# Chapter 12
# Drug Addiction

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**Abstract** Drug addiction is a pervasive worldwide problem characterized by compulsive drug use that continues despite negative consequences and treatment attempts. Historically, the biological basis of drug addiction has focused principally on neuronal activity. However, despite their pivotal role in the underlying pathology of drug addiction, neurons are not the only central nervous system (CNS) component involved. The role of additional cell types, especially the CNS immunocompetent microglial cells, in the development of tolerance and related neuroplastic changes during drug taking, addiction, and withdrawal is also emerging. Within this perspective, this chapter reviews the roles of microglial cells in several aspects of drug addiction and its behavioural consequences, including reward, tolerance, dependence, and withdrawal. The cellular and molecular mechanisms which are particularly recruited will be emphasized. Lastly, we will also summarize the development of pharmacological modulators of microglial activation that offer novel treatment strategies and highlight the need to better understand the roles of microglia in the context of drug addiction.

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**Bullet Points**

- Microglial cells contribute to several aspects of drug addiction and its behavioural consequences, including reward, tolerance, dependence, and withdrawal.
- The cellular and molecular mechanisms utilized by microglia that contribute to drug addiction include microglial Toll-like receptors, NMDA receptors, and purinergic signaling.
- Targeting microglial activation may lead to the development of pharmacological modulators that seek to alter the behavioural consequences associated with drug addiction.
- A better understanding of microglial involvement in drug addiction is therefore needed.

## 12.1 Introduction

Drug abuse can lead to drug addiction, which is the compulsive use of a substance, despite its negative effects. Drug addiction represents one of the major medical, social, and economic burdens of human behavior. Abused drugs range from legally available recreational drugs such as alcohol and legally prescribed opioids such as morphine, to illicit "street" and "party" drugs such as heroin, cocaine, cannabis, and methamphetamine (Coller and Hutchinson 2012). In 2011, there were 22.5 million current illicit drug users aged 12 or older in the United States, comprising 8.7 % of the population (Fig. 12.1a) (Substance Abuse and Mental Health Services Administration 2012). The highest rate of illicit drug use was among the 18–20 year olds (23.8 %, Fig. 12.1b). The number of persons with drug dependence or abuse in 2011 was 20.6 million (Fig. 12.1c). Among the people with illicit drug dependence or abuse, marijuana, opioid analgesics, and cocaine were the 3 most used illicit drugs (Fig. 12.1d). These statistics highlight the need for research aimed at characterizing the molecular mechanisms responsible for drug addiction in an effort to develop more effective treatment regimens.

The physiochemical properties of abusive drugs, and the brain structures they affect, are diverse. Consequently, the neurochemical mechanisms responsible for drug addiction vary depending on the drug. However, all drugs of abuse share the common trait that they summate neuronally to produce elevated signaling in the cortico-mesolimbic reward pathway, which behaviorally presents as a rewarding and reinforcing drive after repeated exposure (Coller and Hutchinson 2012). This pathway begins at the ventral tegmental area (VTA), a midbrain cluster of dopaminergic neurons which project principally to a basal forebrain nucleus known as the nucleus accumbens (NAcc). The reward experienced from all addictive drugs has classically

