induces unique patterns of glial activation throughout the brain. Chronic alcohol exposure for a period of 3–6 months increases GFAP, astrocyte volume, and S100b expression in the CA1 of the HPC, and increases GFAP+ astrocytes in frontal, parietal, and temporal cortex (Tagliaferro *et al.*, 2002; Udomuksorn *et al.*, 2011). Semi-chronic alcohol exposure (10 days) increases GFAP and the chemokine CCL2 in the adult rat cortex, cerebellum, and HPC, but not the cytokines TNFα or IL-6 (Kane *et al.*, 2014). This alcohol exposure paradigm also increases IBA1 expression in the cortex and HPC, along with increasing Nf-κβ expression (Qin & Crews, 2012). The NAc, BLA, and PFC all express different glial-enriched transcript profiles following alcohol exposure (Osterndorff-Kahanek *et al.*, 2015). Importantly a genetic knockout of TLR4 blocks chronic alcohol-induced changes to glial networks (Alfonso-Loeches *et al.*, 2010).

Psychostimulants also promote region-specific changes to microglia and astrocytes. Repeated amphetamine exposure increases GFAP in the dorsal CPu but not the NAc or PFC (Armstrong *et al.*, 2004). Cocaine increases IBA1 and microglial reactivity in the NAc, and increases GFAP in the HPC (Fattore *et al.*, 2002; Lewitus *et al.*, 2016). Psychostimulants also induce circuit-specific functional consequences. Acute cocaine increases IL-1B and TNFα in the NAc, PFC, and VTA (Cearley *et al.*, 2011; Northcutt *et al.*, 2015), whereas methamphetamine increases TNFα and IL-6 in the HPC and PFC (Gonçalves *et al.*, 2008). Together, these data highlight important drug-induced alterations in glial markers and cytokine levels that vary based on drug class, as well as by region and glial cell type.

Drugs of Abuse Alter Glial Regulation of Circuit Communication—Drug-induced changes to glia also affect how brain regions *communicate* with each other. Astrocytes in the striatum respond to D1-receptive or D2-receptive cells in a mutually exclusive manner. D1-responsive astrocytes increase calcium levels in response to D1 receptor stimulation and not

to D2 receptor stimulation, and vice versa (Martin *et al.*, 2015). Similarly, increased calcium levels trigger astrocytes to release glutamate to modulate excitability of neurons in a selective manner, with calcium waves in D2-responsive astrocytes only increasing excitatory post synaptic currents in D2 responsive, but not D1 responsive, neurons (Martin *et al.*, 2015). This foundational work demonstrates that astrocytes modulate neurons in a circuit specific (D1 vs. D2) manner. Future work should investigate how astrocyte circuit-specific modulations affect drug-associated behaviors.

LTP is a persistent increase in synaptic strength, and nearly all drugs of abuse induce LTP within the VTA that is associated with drug intake. Increasing evidence demonstrates that cytokines and chemokines influence LTP in a cell-type specific manner. Hippocampal LTP induces IL-1β and IL-6 release, and blocking their signaling inhibits or supports LTP maintenance, respectively (Del Rey *et al.*, 2013). Bath application of IL-10 in the VTA increases firing of dopaminergic, but not GABAergic, neurons (Williams *et al.*, 2017). Signaling of TGF-β, an anti-inflammatory astrocyte-derived chemokine, also regulates excitatory and inhibitory synaptic balance of dopamine neurons (Luo *et a*l., 2016). Action potential-independent vesicular GABA release in a subset of mouse central amygdala neurons is decreased by IL-1β, an effect that is reversed by co-administration of alcohol (Bajo *et al.*, 2015). Although these initial findings are promising, there needs to be greater analysis of how glial activation, and subsequent release of cytokines and chemokines, alter complex circuit dynamics and the communication between reward-associated regions.

Glial mechanisms underlying behavioral changes after drug exposure

A rapidly growing body of literature has highlighted the potential role of glia in modulating behaviors associated with a variety of drugs, including opioids, alcohol, and stimulants. Animal behavioral models can provide critical information on the neurobiological underpinnings of drug dependence with high face and construct validity, although a full description of these paradigms is beyond the scope of this review, but see (Koob & Le Moal, 2008; Crabbe *et al.*, 2011). Importantly, the knowledge gained from these preclinical models has the potential to guide the development of glial-targeted therapeutics in humans.

Since exposure to alcohol, opioids, and psychostimulants activates the neuroimmune system, preventing glial activation may prove to be a valuable therapeutic approach. Indeed, as shown in Table 3, the predominant effect of microglial inhibitors is to attenuate the rewarding and reinforcing properties of drugs of abuse. Minocycline is a blood-brain barrier permeable tetracycline antibiotic and microglial inhibitor that has been shown to have many inhibitory effects on opioid-associated behaviors. Minocycline and ibudilast, another glial inhibitor, inhibit morphine-induced dopamine release and the development of morphine CPP (Hutchinson *et al.*, 2008; Bland *et al.*, 2009). Naloxone-precipitated withdrawal in mice chronically treated with morphine is also attenuated by minocycline and ibudilast, and spontaneous withdrawal-induced weight loss and hyperactivity is reduced by glial inhibition. These changes are accompanied by reductions in morphine-induced CD11b and GFAP expression, measures of microglial and astrocytic activation, respectively. Interestingly, the analgesic effects of morphine and oxycodone are potentiated by ibudilast or minocycline, without any change in plasma morphine levels (Hutchinson *et al.*, 2008; Hutchinson *et al.*,

2009), suggesting that ibudilast may extend the intended analgesic effects of morphine, while dampening the unwanted rewarding effects. Alcohol consumption is also reduced by minocycline and ibudilast in the two-bottle choice, drinking-in-the-dark, and chronic intermittent access paradigms (Agrawal *et al.*, 2011; Bell *et al.*, 2015; Syapin *et al.*, 2016). Ibudilast's inhibitory effect on alcohol consumption is seen in multiple strains of mice and rats, including strains selectively bred for high alcohol consumption (Bell *et al.*, 2015). Minocycline has also been shown to protect against ethanol-induced damage in the early postnatal brain, decreasing or blocking caspase-3 activation and expression of the pro-inflammatory markers monocyte chemoattractant protein 1 (MCP-1) and CCR2. Minocycline also protects against ethanol-induced activation of microglia, as measured by IBA1 and CD11b, and lysosomal phagocytic marker CD68 expression (Wang *et al.*, 2017).

