Int J Physiol Pathophysiol Pharmacol 2017;9(6):165-177
www.ijppp.org /ISSN:1944-8171/IJPPP0069071

Original Article

Inflammasome in drug abuse

Enquan Xu[1], Jianuo Liu[1], Xiaobei Wang[2], Huangui Xiong[1]

[1]Neurophysiology Laboratory, Department of Pharmacology and Experimental Neuroscience, University of Nebraska Medical Center, Omaha, NE 68198-5880, USA; [2]College of Pharmacy, University of Nebraska Medical Center, Omaha, NE 68198-6125, USA

Received November 13, 2017; Accepted December 9, 2017; Epub December 25, 2017; Published December 30, 2017

Abstract: Drug abuse disorders refer to a set of related negative health implications associated with compulsive drug seeking and use. Because almost all addictive drugs act on the brain, many of them cause neurological impairments after long-term abuse. Neuropathological studies have revealed a widespread impairment of the cellular elements. As the key components to limit the damage of neural cells, CNS immune system is also found affected by these drugs, directly or indirectly. It has been shown that drugs of abuse alter neuroimmune gene expression and signaling. Growing studies on neuroimmune factors further demonstrate their indispensable role in drugs-induced neurotoxicity. As an important proinflammatory intracellular receptor, inflammasome is activated in many neurodegenerative diseases in response to a broad range of damage-associated molecular patterns (DAMPs) signals. In the cases of drug abuse, especially in those with comorbid of HIV infection and sustained pain, inflammasome activation significantly promotes the neuroinflammation-associated toxicities. To understand inflammasome in drug-associated neurotoxic activity, we reviewed the role played by inflammasome in drug abuse-induced microglial neurotoxicity and evaluated the potential of imflammasone as a therapeutic target for drug abuse disorders based on recent development of various selective small-molecular inflammasome inhibitors.

Keywords: Drug abuse, microglia, neuroinflammation, NLRP3, proinflammatory cytokines

An overview of inflammasome in the central nervous system

In a healthy central nervous system (CNS), due to the physical isolation of blood brain barrier (BBB), the peripheral immune cell infiltration and free passage of blood molecules are highly restricted [1-4]. For this reason, the CNS resident innate immune system is primarily response to invading pathogens and/or tissue damage [5]. Increasing attention has in recent years focused on Nod-like receptors (NLRs), an cytoplasmic pattern recognition receptor (PRRs) that is responsible for processing and release of IL-1β and IL-18 [6]. Up to now, 22 members of NLRs have been found in human and 34 in mice, which can be divided into four subfamilies based on their different N-terminal regions (NLRA, NLRB, NLRC, and NLRP) [7, 8]. As important cytosolic sensors, the NLRs are responsible for the reorganization of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) [9].

Many studies have been carried out to determine the roles of NLRs in neuropathogenesis. The subfamily NLRPs (1, 3, 10, 12) have been shown to be associated with the development of neurodegenerative diseases including multiple sclerosis (MS), Alzheimer's disease (AD) and Parkinson's disease (PD). Kong et al analyzed the database of an RNA-sequencing transcriptome and splicing database on the expression levels of NLRs in brain neural cells and they found among all cell types in the brain expressing NLRs, the NLRP3 is primarily expressed in the microglia [8]. As drug abuse-induced inflammasome activation is primarily medicated by NLRP3, this review focuses on the role of NLRP3 in microglia.

The NLRP3 inflammasomes are multimeric protein complexes composed of cytosolic sensor NLRP3, bridge protein apoptosis-associated speck-like protein containing a CARD (ASC), and cysteine protease caspase-1 [10]. In response to stimuli, NLRP3 will recruit the ASC protein and serve as caspase-1-activating scaffold. The

inactive pro-caspase-1 will oligomerize and be autoproteolytically cleaved into the active form [10]. Activation of caspae-1 will directly induce the processing and release of IL-1β and IL-18.

In contrast to other NLRs that require direct binding with their activating ligand, the interaction of ligands and NLRP3 is more complex [11]. There is an expanding list of NLRP3 ligands, which are mostly structure and function unrelated [12]. Currently, the exact mechanisms of how the NLRP3 protein interact with such a wide variety of ligands are still largely unknown. However, certain common patterns of activating stimulus have been identified. It is widely accepted that the full activation of NLRP3 requires two independent signals. The first priming signal is stimulated by NF-κB pathway to upregulate the major component (NLRP3) and substrate (pro-IL-1β) of inflammasome [13]. Emerging evidences indicate that the priming process is more complicated, which involves post-translation regulation of NLRP3 and ASC protein [14-18]. As a prototypical first activation signal, LPS was found to prime NLRP3 both in transcriptional and post-translational manners [19]. For this reason, even in researches related to CNS sterile inflammation, LPS is also widely applied as the first priming signal to investigate inflammasome activation [20-22]. After priming, distinct signals are required to active NLRP3 and thus promote the assembly of inflammasome complex. The potential involved pathways have been extensively studied and three activation models were introduced: potassium flux through ion channels, lysosomal membrane destabilization and release of cathepsin, and mitochondrial damage [10].

In the context of traumatic injury, neurodegenerative disease, and long-term drug abuse induced neurotoxicity, the CNS resident immune system primarily deals with self-derived "sterile insults" released from damaged neuron or other glial cells. The result of the immune cells activation can be either beneficial or detrimental. It is important to determine the timing of engagement and specific downstream inflammatory pathways that directly associated with neuropathology. Otherwise, non-specific immune suppressive therapy will also eliminate the inflammatory cascade that supports healing. Thus, our ultimate research goal is to promote the resolution of inflammation by dampening the specific signaling pathways tied to neurotoxicity. Abundant evidence indicate that NLRP3 inflammasome is actively involved in chronic sterile CNS inflammation and lead to detrimental consequences [20, 21, 23-29]. The criticle role of NLRP3 inflammasome in promoting neuronal damage was further demonstrated in traumatic-induced injury [30]. In both neurodegenerative disease and acture neuronal injury, the DAMPs will be released and quickly initiate a cascade of danger-associated intracellular signaling, which activates the NLRP3 inflammasome that orchestrate the inflammatory signaling in response to neuronal danger signals. One of the most potent effect of NLRP3 inflammasome activation is through processing and release of IL-1β and IL-18 that are critical amplifiers of the innate immune response to CNS damage. The excessive release of pro-inflammatory cytokine exacerbates excitotoxicity-induced neuronal damage [31-33], and in turn, promote the DAMPs-induced inflammasome activation. This vicious cycle can be further fueled by neuropathogenic factors such as amyloid-β or α-synuclein. In general, NLRP3 inflammasome signaling is a specific inflammatory pathway tightly associated with initiation and maintenance of neurotoxicity. Thus, targeting inhibition on NLRP3 inflammasome activation might be a promising therapeutic strategy.

Recently, novel specific small-molecular inhibitor (MCC950) has been developed and successfully reduced the IL-1β production in vivo [34]. The specific inhibition of NLRP3 inflammasome not only delayed the onset of experimental autoimmune encephalomyelitis (EAE), but also attenuated the severity of disease. Administration of MCC950 to APP/PS1 mice (animal model of Alzheimer's disease) significantly reduced the amyloid-β accumulation and thus, significantly improved the cognitive function. The enhanced clearance of amyloid-β is achieved by increased phagocytosis of microglia, which dampen the neuroinflammation-induced neurotoxicity [35]. In addition to small-molecular inhibitor, microRNA that negatively regulates NLRP3 expression was also identified [36]. Overexpression of miR-223 reduced the erythrocyte lysis associated microglia activation and neuronal damage, which suggests a protective role of miR-223 in intracerebral hemorrhage.

Like traumatic injury and neurodegenerative disease, both neuronal autonomous and non-autonomous cell death contribute to the long-term drug abuse-induced neurotoxicity. The microglial NLRP3 is activated in response to DAMPs release, promoting the proinflammatory-induced secondary neuronal damage. By introducing specific NLRP3 inhibition strategy, the auto-amplification loop between neuronal damage and sterile inflammation may be cut off. In next part, we will review the current research evidence that supports the roles of NLRP3 activation in neuronal dysfunction induced by long-term abuse of drugs.