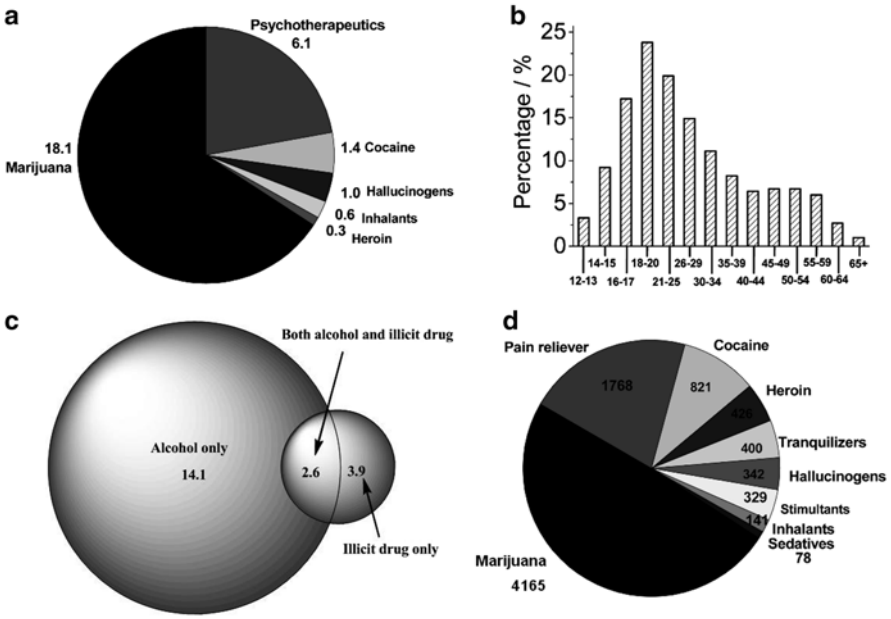



**Fig. 12.1** Prevalence of drug addiction. (**a**), illicit drug use among persons aged 12 or older in 2011 in US. Numbers are in millions. Data are derived from 2011 National Survey on Drug Use and Health (NSDUH). (**b**), age distribution of illicit drug use among persons aged 12 or older in 2011 in US. (**c**), drug dependence or abuse in 2011 among persons aged 12 or older. Numbers are in millions. (**d**), specific illicit drug dependence or abuse among Persons aged 12 or older in 2011. Numbers are in thousands

been thought to be associated, at least in part, with increases in extracellular dopamine from the VTA projections to the NAcc (Hyman et al. 2006). In addition to its hedonic effect, dopamine release at the nucleus accumbens core has been reported to be involved with motivation for drug seeking provided by drug learning memory (Li et al. 2011). Increased accumbal dopamine, in addition to activity in mesocortical circuits, has also been associated with the salience for drug use (Goldstein and Volkow 2002). When not exploited by drug use, this reward circuit reinforces behaviors that are essential for survival. Dopamine release at the nucleus accumbens can be triggered by eating or procreating, thereby contributing to reinforcement and ensuring these behavioural processes are repeated (Ikemoto and Panksepp 1999).

In addition to the VTA and NAcc, the amygdala and hippocampus are critical components of the cortico-mesolimbic reward pathway involved in drug addiction. The amygdala consists of nuclei located within the medial temporal lobes acting as a key stress system through interaction with the hypothalamic-pituitary-adrenal (HPA) axis (Lowry 2002). It is hypothesized that environmental stressors emerging from drug dependence and withdrawal activate the central and basolateral amygdala (BLA) and, once activated, create negative emotional states which are capable of motivating drug-seeking behavior (Koob 2009). The BLA also provides input to the ventral subiculum (vSub), a component of the hippocampus that projects to a

variety of limbic-related structures and modulates a variety of context-dependent processes involved in drug addiction, including drug sensitization and stress (Groenewegen et al. 1987; Herman and Mueller 2006; Sinha 2001). When activated directly by environmental stressors, or via the BLA, the vSub has been shown to induce a hyperdopaminergic response to amphetamine at the NAcc, and is hypothesized to be a key component responsible for drug sensitization (Belujon and Grace 2011; Lodge and Grace 2008).

The cycle of drug addiction varies greatly depending upon the individual and the type of drugs used. However, it generally involves three key stages: initiation, maintenance, and relapse. Initiation is a strong compulsion to continue drug use which is fueled by the motivation to obtain drug reward and is associated with increased activity in the cortico-mesolimbic dopamine reward pathway and associated neural networks (Coller and Hutchinson 2012). With repeated use, this motivation evolves into maintenance, which is characterized by drug dependence and desire to avoid negative withdrawal symptoms. Should an individual become abstinent, cravings and environmental cues can trigger drug relapse and begin the cycle of drug addiction once more.

Typically, animal models for drug initiation and relapse are measured by a self-administration behavioral paradigm, while the degree of reward can be measured by conditioned place preference (CPP) (Cooper et al. 2008). Briefly, the self-administration behavioral paradigm involves inducing an operant response in an animal by pairing a positive reinforcer with an operant behavior. In a drug self-administration behavioral paradigm, the operant behavior is typically a lever press while the positive reinforcer is the administration of an addictive drug. After several pairings, the animal will continue to elicit the operant behavior in the absence of the reinforcer. In this paradigm, initiation and relapse are measured as the degree of operant behavior exhibited by the animal following conditioning. CPP operates by placing the animal in a box subdivided into two sides having distinct colors and patterns. After a pre-exposure test, animals in the experimental group repeatedly receive a drug of abuse on only one side of the box. This conditions the animal to having a preference for the side where it received the drug. In this way, reward is measured as the increase in the amount of time the animal spent on the side of the box where it received the drug relative to the amount of time spent in that same side prior to drug exposure (Tzschentke 2007).