Cocaine-associated behaviors are also impacted by minocycline and ibudilast. Minocycline attenuates cocaine-induced dopamine release and CPP (Northcutt *et al.*, 2015), whereas ibudilast decreases cocaine-induced locomotor sensitization in both male and female rats (Poland *et al.*, 2016). Both minocycline and ibudilast decrease methamphetamine self-administration, locomotor sensitization, reinstatement of drug-seeking, and the neurotoxic effects of methamphetamine. This effect is strongest when minocycline is administered before methamphetamine exposure, which may suggest that the timing of glial inhibition plays an important role in its effect (Zhang *et al.*, 2006; Mizoguchi *et al.*, 2008; Beardsley *et al.*, 2010; Snider *et al.*, 2012; Snider *et al.*, 2013).

Manipulations of TLR4 function Modulate Drug-Associated Behaviors—TLRs are also a critical interface between drugs and glial activation that promotes drug consumption. TLRs are expressed on both astrocytes and microglia, but have higher expression on microglia. The TLR4, in particular, has garnered much interest in the addiction research field in the past decade (Figure 1). Blockade of TLR4s appears to generally decrease opioid-associated behaviors and accompanying neurochemical responses (Table 3). TLR4 antagonists prevent morphine-induced dopamine release in the NAc, a hallmark neurochemical signal of drug reward, as well as interfere with the acquisition and maintenance of morphine CPP and reduce self-administration of the opioid remifentanil (Hutchinson *et al.*, 2012; Chen *et al.*, 2017). This may be due to TLR4-mediated activation of the signaling pathway Signal Transducers and Transcription Activator 3 (STAT3) within the VTA, which has been implicated in regulating reward and motivated behavior (Chen *et al.*, 2017). Similarly, chronic, but not acute, TLR4 blockade prevents incubation of heroin craving during withdrawal, suggesting that TLR4 antagonism does not directly alter the reinforcing properties of heroin but modulates withdrawal-associated synaptic remodeling (Theberge *et al.*, 2013). Tanda et al. (2016) have reported that the TLR4 antagonists, (+)-naloxone and (+)-naltrexone, have no effect on heroin-induced dopamine release, suggesting that TLR4 may alter behavior through mechanisms outside of dopamine release.

Pharmacological and genetic manipulations of TLR4s have also implicated these receptors in alcohol-associated behaviors (Table 4). However, TLR4 signaling may have opposing modulatory roles on ethanol consumption as compared to ethanol-associated behaviors. Indeed, alcohol self-administration increases expression of the TLR4 protein in the VTA of alcohol-preferring rats (June *et al.*, 2015). Mice lacking the TLR4 protein or the TLR4

adapter protein, MyD88, display lower sensitivity to the sedative and motor-impairing effects of alcohol compared to wild-type mice (Wu *et al.*, 2012; Harris *et al.*, 2017), whereas MYD88 knockout increases ethanol consumption (Blednov *et al.*, 2017). TLR4 activation in the VTA and central amygdala, in particular, may be an important signal for binge alcohol consumption, as site-selective knockdown of TLR4 in these regions using siRNA reduces binge drinking in alcohol-preferring rats (Liu *et al.*, 2011; June *et al.*, 2015). Furthermore, genetic knockout of TLR2 or CD14, suppresses ethanol intake (Blednov *et al.*, 2017). In contrast, some have reported no effect of pharmacological or genetic manipulation of TLR4 on excessive alcohol consumption in the two-bottle choice, drinking-in-the-dark, or chronic intermittent access paradigms (Blednov *et al.*, 2017; Harris *et al.*, 2017). Blednov et al. (2017) demonstrated that TLR4 mutant mice have unaltered alcohol consumption behavior, However, Blednov et al. (2017) reported significant effects of TLR4 knockout on total fluid consumption. This may suggest that TLR4 activity modulates general consummatory behavior. Similarly, although the TLR4 inhibitor, T5342126, decreased ethanol drinking in dependent mice, it also had non-specific effects on saccharin intake and locomotor activity (Bajo *et al.*, 2016).. IL-1 receptor antagonists also reduce binge-like consumption of alcohol, suggesting that cytokine expression modulates the reinforcing effects of alcohol (Marshall *et al.*, 2016). Taken together these studies demonstrate that immune-derived factors and receptors modulate alcohol consumption in certain paradigms, but the mechanisms underlying this are unclear.