Inflammasome in alcohol abuse

According to the 2015 National Survey on Drug Use and Health (NSDUH), 6.2 percent of people that ages 18 and older suffered from the Alcohol Use Disorder (AUD) in the USA. There was around 88000 people died with alcohol-related reason (Alcohol and Public Health: Alcohol-Related Disease Impact. Centers for Disease Control and Prevention). Because alcohol molecule is small and amphipathic, it can freely cross the BBB. Long-term abuse of alcohol has been reported to be associated with many negative impacts on normal neurological function, including habit formation, decision making, stress, and reward [37]. Animal studies indicated that chronic use of alcohol induced microglia and astrocyte activation accompanied with increased proinflammatory cytokines, which inhibited the neurogenesis and induced long-term behavioral alterations [38-42]. Two important alcohol related neurotoxic mechanisms have been identified including the release of proinflammatory factors and the ROS activation [43]. Overactivated microglia are considered primarily responsible for elevated neuoinflammatory signaling and contribute to development of neurological deficit [44, 45]. In contrast, anti-neuroinflammatory drug minocycline and doxycycline significantly decreased the alcohol consumption [46, 47]. These promising therapeutic effects of anti-neuroinflammatory strategy push researchers to further investigate the molecular mechanisms of alcohol-induced neuroinflammation.

IL-1 related signaling pathway has been well-studied in alcohol-associated neurological impairment. In human postmortem brain tissue, expression of IL-1β and inflammasome proteins (NLRP3 and NLRP1) are significantly upregulated in hippocampal area [48]. The administration of IL-1 receptor antagonist significantly reduced the acute alcohol-induced sedation and promote the recovery from alcohol-induced motor impairment [49]. It is well-accepted that IL-1 signaling is regulated both by TLR4-NF-κB pathway and casepase-1-induced maturation process, which is priming and activation signal of inflammasome [50]. For this reason, the roles of inflammasome in alcohol-induced toxicity were extensively studied. As the important priming signal of inflammsome, TLR4 was found upregulated in microglia and played a pivotal role in alcohol-induced proinflammatory cytokine production and psychological impairments [38, 51-54]. In addition to TLR4, another important inflammasome priming signal, high mobility group box-1 (HMGB1), has also been found released from damaged neuron [55, 56]. Further studies indicate that alcohol induced the acetylation and phosphorylation on HMGB1, which is highly associated with its release [57-59]. Correspondingly, the expressional level of receptors (TLR2, TLR4, TLR9, and RAGE) of HMGB1 were also found elevated in response to alcohol consumption [59]. There is evidence indicating that NLRP3 activation in macrophage/monocyte will, in turn, stimulate the release of HMGB1 to amplify the inflammatory signaling [60]. However, whether such vicious cycle exists in alcohol-induced neuroinflammation has not been investigated. As for the second activation signal of the inflammasome, current studies suggest that alcohol-induced mitochondrial dysfunction and oxidative stress contribute to the inflammasome activation in astrocyte and neuronal progenitor cells [61, 62]. Taken together, both the first priming signal and the second activation signal were proved to be induced by alcohol. To fully characterize the inflammasome activation in alcohol-induced neuroinflammation, a study performed on mice with both pharmacological and genetic manipulation of an essential component of the NLRP3 inflammasome. After administrated with 5% of ethanol for 5 weeks, mice in control group increased in expressional level of NLRP1, NLRP3, ASC, and proinflammatory cytokines, suggesting a functional role of alcohol as the first priming signal. On the other hand, the increased caspase-1 activity and release of IL-1β proved that alcohol

could also work as the second signal and complete the NLRP3 inflammasome activation [59]. In the mice genetically deleted with TLR4, NLRP3, or ASC less, ethanol administration failed to induce the activation of caspase-1 and production of IL-1β. Furthermore, the caspae-1 activity and IL-1β were found no increase in NLRP3-KO or ASC-KO mice [59]. Based on these evidence, inflammasome-IL-1β signaling cascade plays a critical role in alcohol-induced neuroinflammation. Following studies focused on the inflammasome activation and its association with alcohol-induced neuronal damage and function impairments. The ethanol-induced inflammasome activation impairs the differentiation of neuronal progenitor cells into the mature neuron and thus, inhibits the neurogenesis in the hippocampal area [41, 48, 61]. By pharmacological blockade of inflammasome activation, the alcohol-induced impairment of neurogenesis could be reversed [48]. Although rIL-1ra successfully prevented the alcohol-induced inflammasome activation and proinflammatory cytokines release, its protective role in neurogenesis is still largely unknown. In future, more experiments with NLRP3-KO or ASC-KO mice should be tested whether inhibition of inflammasome could reduce the consumption of alcohol or reverse the neuroinflammation-associated neuronal damage.

Gut-liver-brain axis is another promising but still less studied direction in inflammasome research on alcohol abuse. Because alcohol consumption could impair the integrity of BBB, it has been hypothesized that the peripheral endotoxin might cross into the brain [63, 64]. On the other hand, it is well-accepted that alcohol disrupts the intestinal epithelium integrity, which promotes the translocation of intestinal microbiome [65-67]. Thus, it is reasonable to hypothesize that alcohol increases endotoxin levels in the circulation and promotes the entrance of endotoxins into the brain. However, recent evidence suggests that endotoxin level did not change after 5 weeks of alcohol administration, which ruled out this possibility [59]. This might attribute to the fact that, in a healthy individual, the circulated endotoxin would be kept in check by the interaction of multiple organs [68]. However, it is still unknown if translocated endotoxin could escape the surveillance of immune system in a late stage of alcohol abuse, which is featured by a persistent systemic inflammation and liver detoxification impairment. In that case, it is still possible that gut-derived endotoxin will cross into the brain and prime the alcohol-induced inflammasome activation. Another potential connection between gut and brain in the context of alcohol abuse is through the liver damage. It has been found that alcohol-induced translocation of endotoxin will initiate the inflammasome-related proinflammatory response in liver [69]. The activation of inflammasome not only plays a central role in alcoholic liver disease but also promotes the releasing of liver DAMPs into the blood [70, 71]. Because the liver is considered as a major source of inflammatory cytokines that release into the serum [72], liver inflammasome activation induced by gut-derived endotoxin will further amplify the systematic inflammation. Once these circulated proinflammatory cytokines enter the brain tissue and activate the NF-κB pathway, the CNS inflammasome will be primed to be activated [73]. Lastly, whether gut-liver-brain axis was achieved by the liver-potentiated generalized immune response and subsequent neuroinflammation is worth investigating. The inflammasome activation both in liver and brain might serve as a converging signaling that provides a promising therapeutic target to dampen the inflammatory cross-interaction in systematic level.

Inflammasome in psychostimulants abuse

Cocaine and methamphetamine (Meth) are two primary abused psychostimulants in the United States. Cocaine is a psychostimulant drug that binds to dopamine transporter and inhibits its reuptake of synaptic dopamine. Compared to cocaine, methamphetamine not only blocks the reuptake but also promotes the dopamine release. They are both highly addictive and neurotoxic after long-term abuse. Accumulating evidence suggest that neuroinflammation underlies the mechanisms of neurotoxicity and provides a promising target. To better understand the mechanisms of cocaine- and Meth-related neuroinflammation, latest researches on inflammasome activation will be reviewed. Also, whether inflammasome-related signaling serves as a converging pathway that mediates the synergistic neurotoxic effect of HIV-1 infection with stimulant abuse will also be discussed.