The initiation, maintenance, and relapse stages of drug addiction involve complex changes in the neurocircuitry of reward and stress, both of which are capable of reinforcing drug-seeking behavior (Kovacs 2012). Consequently, an overwhelming proportion of the research on the molecular and cellular basis of addiction has focused on neuronal activity. Likewise, pharmacological therapies for treating drug addiction have been directed toward molecular pathways in neurons (Miguel-Hidalgo 2009). This research has provided a plethora of knowledge on the neuronal circuits altered in drug addiction and provided valuable insights into how their alterations determine specific aberrant behaviors associated with drug addiction (Miguel-Hidalgo 2009). However, despite their pivotal role in the underlying pathology of drug addiction, neurons are not the only central nervous system (CNS)

component regulating neurotransmission. The role of additional cell types, especially the CNS immunocompetent microglial cells, in the development of tolerance and related neuroplastic changes during drug taking, addiction, and withdrawal is also emerging (Hutchinson et al. 2007, 2011; Milligan and Watkins 2009; Watkins et al. 2005, 2009).

Microglia are derived from an erythromyeloid precursor cell of the embryonic hematopoiesis in the yolk sac. As such, they are the resident cells of the innate immune system in the brain (Kierdorf et al. 2013; Neumann and Wekerle 2013). The new role of microglia as neuronal modulators of drug reward has emerged from the opioid's ability to activate microglia and promote the release of pro-inflammatory cytokines (Wang et al. 2012). These cytokines were recently identified as a potential source of microglia-to-neuron signaling stimulating microglia to increase neuronal excitability, thus resulting in a number of pathological conditions. These conditions include maintaining and enhancing pain states, reducing the efficacy of opioid for pain control, and developing analgesic tolerance and opioid withdrawal and dependence (Hutchinson et al. 2007, 2011; Milligan et al. 2009; Watkins et al. 2005, 2009). Since these phenomena are related to the process of addiction, this new role of microglia highlights the need to better understand the mechanisms of drug abuse from a microglial perspective (Coller and Hutchinson 2012). The goal of this chapter is to summarize the cellular and molecular mechanisms utilized by microglia that contribute to drug addiction and review the development of pharmacological microglia modulators that seek to alter the behavioral consequences associated with drug addiction.

## 12.2 Microglia Mechanisms of Drug Abuse

Microglia cells express markers of macrophage lineage and many receptors related to inflammatory processes (Kettenmann et al. 2011), and become activated when exposed to xenobiotics (Hutchinson et al. 2011). Activation of microglia by addictive drugs results in a change in cell morphology from a quiescent to an activated macrophage-like phenotype and initiates an innate immune response characterized by the release of pro-inflammatory cytokines such as interleukin-1 β (IL-1β), tumor necrosis factor α (TNFα), as well as nitric oxide (NO), within the prefrontal cortex, NAcc, VTA, amygdala, and hippocampus (Hutchinson et al. 2012; Miguel-Hidalgo 2009; Zhang et al. 2012). Pro-inflammatory cytokines down-regulate glutamate transporter (Watkins et al. 2009) and GABA receptor expression (Stellwagen et al. 2005), up-regulate AMPA and NMDA receptor expression and function (De et al. 2003), and enhance neurotransmitter release and synaptic transmission (Beattie et al. 2002; Pascual et al. 2012; Youn et al. 2008). Pro-inflammatory cytokines could therefore amplify the changes in neuronal activity induced by drugs of abuse, at multiple points within the associated circuitry. As the primary immune sentinels of the CNS, microglia engulf and remove apoptotic neurons and cellular debris. Microglia make direct contacts with synaptic elements and could also directly modulate synaptic activity by regulating synaptic numbers through sculpting and

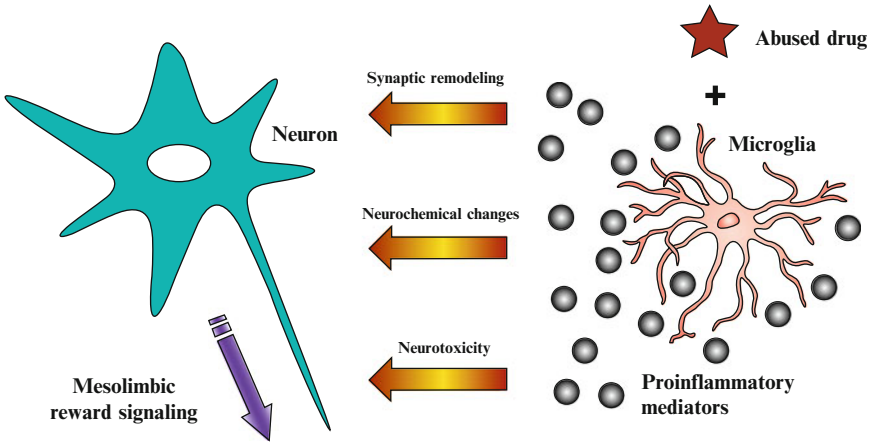



**Fig. 12.2** Schematic illustration of the microglia–neuron interactions which could be implicated in drug addiction. Abuse drugs cause microglia activation, leading to a profound pro-inflammatory immune signaling within the reward circuitry of the brain. In particular, pro-inflammatory immune signaling augments the mesolimbic dopamine signaling possibly through increased synaptic remodeling, neurochemical changes in excitatory transmission, and phagocytosis of apoptotic neurons and cellular debris