The exact role of TLR4 activation in modulating cocaine-associated behaviors is ambiguous, but TLR4 receptors are clearly important for cocaine reinforcement. The TLR4 antagonists, (+)-naloxone and (+)-naltrexone, have been shown to inhibit cocaine-induced dopamine release in the NAc and attenuate cocaine CPP and self-administration (Northcutt *et al.* 2015). Similarly, pharmacological blockade of TLR4s in the VTA reduces drug-primed reinstatement of cocaine-seeking, while activation of VTA TLR4s is sufficient to produce modest reinstatement of cocaine-seeking (Brown *et al.*, 2018). However, another study using parameters similar to those in Northcutt *et al.* (2015) reported an opposite effect of (+)-naltrexone and (+)-naloxone on cocaine-induced dopamine release (Tanda *et al.*, 2016). Interestingly, both groups reported that TLR4 blockade attenuated cocaine CPP and self-administration, although Tanda et al. (2016) reported that the ability of (+)-naltrexone and (+)-naloxone to attenuate cocaine self-administration required high doses that were accompanied by alterations in responding for food reward, which may suggest nonspecific effects of these antagonists. In contrast, genetic manipulation of TLR4 signaling lowered cocaine responding. In C3H/HEJ mice that have impaired TLR4-Nf-κβ signaling, cocaine reinforcement and motivation is lower compared to wild-type mice, without any change in sucrose self-administration (Northcutt *et al.*, 2015). Complete knockout of TLR4 decreases sensitivity to the rewarding effects of cocaine, requiring higher doses to develop significant place preference compared to wild-type animals, and TLR4 knockout prevents the persistent enhancement of cocaine preference that is seen in wild-type controls (Kashima & Grueter, 2017). The authors suggest that alterations in cocaine reward learning in TLR4 knockouts is due to deficits in NMDAR-dependent long-term depression in the NAc core. TLR4 signaling may play a less prominent role in methamphetamine addiction, as TLR4 blockade has no effect on incubation of methamphetamine craving (Theberge *et al.*, 2013). However, a

growing body of literature suggests that glial activation and subsequent TLR4 signaling positively regulates opioid-, alcohol-, and cocaine-associated behaviors.

Manipulating Astrocytic Function Critically Alters Drug-Seeking—Astrocytes may also be important regulators of alcohol consumption. Disruption of astrocytic networks with gap-junction hemichannel blockers increases the motivation for alcohol self-administration, whereas activation of astrocytes using G_q-coupled DREADDs under the control of a GFAP promoter reduces alcohol self-administration (Bull *et al.*, 2014). Similarly, stimulation of G_q-coupled DREADDSs on astrocytes in the NAc normalizes extracellular glutamate levels during cocaine abstinence through stimulation of release-regulating group II metabotropic glutamate autoreceptors to decrease cue-induced reinstatement of cocaine seeking (Scofield *et al.*, 2015).

As discussed previously, astrocyte control of glutamate spillover is critically impaired following drug exposure (Scofield & Kalivas, 2014; Roberts-Wolfe & Kalivas, 2015; Scofield *et al.*, 2016a). Psychostimulants, alcohol, and opioids all decrease expression of GLT-1, an astrocyte-specific glutamate transporter that clears glutamate from the synapse (Smith *et al.*, 2015). Ceftriaxone, a β-lactam antibody that restores expression of GLT-1, decreases morphine tolerance and heroin-induced reinstatement of drug seeking (Rawls *et al.*, 2007; Rawls *et al.*, 2010; Shen *et al.*, 2014), and has been found to reduce alcohol consumption and reduce 'relapse-like drinking' (Qrunfleh *et al.*, 2013; Sari *et al.*, 2013a; Sari *et al.*, 2013b; Alhaddad *et al.*, 2014; Rao & Sari, 2014; Das *et al.*, 2015). Ceftriaxone can also inhibit cocaine and methamphetamine seeking (Sari *et al.*, 2009; Knackstedt *et al.*, 2010; Abulseoud *et al.*, 2012) and prevents the reinstatement, but not the acquisition, of nicotine CPP (Alajaji *et al.*, 2013). N-acetylcysteine (NAC), which restores glutamate homeostasis, also blocks drug-induced decreases in GLT-1, decreases nicotine self-administration (Gipson *et al.*, 2013), and blocks reinstatement of nicotine, cocaine, and heroin seeking (Madayag *et al.*, 2007; Zhou & Kalivas, 2008; Amen *et al.*, 2011; Moussawi *et al.*, 2011; Gipson *et al.*, 2013; Reissner *et al.*, 2015). In addition, mice expressing a dominant-negative SNARE protein selectively in astrocytes do not exhibit cocaine-induced reinstatement of CPP or cue-induced reinstatement of cocaine-seeking (Turner *et al.*, 2013). Taken together, these studies demonstrate that astrocytes, and GLT-1 in particular, may be critical for reinstatement of drug seeking for multiple drugs of abuse.

Clinical implications and potential therapeutic targets

Neuroimmune activation has been found in individuals with substance use disorders, and the immune system represents a promising target for addiction therapeutics. Importantly, genome-wide association studies have demonstrated significant correlations between single nucleotide polymorphisms in cytokine genes, in particular IL-1β and IL-10, with alcohol, opioid, and cocaine use disorders (Pastor *et al.*, 2005; Marcos *et al.*, 2008; Liu *et al.*, 2009; Smith & Humphries, 2009; Coller & Hutchinson, 2012). These genetic correlations suggest that glia may contribute to human addiction. In addition, the patterns of drug-associated alterations in glia that are observed in rodent models are also seen in humans. Indeed, chronic psychostimulant use can lead to astrogliosis and microgliosis, as measured by positron emission tomography or histopathological assessment at autopsy (Sekine *et al.*,

2008; Büttner, 2011). However, some reports have found smaller or minimal effects of chronic methamphetamine use on astrocytes and microglia (Kitamura *et al.*, 2010; Clark *et al.*, 2013). Alcohol-dependent individuals have increased number of glial-associated markers, including IBA1, TLRs, MCP-1, and IL-1β (He & Crews, 2008). These glial changes are highly correlated with lifetime alcohol consumption and the age of drinking onset, with earlier initiation or greater use being associated with higher expression of TLR4s (Crews & Vetreno, 2016). In contrast to microglial-associated makers, alcohol-dependent individuals also display significant decreases in astrocyte number and GFAP intensity in the hippocampus and dorsolateral PFC (Korbo, 1999; Miguel-Hidalgo *et al.*, 2002). Together, these data highlight critical interactions between drug use and the neuroimmune system in humans, and create an important backdrop for the use of glial inhibitors as potential cessation aids. Indeed, a growing body of clinical data has demonstrated the effectiveness of glial-targeted therapeutics in substance abuse (Table 3). These studies are small in number and typically have small sample sizes (i.e., <50 participants), but the results are promising.