In mice administrated with cocaine, NF-κB signaling was found to be activated and associated with structural changes that mediate the

drug reward-based learning [74, 75]. In consistent with this, upregulation of multiple proinflammatory mediators and microglia activation were detected in cocaine abused animal models [76-78]. It has been suggested the proinflammatory signaling activation is due to the dysregulated redox status after cocaine administration [79-81]. Although with the activation of the first signal (NF-κB signaling) and the second signal (oxidative stress), there is little study indicate the inflammasome activation in the brain after exposure to the cocaine. In cultured microglia, cocaine stimulates the proinflammatory release mediated through the ER stress-autophagy and ER stress-TLR2 axes [82, 83]. However, given the fact that ER stress is also a well-accepted activating signal for NLRP3 inflammasome activation [84, 85], there is no further study performed to investigate the role of the inflammasome in cocaine-induced microglial activation.

On the other hand, cocaine downregulates the expression of tight junction while it upregulates the expression of the brain endothelial adhesion molecules and CCL2 [86, 87]. Therefore, cocaine not only impairs the integrity of BBB but also promotes the transmigration of monocyte. In HIV-1 infected patients, cocaine abuse enhances the transmigration of HIV-1 infected monocyte/macrophage [88]. Emerging evidence suggest that HIV-1 virus proteins could prime and activate the NLRP3 inflammasome [89-91]. For this reason, it is worth to investigate whether cocaine could potentiate the HIV-1-induced inflammasome activation. Current evidence indicates that cocaine potentiates the ROS activation in HIV-1-infected macrophage, which is accompanied with the upregulation of inflammasome forming genes [92]. Although the ROS induction was considered as the second activation signal, the direct evidence of increased activity of caspase-1 and processing of IL-1β is still needed. It also suggests NLRP1-caspase-5 axis may contribute to the synergistic effect induced by cocaine and HIV-1 infection. Taken together, current evidences suggest that inflammasome may serve as a converging signaling that mediates the synergistic proinflammatory effect in the macrophage. In future, whether cocaine-induced ER stress could also potentiate the HIV-1-induced inflammasome activation in microglia is worth investigating.

Chronic abuse of Meth is a feature with neurotoxicity marked by diminished dopamine concentration, low level of the dopamine transporter, and neuroinflammation [93-97]. Although with abundant evidence against Meth-induced microglial activation as reviewed above, little study performed on microglia focused on inflammasome. However, the HMGB1 was found to be upregulated in Meth-administrated rats and induced the IL-1β production [98]. The author proposed that HMGB1 is released from the Meth-stressed neuron. It has been demonstrated that neuronal-derived HMGB1 is recognized by microglia as the danger-associated signal and primes the NLRP3 inflammasome [99]. As for the second activation signal, there are accumulating evidence suggesting that Meth application induces the mitochondrial damage and ROS production [100-103]. Therefore, in vivo, after priming with neuronal released HMGB1, Meth is very likely to activate the microglial NLRP3 inflammasome through the mitochondrial ROS pathway [104].

Inflammasome in morphine abuse

Morphine is originally isolated from poppy straw or opium poppy [105]. It is on the World Health Organization's list of Essential Medicines as an effective analgesic medicine [106]. Up to now, morphine is still one of the most effective drugs to treat both acute and chronic severe pain. However, the clinical application of morphine is limited by its long-term deleterious effects including addiction and withdrawal symptoms [107-109]. In chronic pain management, the tolerance of morphine will occur on repeated use of the drug, which increases the dose of the drug to achieve the same extent of pain relief [110]. The increased amount of drug, in turn, promotes the development of drug dependence. To prevent the diminished morphine responsiveness in a patient, multiple hypotheses were raised including μ-opioid receptor desensitization, synaptic plasticity changes, and morphine-induced microglial activation and proinflammatory cytokines release [111-115]. In this review, we primarily focus on the role of inflammasome-induced cytokines release in morphine tolerance.

Traditionally, morphine was defined as an immunosuppressive drug that subjects prolonged abuse under susceptibility of various infectious diseases [116, 117]. Although many basic functions of both innate and adaptive immune system such as phagocytic activities of macrophages or T, B-cell antibody response

were inhibited by morphine, the production of proinflammatory cytokines is, in general, increased by morphine administration [118]. Further studies discovered that morphine promoted the translocation of intestinal bacteria that induces the sepsis in mice [119-121]. Priming with morphine significantly potentiated proinflammatory responses in LPS administrated rats and promoted the progression of sepsis to septic shock [122]. All these evidence provide an alternative explanation for morphine-induced vulnerability to infection, which is achieved by impairment of pathogen clearance capability while augmenting pathogenic inflammatory response. Taken together, the immune modulation of morphine is much more complicated than we thought.

Recently, emerging evidence indicate that while morphine administration systematically dampens the activation of the peripheral immune system, local CNS resident immune cells, microglia are overactivated [123-125]. Because cytokines released from activated microglia might alter the neuronal adaption to morphine [126, 127], the role of neuroinflammation in the development of morphine tolerance was extensively investigated. Further studies confirmed that morphine could non-stereoselectively bind to the accessory protein of TLR4, triggering the TLR4 oligomerization and proinflammation [123]. It has been demonstrated that while μ-opioid receptor expressed on microglia mediated analgesic effect of morphine [124], the microglial TLR4 is critical to the development of morphine tolerance [128, 129]. Inspired by this, following studies targeted microglial activation and subsequent cytokines release and successfully blocked the chronic morphine-induced tolerance [130, 131]. Among those of morphine-induced cytokines, IL-1β stands out for its strong anti-analgesic effects against morphine and important role in morphine tolerance [132, 133]. Administration of morphine significantly stimulate the IL-1β release mediated by TLR4 signaling, and IL-1 receptor antagonization substantially reverses the morphine tolerance [115, 134]. Because NLRP3 activation is primed by TLR4 activation and lead to the release of IL-1β, the NLRP3 inflammasome is receiving more and more attention in research field of morphine tolerance.

After chronic administration of morphine (10 mg/kg, twice a day for 7 days), the western blot analysis of spinal cord indicated an increase of processing of IL-1β, while the pro-IL-1β was not significantly changed [135]. In consistent with this, the activation form of caspase-1 was also significantly elevated while pro-enzyme form remains constant [135]. To confirm the morphine-induced NLRP3 inflammasome activation in vitro, the authors also performed the experiments in BV-2, a cell-line of microglia. After treatment of 200 μM morphine, with or without the additional ATP signal, both the NLRP3 and pro-IL-1β were significantly upregulated. Combined with the second activation signal provided by ATP, they detected a potent processing and release of IL-1β and caspase-1 in supernatant [135]. All results indicate that morphine could work as the first priming signal, which allow microglia response to the second neuronal danger-associated signal (ATP). In addition to this, they also found that morphine induced a strong production of mitochondrial ROS that might work as a second activation signal. After pre-administration of procyanidins, a potent free radical scavenger, both the morphine-induced activation of mitochondrial ROS and NLRP3 inflammasome were blocked [135]. According to this study, it seems that morphine could both prime and activate the NLRP3 inflammasome simultaneously. In contrast to this, a study focused on spinal dorsal horn microglia indicated that morphine only prime the NLRP3 inflammasome through the TLR4-NF-κB pathway, while the second signal was induced by DAMPs released from injured neuron [136]. In their rat model with priming of a short course of morphine, a second prolonged enduration of chronic constriction injury (CCI)-allodynia was found to be mediated by microglial NLRP3 inflammasome. In a combination of morphine and CCI, the level of microRNA-223 that negatively regulated the expression of NLRP3 was significantly decreased. The other essential components of NLRP3 inflammasome were all upregulated (TLR4, NLRP3, pro-Casepae-1, pro-IL-1β). As for the P2X7R, the critical receptor sensing the ATP released from an injured neuron was also elevated. Taken together, morphine induces a persistent sensitization status of microglia that prones to be activated in response to neuronal DAMPs. After the introduction of P2X7R selectively inhibitor, the morphine-induced development of sensiti-

