HHS Public Access

Author manuscript

Brain Behav Immun. Author manuscript; available in PMC 2017 October 24.

Published in final edited form as:

Brain Behav Immun. 2011 June ; 25(Suppl 1): S21–S28. doi:10.1016/j.bbi.2011.01.005.

Stress- and glucocorticoid-induced priming of neuroinflammatory responses: Potential mechanisms of stress-induced vulnerability to drugs of abuse

Matthew G. Frank[*], **Linda R. Watkins**, and **Steven F. Maier**

Department of Psychology and Neuroscience, Center for Neuroscience, University of Colorado, Boulder, CO, USA

Abstract

Stress and stress-induced glucocorticoids (GCs) sensitize drug abuse behavior as well as the neuroinflamatory response to a subsequent pro-inflammatory challenge. Stress also predisposes or sensitizes individuals to develop substance abuse. There is an emerging evidence that glia and glia-derived neuroinflammatory mediators play key roles in the development of drug abuse. Drugs of abuse such as opioids, psychostimulants, and alcohol induce neuroinflammatory mediators such as pro-inflammatory cytokines (e.g. interleukin (IL)-1β), which modulate drug reward, dependence, and tolerance as well as analgesic properties. Drugs of abuse may directly activate microglial and astroglial cells via ligation of Toll-like receptors (TLRs), which mediate the innate immune response to pathogens as well as xenobiotic agents (e.g. drugs of abuse). The present review focuses on understanding the immunologic mechanism(s) whereby stress primes or sensitizes the neuroinflammatory response to drugs of abuse and explores whether stress- and GC-induced sensitization of neuroimmune processes predisposes individuals to drug abuse liability and the role of neuroinflammatory mediators in the development of drug addiction.

Keywords

Stress; Glucocorticoid; Neuroinflammation; Cytokine; Addiction

1. Introduction

Our charge was to discuss whether neuroimmune processes might play a role in the potentiation of drug reward and drug seeking produced by stressors. Stress and stress-induced glucocorticoids (GCs) sensitize drug abuse behavior as well as the neuroinflammatory response to subsequent pro-inflammatory challenges. The central notion to be explored here is that experience of life stressors may induce a vulnerable phenotype characterized, in part, by a neuroimmune microenvironment sensitized to pro-inflammatory stimuli. We will develop the thesis that drugs of abuse (e.g. methamphetamine, morphine and alcohol) may function, in part, as pro-inflammatory stimuli or danger signals to induce neuroinflammatory responses. Therefore, upon exposure to drugs of abuse, a

[*]Corresponding author. Address: Department of Psychology and Neuroscience, Center for Neuroscience, Campus Box 345, University of Colorado at Boulder, Boulder, CO 80309-0345, USA. Fax: +1 303 492 2967. matt.frank@colorado.edu (M.G. Frank).

neuroinflammatory cascade ensues, which facilitates drug reward, dependence, and tolerance, as well as modulation of drug-induced analgesia. A central question to be explored here is whether exposure to stressors sensitizes the neuroinflammatory response to drugs of abuse, thereby potentiating the drug-related phenomena noted above. We will present converging evidence that stress-induced sensitization of neuroinflammatory processes may be a key mechanism(s) by which stress predisposes individuals to developing drug abuse. Understanding the central mechanisms by which stress sensitizes ("primes") the neuroinflammatory response to drugs of abuse may be the key to understand how stress can promote the development of addiction, and as such is the focus of the present review. Clearly, there is an extensive literature that elaborates upon the effects of stress on peripheral immune function. However, the scope of the present review will be restricted to stress-induced modulation of central nervous system (CNS) innate immunity and its potential role in the development of drug addiction.

2. CNS innate immunity, inflammation and microglia

CNS innate immunity and its primary immune effector cell, microglia, are the key immunologic substrates for understanding how stress and GCs potentiate neuroinflammatory responses to drugs of abuse. Therefore, a brief orientation on microglia will be provided.