One of the earliest clinical studies examining the influence of glial inhibitors on drug-associated behaviors showed that minocycline reduces the positive subjective effects of d-amphetamine, but has no effect on self-administration of d-amphetamine (Sofuoglu *et al.*, 2011). However, this was tested in healthy non-users and may not have broader applicability to dependent users. It is not known whether minocycline influences consumption and/or craving and relapse in dependent cocaine or amphetamine users. Ibudilast's effects on psychostimulant use is similarly understudied, although it has been shown to reduce the positive subjective effects of methamphetamine in dependent individuals (Worley *et al.*, 2016) and to decrease cocaine craving in non-treatment seeking opioid-dependent individuals (Metz *et al.*, 2017). Phase 2 clinical trials for 100 mg dosing of ibudilast in methamphetamine-dependent individuals are expected to begin in 2018 (NCT01860807).

Ibudilast is the most studied therapeutic to decrease opioid use, and shows promise as a pharmacotherapy to ameliorate withdrawal symptoms in opioid users. Indeed, in non-treatment seeking heroin-dependent volunteers, ibudilast lowered ratings of 'anxious', 'perspiring', 'restless', and 'stomach cramps' (Cooper *et al.*, 2016), and also produced a slight reduction in the subjective 'liking', reinforcing effects, and ratings of craving for oxycodone in non-treatment seeking opioid-dependent volunteers (Metz *et al.*, 2017). In parallel with what has been seen in animal models, ibudilast potentiated the analgesic effects of oxycodone in opioid-dependent volunteers (Cooper *et al.*, 2017; Metz *et al.*, 2017). These effects suggest that ibudilast may be a useful alongside opioid treatment to prevent dependence and aversive side effects. Ibudilasts' effect on the subjective properties of oxycodone was inconsistent, but there was a general trend for ibudilast treatment to decrease craving for heroin, cocaine, and tobacco (Cooper *et al.*, 2017; Metz *et al.*, 2017). As such, Ibudilast may be useful as a maintenance therapy to prevent craving for a variety of drugs. Ibudilast may also decrease cue- and stress-induced reinstatement of alcohol craving, as well as decrease the subjective effects of alcohol in individuals with alcohol use disorder (Ray *et al.*, 2017).

To date, only one study has examined minocycline's effect on smoking behavior. In a small randomized, double-blind crossover study of non-treatment seeking smokers, Sofuoglu et al.

(2009) found that minocycline does not affect smoking behavior, nicotine metabolism, or nicotine's physiological effects, but it did decrease ratings of 'depressed mood' during withdrawal and reduced cigarette craving during intravenous nicotine self-administration and sample cigarette smoking. However, rather than being mediated by a direct glial mechanism, the authors suggest that minocycline effects on smoking behavior may be due to inhibition of the neuronal nitric oxide (NO) synthase enzyme, ultimately reducing NO production. A recent study by Cooper et al. (2017) found a trend for ibudilast to decrease tobacco craving in opioid-dependent individuals, but this was a secondary endpoint and a small effect. Metz et al. (2017) also showed a significant and dose-dependent decrease in tobacco craving by ibudilast treatment in opioid-dependent individuals, but the sample size was small (n=11).

Although relatively few studies have directly examined neuroimmune therapeutics in the treatment of substance use disorders, current data on ibudilast, in particular, show some promise. Future research should focus on how immune modulators can affect withdrawal, long-term abstinence, and relapse. Research that identifies markers and develops tools to accurately measure glial functioning in humans is also needed, as it is currently difficult to attribute the effectiveness of glial-targeted medications such as ibudilast or minocycline to direct alterations in glia.

Discussion

Interactions between neurons and glia are gaining increasing attention due to their involvement in brain function during health and disease. A growing body of literature demonstrates that glia may be key therapeutic targets that perpetuate addiction. Microglia and astrocytes have distinct and consequential changes following exposure to a wide range of drugs of abuse. The vast majority of drugs of abuse (opioids, alcohol, cocaine, and amphetamines) increase glial markers and promote a pro-inflammatory phenotype. However, It is important to note that drug-induced changes to glia are dependent on drug exposure timing, brain region, animal age, and type of drug. Many of the changes to glia induced by abused drugs are dependent on the immune receptor, TLR4, which identifies TLR4 and its downstream targets as potential therapeutic avenues. However, there are other mechanisms by which drugs of abuse alter glia that are not discussed in detail here, and it is reasonable to predict that more mechanisms underlying drug-induced glial changes will be discovered in the future.

It is paramount that we further investigate the role of glia during the different phases of drug use, from acquisition, to dependence, withdrawal, and reinstatement. This is of particular importance as it has been demonstrated for both alcohol and cocaine that timing of exposure has distinct effects on microglia and astrocytes. These differential responses may contribute to the divergent molecular, cellular, circuit, and behavioral changes observed in these different phases of drug use.