pruning and by modulating synaptic adhesion molecules and glutamate receptor expression (Ji et al. 2013; Kettenmann et al. 2013; Tremblay and Majewska 2011a; Tremblay et al. 2011b) (see Chap. 9 for further information). Collectively, microglia may modulate neuronal circuits of reward and dependence and alter behavioral responses through synaptic remodeling, neurochemical interaction with excitatory transmission, and phagocytosis of apoptotic neurons and cellular debris (Fig. 12.2) (Coller and Hutchinson 2012; Graeber 2010; Kettenmann et al. 2011; Kovacs 2012), although direct evidence for the role of microglia in remodeling the cortico-mesolimbic reward pathway in the context of drug abuse is still missing. Additionally, it should be noted that the activation of central immune signaling pathways induced by drugs of abuse has only been shown as a complimentary mechanism working in conjunction with previously established neuronal circuits underlying drug reward and dependence. Central immune signaling pathways alone will not produce drug addiction behavioral outcomes (Coller and Hutchinson 2012).

### *12.2.1 Toll-Like Receptor 4*

Accumulating evidences show that morphine can activate microglia and that inhibitors of microglia activation are capable of blocking behavioral measures of morphine reward such as CPP and self-administration in animal models (Hutchinson et al. 2007, 2012; Watkins et al. 2009). While microglia express a variety of "classical" opioid receptor subtypes to which morphine can bind, including δ, κ,

and μ-opioid receptors, studies with opioid receptor knockout mice reveal that morphine can act on microglia independently from these receptors. One such study reported that triple opioid receptor knockout mice are still responsive to morphine-induced hyperalgesia (Juni et al. 2007). Furthermore, classical opioid receptors are stereoselective for (−)-opioid agonists (Hutchinson et al. 2010a), yet (+)-opioid agonists, which have no classical opioid receptor activity (Wu et al. 2007), are capable of suppressing (−)-opioid-induced analgesia (Wu et al. 2006). In fact, (+)-opioid agonists are sufficient in themselves for enhancing pain (Hutchinson et al. 2010a). This indicates that (+)-opioid agonists may be capable of inducing microglia activation as well. These findings reveal the existence of a non-classical opioid receptor (Wu et al. 2007) through which morphine might be capable of binding and activating microglia. Subsequently pharmacological blockade and genetic knockout studies have identified the Toll-like receptor 4 (TLR4)/myeloid differentiation factor 2 (MD-2) complex as a non-classical opioid receptor for morphine.

In the CNS, TLR4 is constitutively expressed by microglia, as well as astrocytes (Bsibsi et al. 2002; Lehnardt et al. 2003; Li et al. 2002; Mishra et al. 2006). TLR4 can be activated by microbial associated molecular patterns (MAMP, e.g., lipopolysaccharides (LPS)) (Takeuchi and Akira 2010) and xenobiotic-associated molecular patterns (XAMPs) (Hutchinson et al. 2012). TLR4 works through MyD88 and other adaptor proteins and leads to activation of the IL-1 receptor-associated kinases (IRAKs) and TNF receptor-associated factor-6 (TRAF6), which finally culminates in activation of NFκB and MAPKs and production of pro-inflammatory cytokines (IL-1β, TNFα) as well as NO. (Hameed et al. 2010; Hutchinson et al. 2012; Takeuchi and Akira 2010). Molecular docking and biophysical characterizations show that morphine, morphine-3-glucuronide, methamphetamine, remifentanil, and cocaine bind to the LPS-binding cleft of the TLR4 accessory protein MD-2, and induce pro-inflammatory central immune TLR4 signaling activation (Hutchinson et al. 2007, 2012; Milligan and Watkins 2009).

The p38 mitogen-activated protein kinase (MAPK), which is an important downstream mediator of TLR4 signaling, plays an important role in the acquisition and maintenance of morphine CPP; pharmacological inhibition of p38 by SB203580 in the NAcc microglia blocks morphine-induced CPP (Zhang et al. 2012). Therefore, it is hypothesized that opioids engage inflammatory CNS processes, acting as XAMPs to activate TLR4 signaling, in addition to neuronal targets, thereby potentiating the activity in dopamine reward circuits involved in opioid reward and reinforcement (Hutchinson et al. 2011, 2012).