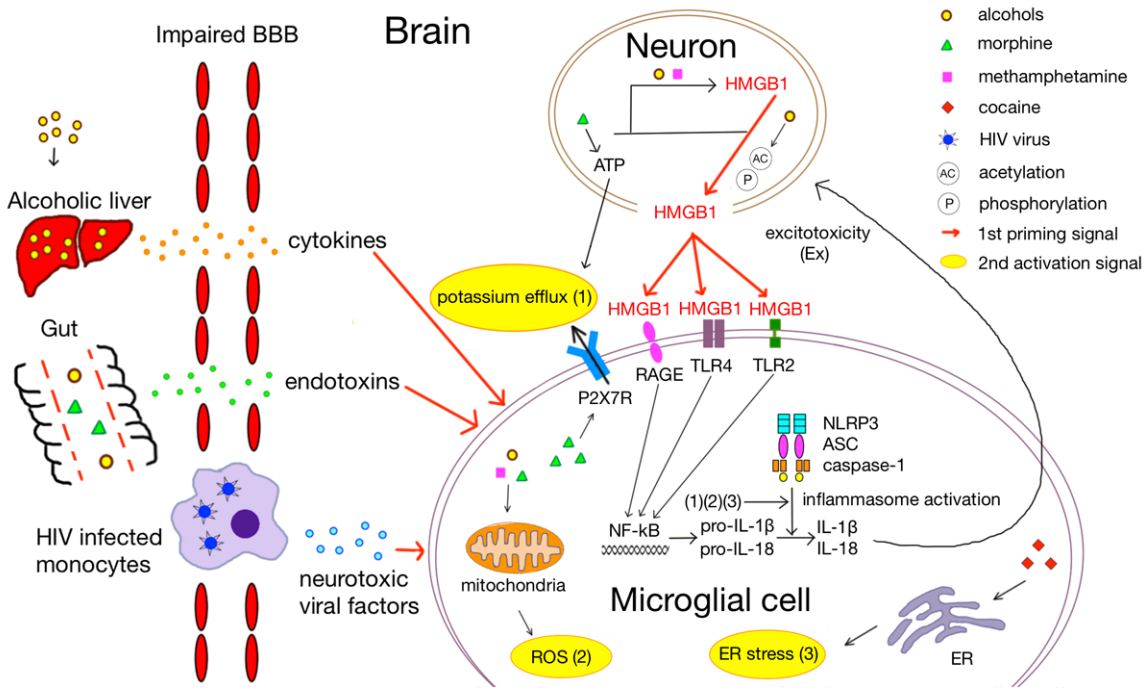

Figure 1. Schematic drawing illustrating drug-abuse effects on inflammasome activation (see text for details).

zation was reversed. It indicates that morphine itself might not be enough to elicit the activation of the NLRP3 inflammasome, but rather potentiated the pre-existing sterile neuroinflammation. Nevertheless, whether the NLRP3 inflammasome selective antagonists could prevent the development of morphine tolerance is highly clinical relevant. It may represent a novel strategy in the maintanance of the strong analgesic effect of morphine while eliminating its chronic side-effects.

In summary, in response to a broad range of damage-associated molecular patterns (DAMPs) signals, microglial inflammasome, as an proinflammatory intracellular receptor, can be activated in the course of drug abuse. Systematic and brain stimuli induced by addictive drugs converge signals on microglial inflammasome (**Figure 1**). Addictive drugs could, on one hand, induce local danger-associated molecular patterns (DAMPs) expression and release from the neuron (HMGB1). On the other hand, the drugs also cause the blood-brain barrier (BBB) damage that allows peripheral cytokines, endotoxins, and HIV-infected monocytes to cross through the BBB. These signals/molecues target the microglia and upregulate the pro-IL-1β and pro-IL-18. The red arrows in **Figure 1** indicate the local and systematic stimuli that work as the first priming signal for microglial inflammasome. The addictive drugs could induce potassium efflux (1), ROS activation (2) and ER stress (3) in microglia, which serve as the second activation signals (highlighted in yellow) for microglial inflammasome. Taken together, addictive drugs induce both the first priming and the second activation signals for the activation of microglial inflammasome, which promotes the maturation and release of IL-1β and IL-18. The excessive release of IL-1β produces neuroinflammation, resulting in neuronal damage.

Acknowledgements

Supported by NIH grant R01NS077873.

Address correspondence to: Dr. Huangui Xiong, Department of Pharmacology and Experiment Neuroscience, University of Nebraska Medical Center, Omaha, Nebraska, 68198-5880, USA. Tel: (402) 559-5140; Fax: (402)559-3744; E-mail: hxiong@unmc.edu

References

[1] Broadwell RD and Sofroniew MV. Serum proteins bypass the blood-brain fluid barriers for

extracellular entry to the central nervous system. Exp Neurol 1993; 120: 245-263.
[2] Bush TG, Puvanachandra N, Horner CH, Polito A, Ostenfeld T, Svendsen CN, Mucke L, Johnson MH and Sofroniew MV. Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after ablation of scar-forming, reactive astrocytes in adult transgenic mice. Neuron 1999; 23: 297-308.
[3] Muldoon LL, Alvarez JI, Begley DJ, Boado RJ, Del Zoppo GJ, Doolittle ND, Engelhardt B, Hallenbeck JM, Lonser RR, Ohlfest JR, Prat A, Scarpa M, Smeyne RJ, Drewes LR and Neuwelt EA. Immunologic privilege in the central nervous system and the blood-brain barrier. J Cereb Blood Flow Metab 2013; 33: 13-21.
[4] Medawar PB. Immunity to homologous grafted skin; the fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. Br J Exp Pathol 1948; 29: 58-69.
[5] Hanamsagar R, Hanke ML and Kielian T. Toll-like receptor (TLR) and inflammasome actions in the central nervous system. Trends Immunol 2012; 33: 333-342.
[6] Strowig T, Henao-Mejia J, Elinav E and Flavell R. Inflammasomes in health and disease. Nature 2012; 481: 278-286.
[7] Motta V, Soares F, Sun T and Philpott DJ. NOD-like receptors: versatile cytosolic sentinels. Physiol Rev 2015; 95: 149-178.
[8] Kong X, Yuan Z and Cheng J. The function of NOD-like receptors in central nervous system diseases. J Neurosci Res 2017; 95: 1565-1573.
[9] Martinon F, Mayor A and Tschopp J. The inflammasomes: guardians of the body. Annu Rev Immunol 2009; 27: 229-265.
[10] Guo H, Callaway JB and Ting JP. Inflammasomes: mechanism of action, role in disease, and therapeutics. Nat Med 2015; 21: 677-687.
[11] Jo EK, Kim JK, Shin DM and Sasakawa C. Molecular mechanisms regulating NLRP3 inflammasome activation. Cell Mol Immunol 2016; 13: 148-159.
[12] Vanaja SK, Rathinam VA and Fitzgerald KA. Mechanisms of inflammasome activation: recent advances and novel insights. Trends Cell Biol 2015; 25: 308-315.
[13] Bauernfeind FG, Horvath G, Stutz A, Alnemri ES, MacDonald K, Speert D, Fernandes-Alnemri T, Wu J, Monks BG, Fitzgerald KA, Hornung V and Latz E. Cutting edge: NF-kappaB activating pattern recognition and cytokine receptors license NLRP3 inflammasome activation by regulating NLRP3 expression. J Immunol 2009; 183: 787-791.
[14] Juliana C, Fernandes-Alnemri T, Kang S, Farias A, Qin F and Alnemri ES. Non-transcriptional priming and deubiquitination regulate NLRP3 inflammasome activation. J Biol Chem 2012; 287: 36617-36622.
[15] Py BF, Kim MS, Vakifahmetoglu-Norberg H and Yuan J. Deubiquitination of NLRP3 by BRCC3 critically regulates inflammasome activity. Mol Cell 2013; 49: 331-338.
[16] Lin YC, Huang DY, Wang JS, Lin YL, Hsieh SL, Huang KC and Lin WW. Syk is involved in NLRP3 inflammasome-mediated caspase-1 activation through adaptor ASC phosphorylation and enhanced oligomerization. J Leukoc Biol 2015; 97: 825-835.
[17] Hara H, Tsuchiya K, Kawamura I, Fang R, Hernandez-Cuellar E, Shen Y, Mizuguchi J, Schweighoffer E, Tybulewicz V and Mitsuyama M. Phosphorylation of the adaptor ASC acts as a molecular switch that controls the formation of speck-like aggregates and inflammasome activity. Nat Immunol 2013; 14: 1247-1255.
[18] Rodgers MA, Bowman JW, Fujita H, Orazio N, Shi M, Liang Q, Amatya R, Kelly TJ, Iwai K, Ting J and Jung JU. The linear ubiquitin assembly complex (LUBAC) is essential for NLRP3 inflammasome activation. J Exp Med 2014; 211: 1333-1347.
[19] Han S, Lear TB, Jerome JA, Rajbhandari S, Snavely CA, Gulick DL, Gibson KF, Zou C, Chen BB and Mallampalli RK. Lipopolysaccharide primes the NALP3 inflammasome by inhibiting its ubiquitination and degradation mediated by the SCFFBXL2 E3 ligase. J Biol Chem 2015; 290: 18124-18133.
[20] Halle A, Hornung V, Petzold GC, Stewart CR, Monks BG, Reinheckel T, Fitzgerald KA, Latz E, Moore KJ and Golenbock DT. The NALP3 inflammasome is involved in the innate immune response to amyloid-beta. Nat Immunol 2008; 9: 857-865.
[21] Shi F, Yang L, Kouadir M, Yang Y, Wang J, Zhou X, Yin X and Zhao D. The NALP3 inflammasome is involved in neurotoxic prion peptide-induced microglial activation. J Neuroinflammation 2012; 9: 73.
[22] Shi F, Yang Y, Kouadir M, Fu Y, Yang L, Zhou X, Yin X and Zhao D. Inhibition of phagocytosis and lysosomal acidification suppresses neurotoxic prion peptide-induced NALP3 inflammasome activation in BV2 microglia. J Neuroimmunol 2013; 260: 121-125.
[23] Gris D, Ye Z, Iocca HA, Wen H, Craven RR, Gris P, Huang M, Schneider M, Miller SD and Ting JP. NLRP3 plays a critical role in the development of experimental autoimmune encephalomyelitis by mediating Th1 and Th17 responses. J Immunol 2010; 185: 974-981.