In addition, the relationship between glia and complex circuit dynamics is just beginning to be recognized. Foundational studies have shown glial-released cytokines alter synaptic number, connectivity, neurotransmitter release, receptor abundance, and neural response to

stimuli. However, the consequence of these cytokine-induced changes to addiction behavior is exceedingly understudied. More recent development of viral, chemogenetic, and optogentic tools to specifically target and modulate glia will aid in answering key questions as to the role of glial mechanisms underlying addiction. Advancing our understanding of glial cells, and our ability to control them, should provide novel therapies for addiction.

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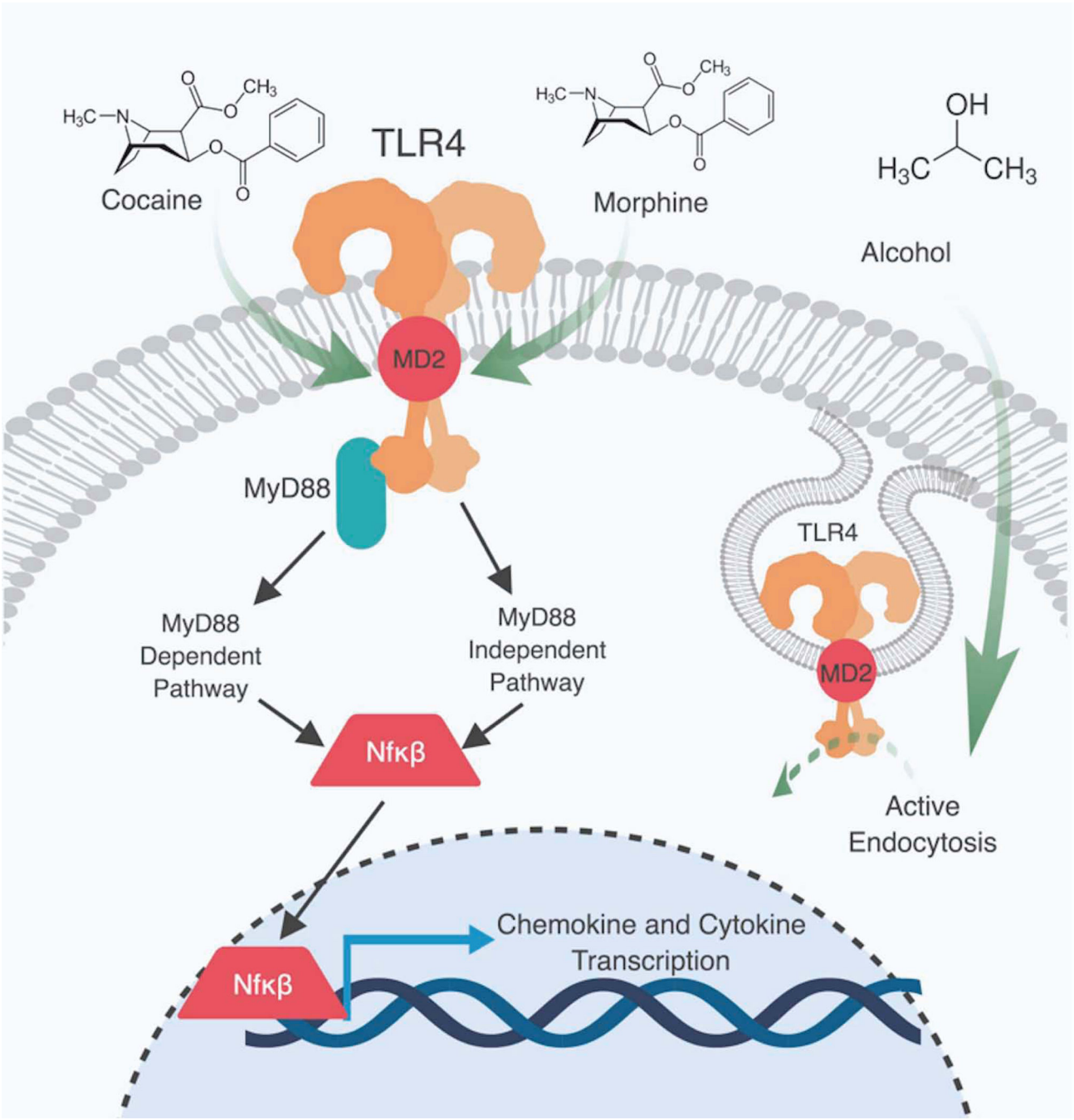

Figure 1:

Toll-like receptor 4 (TLR4) is a central intermediary between abused drugs and glia. Morphine is a direct agonist of TLR4 and binds to a unique binding site in the myeloid differentiation factor 2 (MD-2) domain of the TLR4 receptor. Cocaine is a direct agonist for TLR4 and binds to a domain in the MD-2 region of the receptor, this binding site is the same site of (+)-naltrexone. Alcohol promotes active endocytosis of TLR4 receptors by modulating lipid rafts. TLR4 activation causes the translocation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (Nf-κβ) to the nucleus via the

myeloid differentiation primary response 88 (MyD88) pathway and MyD88-independent pathways.