Acute and chronic alcohol intake also results in region-specific activation of microglia (and astrocytes) in a dose- and time-dependent manner in situ (Coller and Hutchinson 2012; Kovacs 2012; Miguel-Hidalgo 2009). Alcohol induces pro-inflammatory microglia cell activation, including enlargement of their cell bodies and thickening of their processes, as well as over-production of pro-inflammatory factors, such as IL-1β, TNFα, and monocyte chemotactic protein-1 (MCP-1) in prefrontal cortex, cingulate cortex, and VTA (He and Crews 2008; McClain et al. 2011; Miguel-Hidalgo et al. 2002). Recent work in TLR4 genetic knockout mice highlights the essential role of lipid rafts (the microdomains containing an assembly of receptors

and glycosphingolipids), TLR4, and their interaction with MD-2 and CD14 accessory proteins in alcohol-induced neuroinflammation (Alfonso-Loeches et al. 2010; Coller and Hutchinson 2012; Kovacs 2012). Ethanol activates primary microglia cells in culture and induces microglia activation in vivo, via MyD88-dependent and MyD88-independent TLR4 signaling pathways (Coller and Hutchinson 2012; Fernandez-Lizarbe et al. 2009; Lewis et al. 2013; Pandey 2012). Alcohol can also trigger a structural association between TLR4/TLR2, thereby contributing to the inflammatory response in microglia during alcohol abuse (Fernandez-Lizarbe et al. 2013). Blocking TLR4 signaling by genetic knock-out of TLR4 or MyD88 or by the non-opioid TLR4 antagonist (+)-naloxone abolish the behavioral responses related to acute and chronic ethanol exposure (Pandey 2012; Pascual et al. 2011; Wu et al. 2012). Taken together, the above evidences suggest that TLR4 is an important molecular target that may play a significant role in the development of alcoholism.

Neurons have been reported to express TLR4 (and the TLR accessory protein MD-2) at low levels (Diogenes et al. 2011; Ferraz et al. 2011), but their functional impact on the mechanisms of drug addiction is unknown (Hutchinson et al. 2012; Okun et al. 2011). This implies that one may be able to selectively target microglia by modulating TLR4 signaling without affecting CNS neurons, which suggests a novel strategy to separate beneficial analgesic effects of opioids from the unwanted tolerance and rewarding side effects. Several novel TLR4 inhibitors are being developed (Wang et al. 2013); if any of them can cross the blood–brain barrier (BBB), they may have efficacy in prevention or attenuation of drug addiction (Coller and Hutchinson 2012). Pharmacological agents that target any of the associated downstream consequences of TLR4 activation may also prove efficacious for the treatment of drug addiction (Coller and Hutchinson 2012).

### *12.2.2* N-*Methyl-D-Aspartate Receptors*

The NMDA receptor (NMDAR) family is composed of NMDA R1, NMDA R2A-D, and NMDA R3A-B subunits (Domercq et al. 2013). They form heterotetramers that bind to the major excitatory neurotransmitter, glutamate, and work as voltage-gated ion channels. Accumulating evidences support the involvement of glutamatergic neurotransmission, within the dopaminergic reward circuit, among the mechanisms of drug addiction (Kovacs 2012). Morphine has been shown to up-regulate brain-derived neurotrophic factor (BDNF) in microglia, which up-regulates in turn NMDARs (Kovacs 2012; Ueda and Ueda 2009). Activation of microglia NMDARs triggers the release of pro-inflammatory cytokines (TNFα, IL-1β, IL-6, etc.) (Kaindl et al. 2012), which down-regulates glutamate transporters in astrocyte in vivo and leads to a dysregulation of extracellular glutamate. In vitro microglia also produces quinolinic acid following activation (Espey et al. 1997), which promotes microglia glutamate release through activation of their NMDARs (Kovacs 2012). Down-regulation of glutamate transporters and enhanced glutamate release augment CNS excitotoxicity overall, therefore amplifying the abused drug-induced neuronal activity within the reward circuitry and contributing to opioid withdrawal and

dependence (Coller and Hutchinson 2012). In vivo administration of resveratrol, which blocks NMDARs activity and inhibits opioid-induced neuroinflammation in microglia and astrocytes, also attenuates drug tolerance and dependence (Hameed et al. 2010; Tsai et al. 2012). Therefore, NMDARs are considered to play a significant role in drug addiction. However, whether NMDARs activation directly or indirectly controls microglia activation in vivo remains to be determined (Domercq et al. 2013). Therefore, further studies are necessary to characterize in vivo the existence of functional NMDARs in the resident and activated microglia populations, which could respond to the altering glutamate release during drug addiction.