[24] Inoue M, Williams KL, Gunn MD and Shinohara ML. NLRP3 inflammasome induces chemotactic immune cell migration to the CNS in experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 2012; 109: 10480-10485.
[25] Jha S, Srivastava SY, Brickey WJ, Iocca H, Toews A, Morrison JP, Chen VS, Gris D, Matsushima GK and Ting JP. The inflammasome sensor, NLRP3, regulates CNS inflammation and demyelination via caspase-1 and interleukin-18. J Neurosci 2010; 30: 15811-15820.
[26] Meissner F, Molawi K and Zychlinsky A. Mutant superoxide dismutase 1-induced IL-1beta accelerates ALS pathogenesis. Proc Natl Acad Sci U S A 2010; 107: 13046-13050.
[27] Codolo G, Plotegher N, Pozzobon T, Brucale M, Tessari I, Bubacco L and de Bernard M. Triggering of inflammasome by aggregated alpha-synuclein, an inflammatory response in synucleinopathies. PLoS One 2013; 8: e55375.
[28] Heneka MT, Kummer MP, Stutz A, Delekate A, Schwartz S, Vieira-Saecker A, Griep A, Axt D, Remus A, Tzeng TC, Gelpi E, Halle A, Korte M, Latz E and Golenbock DT. NLRP3 is activated in Alzheimer's disease and contributes to pathology in APP/PS1 mice. Nature 2013; 493: 674-678.
[29] Hafner-Bratkovic I, Bencina M, Fitzgerald KA, Golenbock D and Jerala R. NLRP3 inflammasome activation in macrophage cell lines by prion protein fibrils as the source of IL-1beta and neuronal toxicity. Cell Mol Life Sci 2012; 69: 4215-4228.
[30] de Rivero Vaccari JP, Bastien D, Yurcisin G, Pineau I, Dietrich WD, De Koninck Y, Keane RW and Lacroix S. P2X4 receptors influence inflammasome activation after spinal cord injury. J Neurosci 2012; 32: 3058-3066.
[31] Hara H, Friedlander RM, Gagliardini V, Ayata C, Fink K, Huang Z, Shimizu-Sasamata M, Yuan J and Moskowitz MA. Inhibition of interleukin 1beta converting enzyme family proteases reduces ischemic and excitotoxic neuronal damage. Proc Natl Acad Sci U S A 1997; 94: 2007-2012.
[32] Pearson VL, Rothwell NJ and Toulmond S. Excitotoxic brain damage in the rat induces interleukin-1beta protein in microglia and astrocytes: correlation with the progression of cell death. Glia 1999; 25: 311-323.
[33] Takahashi JL, Giuliani F, Power C, Imai Y and Yong VW. Interleukin-1beta promotes oligodendrocyte death through glutamate excitotoxicity. Ann Neurol 2003; 53: 588-595.
[34] Coll RC, Robertson AA, Chae JJ, Higgins SC, Munoz-Planillo R, Inserra MC, Vetter I, Dungan LS, Monks BG, Stutz A, Croker DE, Butler MS, Haneklaus M, Sutton CE, Nunez G, Latz E, Kastner DL, Mills KH, Masters SL, Schroder K, Cooper MA and O'Neill LA. A small-molecule inhibitor of the NLRP3 inflammasome for the treatment of inflammatory diseases. Nat Med 2015; 21: 248-255.
[35] Dempsey C, Rubio Araiz A, Bryson KJ, Finucane O, Larkin C, Mills EL, Robertson AA, Cooper MA, O'Neill LA and Lynch MA. Inhibiting the NLRP3 inflammasome with MCC950 promotes non-phlogistic clearance of amyloid-beta and cognitive function in APP/PS1 mice. Brain Behav Immun 2017; 61: 306-316.
[36] Bauernfeind F, Rieger A, Schildberg FA, Knolle PA, Schmid-Burgk JL and Hornung V. NLRP3 inflammasome activity is negatively controlled by miR-223. J Immunol 2012; 189: 4175-4181.
[37] Koob GF and Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology 2010; 35: 217-238.
[38] Fernandez-Lizarbe S, Pascual M and Guerri C. Critical role of TLR4 response in the activation of microglia induced by ethanol. J Immunol 2009; 183: 4733-4744.
[39] Qin L, He J, Hanes RN, Pluzarev O, Hong JS and Crews FT. Increased systemic and brain cytokine production and neuroinflammation by endotoxin following ethanol treatment. J Neuroinflammation 2008; 5: 10.
[40] He J and Crews FT. Increased MCP-1 and microglia in various regions of the human alcoholic brain. Exp Neurol 2008; 210: 349-358.
[41] Nixon K and Crews FT. Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. J Neurochem 2002; 83: 1087-1093.
[42] Pascual M, Blanco AM, Cauli O, Minarro J and Guerri C. Intermittent ethanol exposure induces inflammatory brain damage and causes long-term behavioural alterations in adolescent rats. Eur J Neurosci 2007; 25: 541-550.
[43] Chastain LG and Sarkar DK. Role of microglia in regulation of ethanol neurotoxic action. Int Rev Neurobiol 2014; 118: 81-103.
[44] Lee H, Jeong J, Son E, Mosa A, Cho GJ, Choi WS, Ha JH, Kim IK, Lee MG, Kim CY and Suk K. Ethanol selectively modulates inflammatory activation signaling of brain microglia. J Neuroimmunol 2004; 156: 88-95.
[45] Zhao YN, Wang F, Fan YX, Ping GF, Yang JY and Wu CF. Activated microglia are implicated in cognitive deficits, neuronal death, and successful recovery following intermittent ethanol exposure. Behav Brain Res 2013; 236: 270-282.
[46] Agrawal RG, Hewetson A, George CM, Syapin PJ and Bergeson SE. Minocycline reduces ethanol drinking. Brain Behav Immun 2011; 25 Suppl 1: S165-169.
[47] McIver SR, Muccigrosso MM and Haydon PG. The effect of doxycycline on alcohol consump-