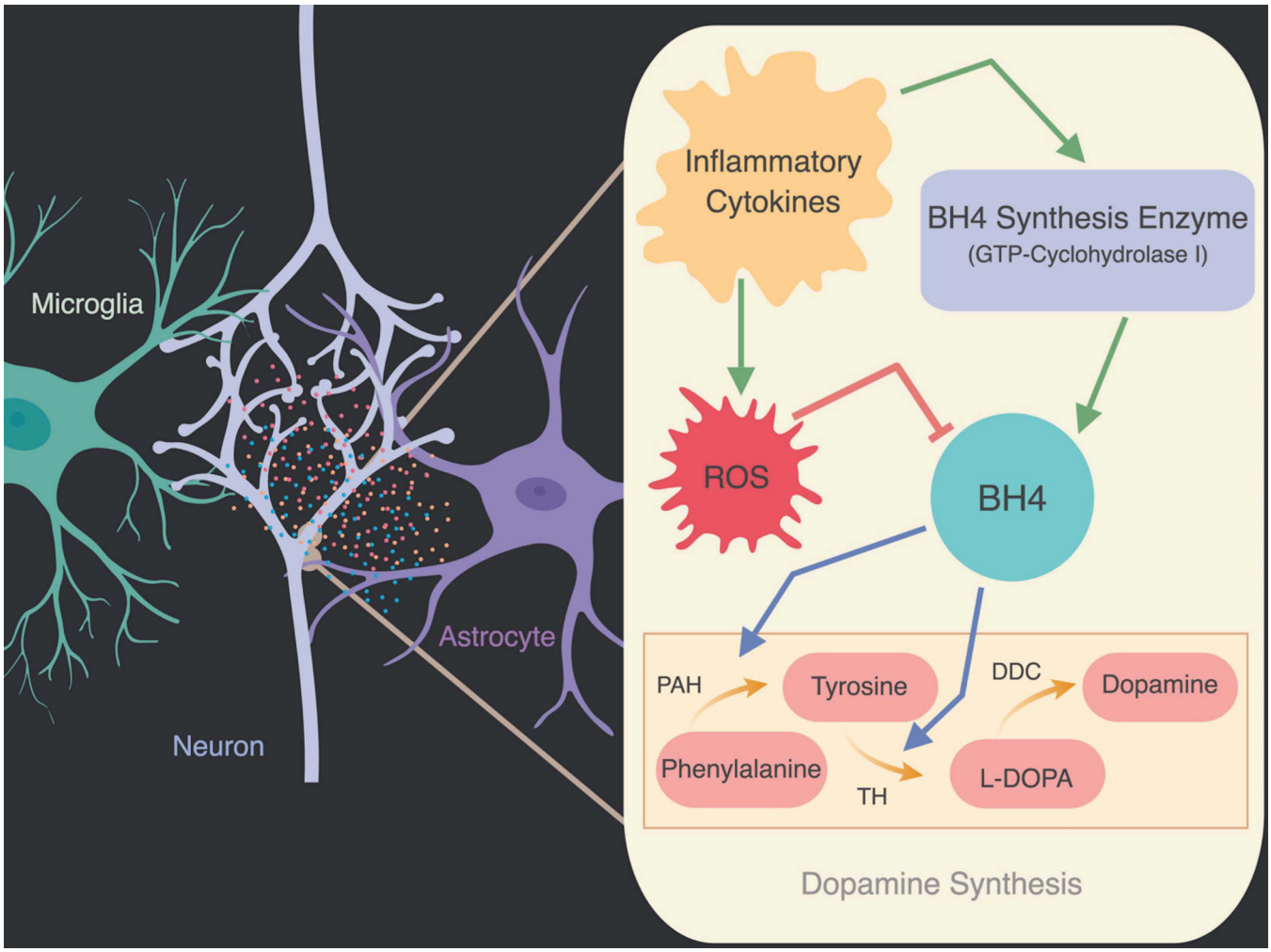

Figure 2:

Dopamine synthesis relies on the conversion of tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase (TH). Phenylalanine is converted to tyrosine by phenylalanine hydroxylase (PAH). Both TH and PAH require tetrahydrobiopterin (BH4) as a cofactor. Although inflammatory cytokines induce GTP-cyclohydrolase I expression, an enzyme necessary for BH4 synthesis, they also increase free radicals. Reactive oxygen species (ROS) contribute to oxidative stress and reduction of BH4. Inflammatory cytokines can both increase and decrease dopamine synthesis through different pathways. Red arrows indicate inhibitory effects and green arrows indicate excitatory effects on enzymes.

Table 1-

Cytokines and chemokines that contribute to drug use

Cytokine	Acronym / Receptor	Function
Interferon Gamma	IFNγ / IFNϒR1,2	Increases lysosome activity of macrophages; Activates iNOS
Interleukin 1 Alpha	IL-1α / IL-1R1,2	Induces synthesis of proteases; Induces TNFα production
Interleukin 1 Beta	IL-1β / IL-1R1,2	Proinflammatory mediator, involved in apoptosis and proliferation
Interleukin 10	IL-10 / IL-10R1,2	Block NFkβ activity
Interleukin 12	IL-12 / IL-12R-β1,2	Stimulates production of TNFα,IFNγ
Interleukin 4	IL-4 / IL-4Rα	Cooperates with TGFβ, drives mitogenesis
Interleukin 6	IL-6 /IL-6R	Drives chronic inflammation and autoimmunity
Transforming Growth Factor Beta	TGFβ / TGFβR	Inhibits secretion of TNFα, IFNγ and various interleukins
Tumor Necrosis Factor Alpha	TNFα / TNFR1,2	Apoptosis, inflammation
Chemokine	**Acronym / Receptor**	**Function**
C-C motif chemokine ligand 2	CCL2 / CCR2	Recruits immune cells since to sites of inflammation
C-X-C motif chemokine ligand 12	CXCL12 / CXCR4	Induced by TNFα and IL-1
C-X3-C motif ligand 1	Fracktalkine / CX3CR1	Microglial synaptic pruning
C-C motif chemokine ligand 4	CCL4 / CCR5	Mediate pro-inflammatory signals
C-C motif chemokine ligand 17	CCL17 / CCR4;CCR8	Induces chemotaxis in T cells