### *12.2.3 P2X4 Receptor*

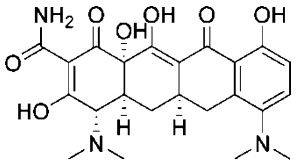
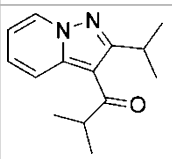
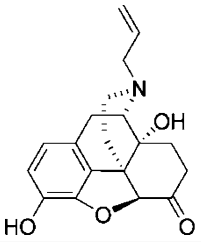
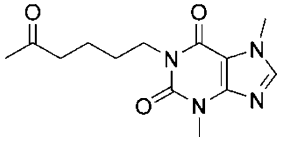
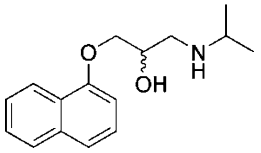
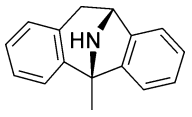
P2X4 receptor is an ATP-gated ion channel encoded by the P2X purinoceptor 4 gene, which occurs naturally as both homo- and hetero-oligomers (Kaczmarek-Hajek et al. 2012). Microglia express ionotropic purinergic receptor P2X4 (Inoue 2006). Multiple splice variants, often with distinct patterns of expression, have been found for the P2X4 receptor (Kaczmarek-Hajek et al. 2012). The expression of the P2X4 receptor is enhanced in microglia when exposed to morphine (Horvath and DeLeo 2009). Chronic morphine administration induces ATP release in the spinal cord dorsal horn (Horvath et al. 2010), which can activate microglia via P2X4 receptors leading to the release of bioactive substances including cytokines such as TNFα, IL-1β, IL-6, etc. (Horvath et al. 2010; Inoue 2006). Pro-inflammatory factors released from activated microglia contribute to the development of opioid reward and dependence (Coller and Hutchinson 2012; Kovacs 2012). Therefore, modulation of P2X4 receptor expression and activity on microglia may prove an attractive target for the prevention or attenuation of opioid-induced side effects (Horvath and DeLeo 2009; Horvath et al. 2010). However, it should be noted that P2X4 is not exclusively expressed by microglia. Neurons and astrocytes also express P2X4. Further studies are thus required to understand how the microglia-astrocyte and neuron-glia interactions at P2X4 receptors affect the dopaminergic reward circuits and the behaviors of drug abuse.

## 12.3 Pharmacological Microglia Modulators as a Way to Alter the Behavioral Effect of Drug Abuse

Drug abuse costs the US economy hundreds of billions of dollars in increased healthcare costs, crime, and lost productivity (Mental Health Services Administration 2012). Drug addiction changes the structure of the brain and its functionality, which in turn affect individual personality and behaviors. The impact of addiction can be far reaching. Cardiovascular disease, high blood pressure, diabetes, liver disease, lung disease, stroke, HIV/AIDS, and other health problems can be affected by drug abuse.

Further, drug abuse not only affects the abuser's existence, but also the lives of his/her family and community members. This includes tearing the family apart, loss of employment, failure in school, increasing violence, child abuse, and other crimes. Therefore, there is great need for the development of pharmacological therapies to reverse the behavioral effects of drug abuse. Microglia release neuroinflammatory mediators in response to illicit drugs and have been shown to mediate sensitization and tolerance (Miguel-Hidalgo 2009; Hutchinson et al. 2007, 2011; Milligan and Watkins 2009; Watkins et al. 2009). Therefore, a better understanding of the underlying central immune signaling could provide novel opportunities for developing anti-addiction therapies that specifically target activated microglia (Table 12.1).

**Table 12.1** Small molecule modulators of microglia activation which could be used for the treatment of drug addiction

| Compound | Structure | Reference |
|---|---|---|
| Minocycline |  | Habibi-Asl et al. (2009), Hutchinson et al. 2008, Zhang et al. (2006) |
| Ibudilast |  | Beardsley et al. (2010), Hutchinson et al. (2009); Ledeboer et al. (2007), Snider et al. (2012, 2013) |
| (+)-Naloxone |  | Hutchinson et al. (2012) |
| Pentoxifylline |  | Ciraulo et al. (2005), Yoshikawa et al. (1999) |
| Propranolol |  | Bernardi et al. (2006); Kampman et al. (2001), Saladin et al. (2013) |
| Dizocilpine |  | Fan et al. (2012), Hameed et al. (2010), Shu et al. (2008)) |