tion and sensitivity: consideration for inducible transgenic mouse models. Exp Biol Med (Maywood) 2012; 237: 1129-1133.
[48] Zou J and Crews FT. Inflammasome-IL-1beta signaling mediates ethanol Inhibition of hippocampal neurogenesis. Front Neurosci 2012; 6: 77.
[49] Wu Y, Lousberg EL, Moldenhauer LM, Hayball JD, Robertson SA, Coller JK, Watkins LR, Somogyi AA and Hutchinson MR. Attenuation of microglial and IL-1 signaling protects mice from acute alcohol-induced sedation and/or motor impairment. Brain Behav Immun 2011; 25 Suppl 1: S155-164.
[50] Davis BK, Wen H and Ting JP. The inflammasome NLRs in immunity, inflammation, and associated diseases. Annu Rev Immunol 2011; 29: 707-735.
[51] Alfonso-Loeches S, Pascual-Lucas M, Blanco AM, Sanchez-Vera I and Guerri C. Pivotal role of TLR4 receptors in alcohol-induced neuroinflammation and brain damage. J Neurosci 2010; 30: 8285-8295.
[52] Pascual M, Balino P, Alfonso-Loeches S, Aragon CM and Guerri C. Impact of TLR4 on behavioral and cognitive dysfunctions associated with alcohol-induced neuroinflammatory damage. Brain Behav Immun 2011; 25 Suppl 1: S80-91.
[53] Wu Y, Lousberg EL, Moldenhauer LM, Hayball JD, Coller JK, Rice KC, Watkins LR, Somogyi AA and Hutchinson MR. Inhibiting the TLR4-MyD88 signalling cascade by genetic or pharmacological strategies reduces acute alcohol-induced sedation and motor impairment in mice. Br J Pharmacol 2012; 165: 1319-1329.
[54] Pascual M, Balino P, Aragon CM and Guerri C. Cytokines and chemokines as biomarkers of ethanol-induced neuroinflammation and anxiety-related behavior: role of TLR4 and TLR2. Neuropharmacology 2015; 89: 352-359.
[55] Wang X, Chu G, Yang Z, Sun Y, Zhou H, Li M, Shi J, Tian B, Zhang C and Meng X. Ethanol directly induced HMGB1 release through NOX2/NLRP1 inflammasome in neuronal cells. Toxicology 2015; 334: 104-110.
[56] Zou JY and Crews FT. Release of neuronal HMGB1 by ethanol through decreased HDAC activity activates brain neuroimmune signaling. PLoS One 2014; 9: e87915.
[57] Bonaldi T, Talamo F, Scaffidi P, Ferrera D, Porto A, Bachi A, Rubartelli A, Agresti A and Bianchi ME. Monocytic cells hyperacetylate chromatin protein HMGB1 to redirect it towards secretion. EMBO J 2003; 22: 5551-5560.
[58] Youn JH and Shin JS. Nucleocytoplasmic shuttling of HMGB1 is regulated by phosphorylation that redirects it toward secretion. J Immunol 2006; 177: 7889-7897.
[59] Lippai D, Bala S, Petrasek J, Csak T, Levin I, Kurt-Jones EA and Szabo G. Alcohol-induced IL-1beta in the brain is mediated by NLRP3/ASC inflammasome activation that amplifies neuroinflammation. J Leukoc Biol 2013; 94: 171-182.
[60] Willingham SB, Allen IC, Bergstralh DT, Brickey WJ, Huang MT, Taxman DJ, Duncan JA and Ting JP. NLRP3 (NALP3, Cryopyrin) facilitates in vivo caspase-1 activation, necrosis, and HMGB1 release via inflammasome-dependent and -independent pathways. J Immunol 2009; 183: 2008-2015.
[61] De Filippis L, Halikere A, McGowan H, Moore JC, Tischfield JA, Hart RP and Pang ZP. Ethanol-mediated activation of the NLRP3 inflammasome in iPS cells and iPS cells-derived neural progenitor cells. Mol Brain 2016; 9: 51.
[62] Alfonso-Loeches S, Urena-Peralta JR, Morillo-Bargues MJ, Oliver-De La Cruz J and Guerri C. Role of mitochondria ROS generation in ethanol-induced NLRP3 inflammasome activation and cell death in astroglial cells. Front Cell Neurosci 2014; 8: 216.
[63] Haorah J, Knipe B, Leibhart J, Ghorpade A and Persidsky Y. Alcohol-induced oxidative stress in brain endothelial cells causes blood-brain barrier dysfunction. J Leukoc Biol 2005; 78: 1223-1232.
[64] Haorah J, Knipe B, Gorantla S, Zheng J and Persidsky Y. Alcohol-induced blood-brain barrier dysfunction is mediated via inositol 1,4,5-triphosphate receptor (IP3R)-gated intracellular calcium release. J Neurochem 2007; 100: 324-336.
[65] Fouts DE, Torralba M, Nelson KE, Brenner DA and Schnabl B. Bacterial translocation and changes in the intestinal microbiome in mouse models of liver disease. J Hepatol 2012; 56: 1283-1292.
[66] Hartmann P, Chen WC and Schnabl B. The intestinal microbiome and the leaky gut as therapeutic targets in alcoholic liver disease. Front Physiol 2012; 3: 402.
[67] Draper LR, Gyure LA, Hall JG and Robertson D. Effect of alcohol on the integrity of the intestinal epithelium. Gut 1983; 24: 399-404.
[68] Wang HJ, Zakhari S and Jung MK. Alcohol, inflammation, and gut-liver-brain interactions in tissue damage and disease development. World J Gastroenterol 2010; 16: 1304-1313.
[69] Ganz M, Csak T, Nath B and Szabo G. Lipopolysaccharide induces and activates the Nalp3 inflammasome in the liver. World J Gastroenterol 2011; 17: 4772-4778.
[70] Petrasek J, Bala S, Csak T, Lippai D, Kodys K, Menashy V, Barrieau M, Min SY, Kurt-Jones EA and Szabo G. IL-1 receptor antagonist ameliorates inflammasome-dependent alcoholic ste-