Table 2-

Brain regional differences in glial response to drugs of abuse

Brain Region	Drug of Abuse	Chemokine/Cytokine	Effect	References
Nucleus accumbens	Morphine	IFN-γ	↑	Shwartz et al. 2014
		CCL4 / CCR4		
		CCL17		
		IL-10		
		GFAP		Marie-Claire et al. 2004
		IBA1		Hutchinson et al. 2009
	Cocaine	IBA1	↑	Lewitus et al. 2016
		IL-1β		Cearley et al. 2011; Northcutt et al. 2015
		TNFα		Cearley et al. 2011; Northcutt et al. 2015
	Amphetamine	GFAP	=	Armstrong et al. 2004
Caudate Putamen	Morphine	GFAP	↑	Marie-Claire et al. 2004
		IBA1		
Cortex	Morphine	IBA1		Hutchinson et al. 2009
	Alcohol	GFAP	↑	Udomuksorn et al. 2011; Kane et al. 2014
		CCL2	↑	Kane et al. 2014
		IL-6	=	
		TNFα		
		IBA1	↑	Qin and Crews 2012
	Cocaine	IL-1β	↑	Cearley et al. 2011; Northcutt et al. 2015
		TNFα		
	Amphetamine	GFAP	=	Armstrong et al. 2004
	Methamphetamine	TNFα	↑	Goncalves et al. 2008
		IL-6		
Hippocampus	Morphine	IL-1β	↑	Shwartz et al. 2014
		IBA1		Hutchinson et al. 2009

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Brain Region	Drug of Abuse	Chemokine/Cytokine	Effect	References
	Alcohol	GFAP	↑	Tagliaferro et al. 2002; Kane et al. 2014
		CCL2	↑	Kane et al. 2014
		IL-6	=	Kane et al. 2014
		TNFα	=	Kane et al. 2014
		IBA1	↑	Qin and Crews 2012
	Cocaine	GFAP	↑	Fattore et al. 2002
	Methamphetamine	TNFα	↑	Goncalves et al. 2008
		IL-6	↑	Goncalves et al. 2008
Ventral Tegmental Area	Morphine	IBA1	↑	Hutchinson et al. 2009
	Cocaine	IL-1β	↑	Cearley et al. 2011; Northcutt et al. 2015
		TNFα	↑	Cearley et al. 2011; Northcutt et al. 2015
Periaqueductal Gray	Morphine	IL-1β	↑	Shwartz et al. 2014
Substantia Nigra	Morphine	IL-6	↑	Shwartz et al. 2014
		IBA1	↑	Hutchinson et al. 2009
Locus coeruleus	Morphine	GFAP	↑	Alonso et al. 2007
Nucleus of the Solitary Tract	Morphine	GFAP	↑	Alonso et al. 2007
Dorsal Raphe	Morphine	IBA1	=	Hutchinson et al. 2009

IFN-γ = interferon gamma; CCL4 = chemokine ligand 4; CCR4 = chemokine receptor type 4; CCL17 = chemokine ligand 17; IL-10 = interleukin 10; GFAP = glial fibrillary acidic protein; IBA1 = ionized calcium binding adaptor molecule 1; CCL2 = chemokine ligand 2; IL-6 = interleukin 6; TNFα = tumor necrosis factor alpha; IL-1β = interleukin 1 beta

Table 3-

Effects of pharmacological manipulation of glia or TLRs on drug-associated behaviors

Drug of Abuse	Inhibitor	Measure - Rodents	Effect	Measure – Humans	Effect	References
Opioids	**Minocycline**	CPP	↓			Hutchinson et al. 2008; 2009
		Spontaneous somatic withdrawal	↓			
		Plasma drug levels	=			
		Analgesia	↑			
	Ibudilast	NAc dopamine release	↓	Ratings of ‘restless’, ‘anxious’, ‘perspiring’, and ‘stomach cramps’	↓	Bland et al. 2009; Cooper et al. 2016; Metz et al. 2017
		Naloxone-precipitated somatic withdrawal	↓	Craving	↓	
		Spontaneous somatic withdrawal	↓	Subjective ‘liking’	↓	Hutchinson et al. 2008; 2009; Cooper et al. 2016; Metz et al. 2017
		Plasma drug levels	=	Reinforcing effects		
		Analgesia	↑	Analgesia	↑	
				Positive subjective effects	=	
	(+)-naloxone	NAc dopamine	↓			Hutchinson et al. 2012; Tanda et al. 2016
		CPP	↓			Hutchinson et al. 2012
		Self-administration	↓			
		Analgesia	↑			
	LPS-RS	Development of CPP	↓			Chen et al. 2017
		Expression of CPP	=			
	(+)-naltrexone	NAc dopamine release	=			Tanda et al. 2016
		Cue-induced reinstatement	↓			Theberge et al. 2013

Drug of Abuse	Inhibitor	Measure - Rodents	Effect	Measure – Humans	Effect	References
	Ceftriaxone	Hyperthermia	↓			Rawls et al. 2007
		Naloxone-precipitated somatic withdrawal	↓			Rawls et al. 2010
		Cue-induced reinstatement	↓			Shen et al. 2014
	N-acetylcysteine	Cue-induced reinstatement	↓			Zhou and Kalivas 2008
		Drug-induced reinstatement				Agrawal et al. 2011
Alcohol	**Minocycline**	Ethanol intake	↓			Syapin et al. 2016
	Ibudilast	Ethanol intake	↓	Cue-induced reinstatement of craving	↓	Bell et al. 2015; Ray et al. 2017
				Stress-induced reinstatement of craving		
				Positive subjective effects		
	(+)-naloxone	Ethanol intake	=			Blednov et al. 2017; Harris et al. 2017
		LORR	↓			Wu et al. 2012
	T5342126	Ethanol intake	↓			Bajo et al. 2016
	Ceftriaxone	Ethanol intake	↓			Sari et al. 2011; 2013a; 2013b; Rao and Sari 2014; Das et al. 2015
		CPP	↓			Das et al. 2015
		'Relapse-like' drinking	↓			Qrunfleh et al. 2013; Alhaddad et al. 2014
Cocaine	**Minocycline**	NAc dopamine release	↓			Northcutt et al. 2015
		CPP	↓			Northcutt et al. 2015
	Ibudilast	Locomotor sensitization	↓	Craving	↓	Poland et al. 2016; Metz et al. 2017
	(+)-naloxone (+)-naltrexone	NAc dopamine release	↓			Northcutt et al. 2015