### 12.3.1 Interleukin-10 (IL-10)

Opioids induce microglia activation, which is characterized by the release of pro-inflammatory molecules that contribute, at least in part, to opiate addiction (Hutchinson et al. 2007, 2012; Milligan and Watkins 2009). Among the pro-inflammatory cytokines released by activated microglia are IL-1, IL-6, and TNFα (Watkins et al. 2005). When IL-1, IL-6, and TNFα are injected into the spinal intrathecal (peri-spinal) space of rats in an effort to mimic the presence of cytokines released by activated microglia, each has been found to enhance pain sensitivity, a phenomenon known as hyperalgesia. Additionally, IL-1 and TNFα induce spontaneous and enhanced activity in the responding neurons (Hermann et al. 2001; Reeve et al. 2000). The ability to counter-regulate chronic pain states through morphine-induced microglia activation has set the stage for new research based upon interactions between drugs, microglia, and their effects on neuronal activity. It has been shown that anti-inflammatory factor IL-10 also attenuates morphine craving or relapse and completely knocks out morphine-seeking behavior (Schwarz et al. 2011). Administration of recombinant IL-10 has been found to reduce rats' cravings for morphine (Schwarz et al. 2011). The value of IL-10 therapies has yet to be explored in drug addiction treatment. Currently, Xalud Therapeutics is using XT-101, a microparticle-delivered non-viral gene therapy that drives the production of IL-10 to normalize microglia activity and treat neuropathic pain, multiple sclerosis (MS), and amyotrophic lateral sclerosis (ALS). While these conditions seem unrelated to addiction, this therapeutic approach may reveal the potential of treating a variety of neurological problems, including opioid addiction, by attenuating the neuroinflammatory response generated by activated microglia.

### 12.3.2 Minocycline

Minocycline is the most lipid-soluble derivative of tetracycline and is known to cross the BBB (Bastos et al. 2012). Minocycline inhibits microglia activation through blockade of NFκB nuclear translocation, inhibition of protein kinase C (PKC), p38 and c-Jun N-terminal kinase (JNK) activities, and suppression of inflammatory cytokines over-production (Bastos et al. 2012). Minocycline suppresses opioid-induced respiratory depression, tolerance, and CPP (Habibi-Asl et al. 2009; Hutchinson et al. 2008; Zhang et al. 2006, 2012). Minocycline was also observed to decrease alcohol consumption in mice thus proposing a new approach for the treatment of alcoholism (Agrawal et al. 2011). Therefore, minocycline may have therapeutic usefulness for reducing addictive behavioral responses associated with drug abuse. However, it should be noted that minocycline also acts on other cell types such as astrocytes, T lymphocytes, and macrophages (Bastos et al. 2012; Brundula et al. 2002; Dutta et al. 2010; Szeto et al. 2011). Most likely the actions of minocycline on peripheral immune cells also play a role in suppressing the unwanted effects of opioids (Bastos et al. 2012).

### 12.3.3 Ibudilast

Ibudilast (formerly AV-411, now MN-166) attenuates innate immune signaling and can cross the BBB (Ledeboer et al. 2007). It has a complex mechanism of action, including inhibition of phosphodiesterases, TLR4, and macrophage migration inhibitory factor (MIF) (Ledeboer et al. 2007). It reduces pro-inflammatory factor over-production, at least in part by inhibiting TLR4 signaling (Ledeboer et al. 2007). Microglia activation is reduced in a dose-dependent manner by ibudilast with reduction in the levels of LPS-induced NO, reactive oxygen species (ROS), IL-1β, IL-6, and TNFα production, and enhanced production of the anti-inflammatory cytokine IL-10 (Ledeboer et al. 2007). Preclinical data show that ibudilast is well tolerated, is effective via oral administration, and reduces astrocyte and microglia activation markers glial fibrillary acidic protein (GFAP) and cluster of differentiation molecule 11b (CD11b) in vitro (Ledeboer et al. 2007), as well as attenuated morphine and methamphetamine addiction (Beardsley et al. 2010; Hutchinson et al. 2009; Snider et al. 2012, 2013). This CNS-directed anti-inflammatory activity of ibudilast is of potential use in the improved efficacy and safety of opioids and other drugs of abuse such as methamphetamine as it decreases drug reward, dependence, withdrawal, and reinforcement (Snider et al. 2012, 2013).

### 12.3.4 (+)-Naloxone

(+)-naloxone is the plus enantiomer of the opioid antagonist drug (−)-naloxone. Unlike "normal and natural" (−)-naloxone, (+)-naloxone has no significant affinity for opioid receptors, but instead binds to the TLR4 accessory protein myeloid MD-2 and acts as a selective antagonist of TLR4 (Hutchinson et al. 2008, 2012). Opioids and alcohol activate microglia by TLR4 signaling and chronic use of these drugs causes release of TNFα and IL-1β as well as other downstream effects (Fernandez-Lizarbe et al. 2009, 2013; Hutchinson et al. 2010b, 2012; Lewis et al. 2013; Pandey 2012; Wu et al. 2012). These pro-inflammatory cytokines are thought to contribute to drug tolerance, dependence, and reinforcement by inhibiting TLR4-dependent microglia activation. In addition, it is thought that (+)-naloxone can counteract these effects (Alfonso-Loeches et al. 2010; Hutchinson et al. 2007, 2012). Since (+)-naloxone does not have any apparent binding affinity for the μ-opioid receptor, the desired opioid analgesic effects could be preserved.