atohepatitis in mice. J Clin Invest 2012; 122: 3476-3489.
[71] Hritz I, Mandrekar P, Velayudham A, Catalano D, Dolganiuc A, Kodys K, Kurt-Jones E and Szabo G. The critical role of toll-like receptor (TLR) 4 in alcoholic liver disease is independent of the common TLR adapter MyD88. Hepatology 2008; 48: 1224-1231.
[72] Szabo G, Mandrekar P, Petrasek J and Catalano D. The unfolding web of innate immune dysregulation in alcoholic liver injury. Alcohol Clin Exp Res 2011; 35: 782-786.
[73] D'Mello C and Swain MG. Immune-to-Brain Communication pathways in inflammation-associated sickness and depression. Curr Top Behav Neurosci 2017; 31: 73-94.
[74] Ang E, Chen J, Zagouras P, Magna H, Holland J, Schaeffer E and Nestler EJ. Induction of nuclear factor-kappaB in nucleus accumbens by chronic cocaine administration. J Neurochem 2001; 79: 221-224.
[75] Russo SJ, Wilkinson MB, Mazei-Robison MS, Dietz DM, Maze I, Krishnan V, Renthal W, Graham A, Birnbaum SG, Green TA, Robison B, Lesselyong A, Perrotti LI, Bolanos CA, Kumar A, Clark MS, Neumaier JF, Neve RL, Bhakar AL, Barker PA and Nestler EJ. Nuclear factor kappa B signaling regulates neuronal morphology and cocaine reward. J Neurosci 2009; 29: 3529-3537.
[76] Renthal W, Kumar A, Xiao G, Wilkinson M, Covington HE, 3rd, Maze I, Sikder D, Robison AJ, LaPlant Q, Dietz DM, Russo SJ, Vialou V, Chakravarty S, Kodadek TJ, Stack A, Kabbaj M and Nestler EJ. Genome-wide analysis of chromatin regulation by cocaine reveals a role for sirtuins. Neuron 2009; 62: 335-348.
[77] Piechota M, Korostynski M, Solecki W, Gieryk A, Slezak M, Bilecki W, Ziolkowska B, Kostrzewa E, Cymerman I, Swiech L, Jaworski J and Przewlocki R. The dissection of transcriptional modules regulated by various drugs of abuse in the mouse striatum. Genome Biol 2010; 11: R48.
[78] Blanco-Calvo E, Rivera P, Arrabal S, Vargas A, Pavon FJ, Serrano A, Castilla-Ortega E, Galeano P, Rubio L, Suarez J and Rodriguez de Fonseca F. Pharmacological blockade of either cannabinoid CB1 or CB2 receptors prevents both cocaine-induced conditioned locomotion and cocaine-induced reduction of cell proliferation in the hippocampus of adult male rat. Front Integr Neurosci 2014; 7: 106.
[79] Dietrich JB, Mangeol A, Revel MO, Burgun C, Aunis D and Zwiller J. Acute or repeated cocaine administration generates reactive oxygen species and induces antioxidant enzyme activity in dopaminergic rat brain structures. Neuropharmacology 2005; 48: 965-974.
[80] Muriach M, Lopez-Pedrajas R, Barcia JM, Sanchez-Villarejo MV, Almansa I and Romero FJ. Cocaine causes memory and learning impairments in rats: involvement of nuclear factor kappa B and oxidative stress, and prevention by topiramate. J Neurochem 2010; 114: 675-684.
[81] Uys JD, Knackstedt L, Hurt P, Tew KD, Manevich Y, Hutchens S, Townsend DM and Kalivas PW. Cocaine-induced adaptations in cellular redox balance contributes to enduring behavioral plasticity. Neuropsychopharmacology 2011; 36: 2551-2560.
[82] Guo ML, Liao K, Periyasamy P, Yang L, Cai Y, Callen SE and Buch S. Cocaine-mediated microglial activation involves the ER stress-autophagy axis. Autophagy 2015; 11: 995-1009.
[83] Costa BM, Yao H, Yang L and Buch S. Role of endoplasmic reticulum (ER) stress in cocaine-induced microglial cell death. J Neuroimmune Pharmacol 2013; 8: 705-714.
[84] Lebeaupin C, Proics E, de Bieville CH, Rousseau D, Bonnafous S, Patouraux S, Adam G, Lavallard VJ, Rovere C, Le Thuc O, Saint-Paul MC, Anty R, Schneck AS, Iannelli A, Gugenheim J, Tran A, Gual P and Bailly-Maitre B. ER stress induces NLRP3 inflammasome activation and hepatocyte death. Cell Death Dis 2015; 6: e1879.
[85] Menu P, Mayor A, Zhou R, Tardivel A, Ichijo H, Mori K and Tschopp J. ER stress activates the NLRP3 inflammasome via an UPR-independent pathway. Cell Death Dis 2012; 3: e261.
[86] Gan X, Zhang L, Berger O, Stins MF, Way D, Taub DD, Chang SL, Kim KS, House SD, Weinand M, Witte M, Graves MC and Fiala M. Cocaine enhances brain endothelial adhesion molecules and leukocyte migration. Clin Immunol 1999; 91: 68-76.
[87] Dhillon NK, Peng F, Bokhari S, Callen S, Shin SH, Zhu X, Kim KJ and Buch SJ. Cocaine-mediated alteration in tight junction protein expression and modulation of CCL2/CCR2 axis across the blood-brain barrier: implications for HIV-dementia. J Neuroimmune Pharmacol 2008; 3: 52-56.
[88] Zhang L, Looney D, Taub D, Chang SL, Way D, Witte MH, Graves MC and Fiala M. Cocaine opens the blood-brain barrier to HIV-1 invasion. J Neurovirol 1998; 4: 619-626.
[89] Walsh JG, Reinke SN, Mamik MK, McKenzie BA, Maingat F, Branton WG, Broadhurst DI and Power C. Rapid inflammasome activation in microglia contributes to brain disease in HIV/AIDS. Retrovirology 2014; 11: 35.
[90] Guo H, Gao J, Taxman DJ, Ting JP and Su L. HIV-1 infection induces interleukin-1beta production via TLR8 protein-dependent and NLRP3

inflammasome mechanisms in human monocytes. J Biol Chem 2014; 289: 21716-21726.
[91] Chivero ET, Guo ML, Periyasamy P, Liao K, Callen SE and Buch S. HIV-1 Tat primes and activates microglial NLRP3 inflammasome-mediated neuroinflammation. J Neurosci 2017; 37: 3599-3609.
[92] Atluri VS, Pilakka-Kanthikeel S, Garcia G, Jayant RD, Sagar V, Samikkannu T, Yndart A and Nair M. Effect of cocaine on HIV infection and inflammasome gene expression profile in HIV infected macrophages. Sci Rep 2016; 6: 27864.
[93] Seiden LS and Sabol KE. Methamphetamine and methylenedioxymethamphetamine neurotoxicity: possible mechanisms of cell destruction. NIDA Res Monogr 1996; 163: 251-276.
[94] Volkow ND, Chang L, Wang GJ, Fowler JS, Leonido-Yee M, Franceschi D, Sedler MJ, Gatley SJ, Hitzemann R, Ding YS, Logan J, Wong C and Miller EN. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. Am J Psychiatry 2001; 158: 377-382.
[95] Schwendt M, Rocha A, See RE, Pacchioni AM, McGinty JF and Kalivas PW. Extended methamphetamine self-administration in rats results in a selective reduction of dopamine transporter levels in the prefrontal cortex and dorsal striatum not accompanied by marked monoaminergic depletion. J Pharmacol Exp Ther 2009; 331: 555-562.
[96] Bowyer JF, Robinson B, Ali S and Schmued LC. Neurotoxic-related changes in tyrosine hydroxylase, microglia, myelin, and the blood-brain barrier in the caudate-putamen from acute methamphetamine exposure. Synapse 2008; 62: 193-204.
[97] Sekine Y, Ouchi Y, Sugihara G, Takei N, Yoshikawa E, Nakamura K, Iwata Y, Tsuchiya KJ, Suda S, Suzuki K, Kawai M, Takebayashi K, Yamamoto S, Matsuzaki H, Ueki T, Mori N, Gold MS and Cadet JL. Methamphetamine causes microglial activation in the brains of human abusers. J Neurosci 2008; 28: 5756-5761.
[98] Frank MG, Adhikary S, Sobesky JL, Weber MD, Watkins LR and Maier SF. The danger-associated molecular pattern HMGB1 mediates the neuroinflammatory effects of methamphetamine. Brain Behav Immun 2016; 51: 99-108.
[99] Weber MD, Frank MG, Tracey KJ, Watkins LR and Maier SF. Stress induces the danger-associated molecular pattern HMGB-1 in the hippocampus of male sprague dawley rats: a priming stimulus of microglia and the NLRP3 inflammasome. J Neurosci 2015; 35: 316-324.
[100] Tian C, Murrin LC and Zheng JC. Mitochondrial fragmentation is involved in methamphetamine-induced cell death in rat hippocampal neural progenitor cells. PLoS One 2009; 4: e5546.
[101] Mashayekhi V, Eskandari MR, Kobarfard F, Khajeamiri A and Hosseini MJ. Induction of mitochondrial permeability transition (MPT) pore opening and ROS formation as a mechanism for methamphetamine-induced mitochondrial toxicity. Naunyn Schmiedebergs Arch Pharmacol 2014; 387: 47-58.
[102] Brown JM and Yamamoto BK. Effects of amphetamines on mitochondrial function: role of free radicals and oxidative stress. Pharmacol Ther 2003; 99: 45-53.
[103] Brown JM, Quinton MS and Yamamoto BK. Methamphetamine-induced inhibition of mitochondrial complex II: roles of glutamate and peroxynitrite. J Neurochem 2005; 95: 429-436.
[104] Zhou R, Yazdi AS, Menu P and Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature 2011; 469: 221-225.
[105] Hagel JM and Facchini PJ. Dioxygenases catalyze the O-demethylation steps of morphine biosynthesis in opium poppy. Nat Chem Biol 2010; 6: 273-275.
[106] Organization WH. The selection and use of essential medicines: report of the WHO expert committee, 2015 (including the 19th WHO model list of essential medicines and the 5th WHO model list of essential medicines for children). World Health Organization 2016.
[107] Martin WR and Fraser HF. A comparative study of physiological and subjective effects of heroin and morphine administered intravenously in postaddicts. J Pharmacol Exp Ther 1961; 133: 388-399.
[108] Tompkins DA, Bigelow GE, Harrison JA, Johnson RE, Fudala PJ and Strain EC. Concurrent validation of the clinical opiate withdrawal scale (COWS) and single-item indices against the clinical institute narcotic assessment (CINA) opioid withdrawal instrument. Drug Alcohol Depend 2009; 105: 154-159.
[109] Wesson DR and Ling W. The clinical opiate withdrawal scale (COWS). J Psychoactive Drugs 2003; 35: 253-259.
[110] Nestler EJ. Under siege: the brain on opiates. Neuron 1996; 16: 897-900.
[111] Bohn LM, Gainetdinov RR, Lin FT, Lefkowitz RJ and Caron MG. Mu-opioid receptor desensitization by beta-arrestin-2 determines morphine tolerance but not dependence. Nature 2000; 408: 720-723.
[112] Appleyard SM, Celver J, Pineda V, Kovoor A, Wayman GA and Chavkin C. Agonist-dependent desensitization of the kappa opioid receptor by G protein receptor kinase and beta-arrestin. J Biol Chem 1999; 274: 23802-23807.
[113] Mao J, Sung B, Ji RR and Lim G. Chronic morphine induces downregulation of spinal glutamate transporters: implications in morphine tolerance and abnormal pain sensitivity. J Neurosci 2002; 22: 8312-8323.