Innate immunity is the first line of defense against infection. Within the CNS, microglia, as part of the myeloid lineage of monouclear phagocytes, constitute the predominant innate immune cell in the brain parenchyma and serve many functions including immunosurveillance of the brain microenvironment for pathogen invasion, cellular debris, apoptotic cells, and alterations in neuroal phenotype (Ransohoff and Cardona, 2010). It is important to note that other mononuclear phagocytes including meningeal, choroid plexus, and perivascular macrophages, which reside outside the brain parenchyma, are also myeloid in origin. These macrophage subtypes also serve a critical role in the brain's innate immune response (Schiltz and Sawchenko, 2003) and may potentially contribute to the processes under discussion, but will not be further addressed here. In the normal healthy CNS, microglia send out processes that sample the local environment at a rate of several times per second (Nimmerjahn et al., 2005) and have been termed "surveillant" microglia (Ransohoff and Cardona, 2010). If a microorganism or danger signal (see below) is encountered, the cell undergoes rapid morphological and functional changes that include the synthesis and secretion of inflammatory mediators including pro-inflammatory cytokines (PICs; e.g. interleukin-1beta, IL-1β), chemokines, nitric oxide (NO), prostaglandins (PG), and reactive oxygen species (ROS). This response induces neuroinflamation. Microglial function is complex, and microglia cannot be regarded as being in only inactive or activated states. It is common to consider whether these cells are activated "classically" (via Toll-like receptors and interferon (IFN)-gamma) or "alternatively" (via IL-4 or IL-13), each of which produces cells with quite different properties. However, recent views (Mosser and Edwards, 2008) suggest that macrophages and microglia can enter a spectrum of activation states, thereby producing varying blends of pro- and anti-inflammatory products depending on how they are further stimulated. Of particular relevance to the present focus, these cells can enter a state that can be called "sensitized" or "primed" (Perry, 2004). Here, macrophages undergo a morphological transformation from "ramified" to "activated", which is characterized by an

enlargement of the soma, a shortening of processes and cell surface up-regulation of myeloid markers (e.g. major histocompatibility complex II; MHCII). Though “activated”, these “sensitized” macrophages do not produce inflammatory or anti-inflammatory products but, if further stimulated, produce exaggerated levels of inflammatory products.

A note on our use of the term inflammation is likely in order. Neuroinflammation involves a number of processes such as infiltration of the CNS by peripheral immune cells, including neutrophils, monocytes, dendritic cells, and various sub-classes of T-cells. However, these processes are generally under the control of inter-cellular signaling molecules including PICs, chemokines, etc. It is common to refer to an increase in these signaling molecules as inflammatory, and that is what is intended here.

At least 50 cell surface antigens distinguish mononuclear phagocytes from other cell types (Ransohoff and Cardona, 2010). Of these, Toll-like receptors (TLRs) are of particular relevance here. First, microglia express many of the TLRs characterized in rodents (Carpentier et al., 2008) (13 in all). Second, stress and GCs up-regulate expression of TLRs (Frank et al., 2010). Lastly, drugs of abuse signal through TLRs (see below for discussion). Briefly, cells of the innate immune system recognize microbial products via a relatively small number (roughly 30) of germ line-encoded receptors that recognize general molecular patterns that characterize different classes of pathogens (Pathogen Associated Molecular Patterns, PAMPs; Janeway and Medzhitov, 2002). For this reason, receptors that recognize PAMPs have been called pattern recognition receptors (PRRs). Of these PRRs, TLRs have been the most extensively characterized (Barton and Kagan, 2009). TLRs are a family of highly conserved membrane or cytosolic (dependent on TLR subtype) proteins that transduce signals through a family of cytosolic Toll adapter proteins that link to downstream signaling cascades (Barton and Kagan, 2009). The ligation of many of the TLR family members ultimately activates NF-κB as well as other transcription factors (Kawai and Akira, 2007), and NF-κB activation is viewed as key to the inflammatory effects of TLR ligation (Salminen et al., 2008). TLR2 and TLR4 have been the most intensively studied PRRs within the CNS, and are densely expressed on microglial cells (Aravalli et al., 2007). They are sometimes reported to be present on astrocytes and sometimes to be absent, and are typically reported to be absent on neurons (Lehnardt et al., 2003). TLR4 recognizes the lipopolysaccharide (LPS) motif that is present in the cell membrane of all Gramegative bacteria, while TLR2 recognizes lipoteichoic acid that characterizes Gram-positive bacteria (Kawai and Akira, 2007). Interestingly, TLR signaling, in particular TLR2 and TLR4, has been co-opted by endogenous signaling molecules which are thought to alert microglia to a variety of internal conditions such as cellular stress, damage or death (Kawai and Akira, 2010). The PRRs on innate immune cells have typically been thought to function to allow discrimination of “self” from “non-self”. However, because endogenous molecules can activate TLR signaling, this view has shifted in recent years to one in which TLRs discriminate “danger” from “non-danger” (Bianchi, 2007). These endogenous molecules are capable of acting like PAMPs, activating innate immune cells via ligation of TLRs and producing inflammatory responses. In 2006, Joost Oppenheim coined the term “alarmins” to represent these substances. Putative alarmins include high mobility group box 1 (HMGB1), S100 proteins, heat shock proteins (HSPs), and hyaluronan (Bianchi, 2007), with HMGB1 having received the most attention to date.