### 12.3.5 Pentoxifylline

Pentoxifylline is a competitive nonselective phosphodiesterase inhibitor which can cross the BBB and reduces cyclic adenosine monophosphate (cAMP) production. This, in turn, decreases release of pro-inflammatory cytokines TNFα and IL-1β and

increases production of the anti-inflammatory cytokine IL-10 in cultured microglia (Yoshikawa et al. 1999). Neuroinflammation is a critical component in the development and maintenance of drug abuse (Kovacs 2012). Therefore, pentoxifylline might be used to block drug-induced pro-inflammatory factors and attenuate drug reward and dependence effects (Ciraulo et al. 2005).

### *12.3.6 Propranolol*

Cultured microglia express β-adrenergic receptor, which are G-protein-coupled receptors for catecholamines, such as adrenaline and noradrenaline (Tanaka et al. 2002). β-Adrenergic receptor signaling activation increases expression of pro-inflammatory IL-1β, TNFα, and IL-6 in the brain, both at the mRNA and protein levels. Propranolol, a β-adrenergic receptor antagonist, antagonizes pro-inflammatory cytokine production in microglia cells (Wang et al. 2010). Preclinical and clinical evidence shows that propranolol can attenuate cocaine reward, dependence, and withdrawal (Bernardi et al. 2006; Kampman et al. 2001; Saladin et al. 2013). The effect of propranolol on the attenuation of cocaine addiction may be, at least in part, mediated by its suppression of microglia inflammation.

### *12.3.7 Dizocilpine (MK-801)*

Dizocilpine (MK-801) is an NMDARs noncompetitive antagonist that reduces pro-inflammatory factors cyclooxygenase-2 (COX-2) and TNFα and prevents microglia activation and neurotoxicity (Thomas and Kuhn 2005). Dizocilpine can reduce methamphetamine-induced activation of microglia in the striatum in vivo (Thomas and Kuhn 2005). Therefore, dizocilpine inhibits addictive behaviors in experimental animals (Fan et al. 2012; Hameed et al. 2010). However, a low dose of dizocilpine (0.05 mg/kg, i.p.) does not impair morphine-induced CPP and delay morphine extinction (Fan et al. 2012). The combined action of a low dose of dizocilpine (0.05 mg/kg, i.p.) and glutamate transporter activation by ceftriaxone (25 mg/kg, i.p.), however, effectively reduces the acquisition of morphine-induced CPP and completely prevents morphine reinstatement (Fan et al. 2012). Dizocilpine injected intrathecally decreases morphine-induced tolerance at the spinal cord level (Hameed et al. 2010; Kest et al. 1993). Dizocilpine's ability to reduce opioid tolerance is, at least in part, attributed to its inhibition of the NMDARs-dependent activation of spinal JNK; this kinase has been shown to be involved in the development of opioid tolerance (Guo et al. 2009).

## 12.4 Conclusions

In an effort to develop a better understanding of drug addiction, the implication of microglia needs to be considered, given the growing evidence that neuroinflammatory processes generated by these non-neuronal cells profoundly impact reward circuitries. Such processes are capable of modulating neuronal physiology and neuronal circuits of reward and dependence (Fig. 12.2) (Coller and Hutchinson 2012; Kettenmann et al. 2011; Kovacs 2012). Among the CNS immunocompetent cells, resident microglia are emerging as key contributors to the behavioral consequences of drug addiction by promoting neuroinflammation through release of proinflammatory cytokines. This finding invites the development of novel medications that treat drug addiction by modulating microglia activation specifically. In this regard, the small molecule modulators of microglia activation discussed in this chapter have excellent, well-established clinical safety records. Combining opioid agonist activity with the ability to block microglia activation in one small molecule may represent a novel strategy to yield powerful analgesia but with reduced abuse potential. For instance, PTI-609 is a novel agent that activates opioid receptors via a novel binding domain (Burns and Wang 2010) and inhibits the release of proinflammatory cytokines while also activating opioid receptors, thus leading to excellent analgesic action with no apparent potential for addiction (Burns and Wang 2010). This rationale may prove extremely beneficial in the future for the development of powerful but safe analgesics. In closing, it should be noted that microglia modulators also show effects on other CNS immunocompetent cells, such as astrocytes, CNS endothelial cells, and peripheral immune cells such as macrophages, monocytes, and lymphocytes, which can infiltrate the parenchyma in pathological contexts (Coller and Hutchinson 2012). Therefore, further research must also consider the contribution of these other CNS immunocompetent cells and peripheral immune cells to drug addiction.

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