Drug of Abuse	Inhibitor	Measure - Rodents	Effect	Measure – Humans	Effect	References
			↑			Tanda et al. 2016
		CPP	↓			Northcutt et al. 2015; Tanda et al. 2016
		Self-administration	↓			Northcutt et al. 2015; Tanda et al. 2016
	Ceftriaxone	Cue-induced reinstatement	↓			Sari et al. 2009; Knackstedt et al. 2010
		Drug-induced reinstatement	↓			Knackstedt et al. 2010
	N-acetylcysteine	Drug-induced reinstatement	↓	Craving	↓	*La Rowe et al. 2006;* Madayag et al. 2007; *Amen et al. 2007*; Moussawi et al. 2011
		Cue-induced reinstatement	↓			Moussawi et al. 2011; Reissner et al. 2015
Methamphetamine	**Minocycline**	Self-administration	↓			Snider et al. 2013
		NAc dopamine release	↓			Zhang et al. 2006
		Locomotor sensitization	↓			Zhang et al. 2006; Mizoguchi et al. 2008
			=			Mizoguchi et al. 2008
	Ibudilast	Hyperlocomotion	↓	Positive subjective effects	↓	Snider et al. 2012; Worley et al. 2016
		Locomotor sensitization	↓			Snider et al. 2012
		Self-administration	↓			Snider et al. 2013
		Drug-induced reinstatement	↓			Beardsley et al. 2010
		Stress-induced reinstatement	↓			
	(+)-naltrexone	Cue-induced reinstatement	=			Theberge et al. 2013
	Ceftriaxone	Drug-induced reinstatement of CPP	↓			Abulseoud et al. 2012
d-Amphetamine	**Minocycline**			Positive subjective effects	↓	*Soguoglu et al. 2011*
				Self-administration	=	

Drug of Abuse	Inhibitor	Measure - Rodents	Effect	Measure – Humans	Effect	References
Nicotine/Tobacco	**Minocycline**			Smoking behavior	=	Sofuoglu et al. 2009
				Metabolism		
				Physiological effects		
				Ratings of 'depressed mood' during withdrawal	↓	
				Cigarette craving		
	Ibudilast			Craving	↓	Cooper et al. 2017; Metz et al. 2017
	Ceftriaxone	Reinstatement of CPP	↓			Alajaji et al. 2013
		Acquisition of CPP	=			
	N-acetylcysteine	Self-administration	↓	Craving	↓	*Schmaal et al. 2011*; Gipson et al. 2013; *Froeliger et al. 2015*
		Reinstatement	↓		=	
				Positive affect	↑	*Froeliger et al. 2015*
				Withdrawal symptoms	=	

References in italics are human clinical studies, all others are preclinical animal studies. CPP = conditioned place preference; DID = drinking-in-the-dark; CIE = chronic intermittent ethanol; LPS-RS = lipopolysaccharide from *Rhodobacter sphaeroides*; 2BC = two-bottle choice; CIE-2BC = two-bottle choice after chronic intermittent ethanol exposure

Table 4-

Effects of genetic manipulation of TLR4 signaling on drug-associated behaviors

Drug of Abuse	Genotype	Measure	Effect	Notes	References
Alcohol	TLR4 $^{-/-}$	LORR	↓		Wu et al. 2012; Harris et al. 2017
		Self-administration	=	In dependent and non-dependent rats	Harris et al. 2017
		2BC	=	Both sexes; reduced total fluid intake in females more	Blednov et al. 2017
		DID		Males; reduced total fluid intake	
		CIE-2BC		Males and females	Harris et al. 2017
	Site specific TLR4 knockdown	2BC	=	NAc knockdown	Harris et al. 2017
		DID	↓	VTA knockdown in P rats	June et al. 2015
				CeA knockdown in P rats	Liu et al. 2011; June et al. 2015
	MYD88 $^{-/-}$	LORR	↓		Wu et al. 2012
		2BC	↑	Males; accompanied by reduction in saccharin preference	Blednov et al. 2017
			↓	Females drinking 20% EtOH; reduced total fluid intake	
		Ethanol preference	=	2BC or DID; males and females	
	TLR2 $^{-/-}$	2BC	↓		
		DID			
	CD14 $^{-/-}$	2BC	↓	Increased total fluid intake	
Cocaine	TLR4 $^{-/-}$	CPP	↓	Lower sensitivity; no persistent CPP	Kashima and Grueter 2017
	TLR4 point mutation (C3H/HeJ)	Self-administration	↓	Acquisition and progressive-ratio testing	Northcutt et al. 2015

LORR = loss of righting reflex; TLR4 = Toll-like receptor 4; 2BC = two-bottle choice; CIE-2BC; two-bottle choice after chronic intermittent ethanol exposure; DID = drinking-in-the-dark; NAc = nucleus accumbens; VTA = ventral tegmental area; CeA = central amygdala; VP = ventral pallidum; MYD88 = myelin differentiation primary response gene 88; P rats = alcohol preferring rats; TLR2 = Toll-like receptor 2; CD14 = cluster of differentiation 14; C3H/HeJ = mouse strain with point mutation preventing TLR-NFκB signaling