[114] Mao J. Opioid tolerance and neuroplasticity. Novartis Found Symp 2004; 261: 181-186; discussion 187-193.
[115] Raghavendra V, Rutkowski MD and DeLeo JA. The role of spinal neuroimmune activation in morphine tolerance/hyperalgesia in neuropathic and sham-operated rats. J Neurosci 2002; 22: 9980-9989.
[116] Vallejo R, de Leon-Casasola O and Benyamin R. Opioid therapy and immunosuppression: a review. Am J Ther 2004; 11: 354-365.
[117] Wei G, Moss J and Yuan CS. Opioid-induced immunosuppression: is it centrally mediated or peripherally mediated? Biochem Pharmacol 2003; 65: 1761-1766.
[118] Odunayo A, Dodam JR, Kerl ME and DeClue AE. Immunomodulatory effects of opioids. J Vet Emerg Crit Care (San Antonio) 2010; 20: 376-385.
[119] Hilburger ME, Adler MW, Truant AL, Meissler JJ Jr, Satishchandran V, Rogers TJ and Eisenstein TK. Morphine induces sepsis in mice. J Infect Dis 1997; 176: 183-188.
[120] Mellon RD and Bayer BM. Evidence for central opioid receptors in the immunomodulatory effects of morphine: review of potential mechanism(s) of action. J Neuroimmunol 1998; 83: 19-28.
[121] Frenklakh L, Bhat RS, Bhaskaran M, Sharma S, Sharma M, Dinda A and Singhal PC. Morphine-induced degradation of the host defense barrier role of intestinal mucosal injury. Dig Dis Sci 2006; 51: 318-325.
[122] Ocasio FM, Jiang Y, House SD and Chang SL. Chronic morphine accelerates the progression of lipopolysaccharide-induced sepsis to septic shock. J Neuroimmunol 2004; 149: 90-100.
[123] Wang X, Loram LC, Ramos K, de Jesus AJ, Thomas J, Cheng K, Reddy A, Somogyi AA, Hutchinson MR, Watkins LR and Yin H. Morphine activates neuroinflammation in a manner parallel to endotoxin. Proc Natl Acad Sci U S A 2012; 109: 6325-6330.
[124] Ferrini F, Trang T, Mattioli TA, Laffray S, Del'Guidice T, Lorenzo LE, Castonguay A, Doyon N, Zhang W, Godin AG, Mohr D, Beggs S, Vandal K, Beaulieu JM, Cahill CM, Salter MW and De Koninck Y. Morphine hyperalgesia gated through microglia-mediated disruption of neuronal Cl(-) homeostasis. Nat Neurosci 2013; 16: 183-192.
[125] Matsushita Y, Omotuyi IO, Mukae T and Ueda H. Microglia activation precedes the anti-opioid BDNF and NMDA receptor mechanisms underlying morphine analgesic tolerance. Curr Pharm Des 2013; 19: 7355-7361.
[126] Kawasaki Y, Zhang L, Cheng JK and Ji RR. Cytokine mechanisms of central sensitization: distinct and overlapping role of interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in regulating synaptic and neuronal activity in the superficial spinal cord. J Neurosci 2008; 28: 5189-5194.
[127] Zhang L, Berta T, Xu ZZ, Liu T, Park JY and Ji RR. TNF-alpha contributes to spinal cord synaptic plasticity and inflammatory pain: distinct role of TNF receptor subtypes 1 and 2. Pain 2011; 152: 419-427.
[128] Eidson LN and Murphy AZ. Blockade of Toll-like receptor 4 attenuates morphine tolerance and facilitates the pain relieving properties of morphine. J Neurosci 2013; 33: 15952-15963.
[129] Hutchinson MR, Zhang Y, Shridhar M, Evans JH, Buchanan MM, Zhao TX, Slivka PF, Coats BD, Rezvani N, Wieseler J, Hughes TS, Landgraf KE, Chan S, Fong S, Phipps S, Falke JJ, Leinwand LA, Maier SF, Yin H, Rice KC and Watkins LR. Evidence that opioids may have toll-like receptor 4 and MD-2 effects. Brain Behav Immun 2010; 24: 83-95.
[130] Jiang C, Xu L, Chen L, Han Y, Tang J, Yang Y, Zhang G and Liu W. Selective suppression of microglial activation by paeoniflorin attenuates morphine tolerance. Eur J Pain 2015; 19: 908-919.
[131] Mika J, Wawrzczak-Bargiela A, Osikowicz M, Makuch W and Przewlocka B. Attenuation of morphine tolerance by minocycline and pentoxifylline in naive and neuropathic mice. Brain Behav Immun 2009; 23: 75-84.
[132] Johnston IN, Milligan ED, Wieseler-Frank J, Frank MG, Zapata V, Campisi J, Langer S, Martin D, Green P, Fleshner M, Leinwand L, Maier SF and Watkins LR. A role for proinflammatory cytokines and fractalkine in analgesia, tolerance, and subsequent pain facilitation induced by chronic intrathecal morphine. J Neurosci 2004; 24: 7353-7365.
[133] Shavit Y, Wolf G, Goshen I, Livshits D and Yirmiya R. Interleukin-1 antagonizes morphine analgesia and underlies morphine tolerance. Pain 2005; 115: 50-59.
[134] Liang Y, Chu H, Jiang Y and Yuan L. Morphine enhances IL-1beta release through toll-like receptor 4-mediated endocytic pathway in microglia. Purinergic Signal 2016; 12: 637-645.
[135] Cai Y, Kong H, Pan YB, Jiang L, Pan XX, Hu L, Qian YN, Jiang CY and Liu WT. Procyanidins alleviates morphine tolerance by inhibiting activation of NLRP3 inflammasome in microglia. J Neuroinflammation 2016; 13: 53.
[136] Grace PM, Strand KA, Galer EL, Urban DJ, Wang X, Baratta MV, Fabisiak TJ, Anderson ND, Cheng K, Greene LI, Berkelhammer D, Zhang Y, Ellis AL, Yin HH, Campeau S, Rice KC, Roth BL, Maier SF and Watkins LR. Morphine paradoxically prolongs neuropathic pain in rats by amplifying spinal NLRP3 inflammasome activation. Proc Natl Acad Sci U S A 2016; 113: E3441-3450.