To be developed in detail below, TLR signaling may be co-opted by drugs of abuse, which raises the interesting notion that drugs of abuse act as xenobiotic agents (i.e. a substance which is found in an organism, but which is not normally produced or expected to be present in it) and may be sensed as danger signals, thereby eliciting a pro-inflammatory response.

3. Stress and stress-induced GCs sensitize neuroinflammatory responses

3.1. Stress

Exposure to stressors and pro-inflammatory agents induces a similar profile of pro-inflammatory "cytokine signatures" in the CNS (Maier, 2003). Stressors can induce two very different types of inflammatory responses in the brain. The first is a rapid, short duration (several hours) increase in inflammatory mediators (Nguyen et al., 1998). The second is a slower developing, longer lasting (days) sensitization (or "priming") of neuroinflammatory responses to subsequently occurring infectious/pathogenic stimuli or stressors (i.e. delayed challenge) (Johnson et al., 2003). These two inflammatory sequelae of stress are very likely produced by different mechanisms (see below). The focus here is on stressor-induced inflammatory priming to a subsequent challenge (i.e. drugs of abuse) that is delayed in time relative to the initial stressor. This is the focus because inflammatory priming may have especially important implications for understanding how stress predisposes individuals to developing addiction to drugs of abuse. Importantly, the mechanism(s) of stress-induced inflammatory priming is almost entirely unknown.

The acute neuroinflammatory responses to stressors are induced rapidly and are short-lived. Brain levels of pro-inflammatory cytokines are already elevated immediately after a moderate duration (~2 h) of stress (O'Connor et al., 2003) and persist for 4–6 h. Indeed, a considerable literature shows that several different kinds of stressors induce pro-inflammatory cytokines in the CNS (Anisman, 2009; Hennessy et al., 2009; Minami et al., 1991; Shintani et al., 1995). These cytokines are very likely microglial in origin. The mechanism(s) by which stressors activate microglia and lead to the production of inflammatory molecules is not well understood, but involves CNS noradrenergic processes (Johnson et al., 2005). Importantly, it is quite clear that GCs are *not* involved in the initiation of this immediate short-term inflammatory response to stress as adrenalectomy *potentiates* the response (Nguyen et al., 1998) and GC injection before the stressor *inhibits* it (Nguyen et al., 2000). That is, stressors induce inflammation *despite* elevations in GCs, not because of the elevations. These same points can be made with regard to the immediate neuroinflammatory response to immune activators such as LPS (Goujon et al., 1996).

While stress-induced brain cytokine increases are transient and short-lived, the neuroimmune microenvironment displays a gradual increase in glial activation markers post-stress, which may underlie the sensitized neuroinflammatory response that occurs to a subsequent pro-inflammatory challenge, such as drugs of abuse. For example, our laboratory has shown that the microglial activation marker MHCII is up-regulated, and the neuronal glycoprotein CD200 (which constitutively constrains microglial activation, see below) is down-regulated 24 h post-stress (Frank et al., 2007). The CNS parenchyma shows very low levels of basal MHCII (Ransohoff and Perry, 2009) as well as high-level expression of CD200 (Barclay et al., 2002), indicative of the quiescent immunophenotype of the normal

brain microenvironment. Therefore, up-regulation and down-regulation of MHCII and CD200, respectively, indicates a stress-induced shift in the activation state of the neuroimmune microenvironment. CD200 functions to inhibit microglia and other CNS macrophages through the CD200 receptor expressed predominately on brain macrophages/microglia (Barclay et al., 2002; Koning et al., 2009), thereby constraining the onset of pro-inflammatory processes. It should be noted that pro-inflammatory cytokines have returned to basal levels by 24 h after stress. However, we have demonstrated that stress potentiates the neuroinflammatory response to a pro-inflammatory challenge (peripheral LPS) occurring 1–4 days post-stress (Frank et al., 2007; Johnson et al., 2003). Further, we found that hippocampal microglia rapidly isolated 24 h post-stress exhibited up-regulated MHCII expression and potentiated pro-inflammatory responses to LPS *in vitro,* indicating that the prior stressor had sensitized microglia to a later pro-inflammatory stimulus (Frank et al., 2007).

Taken together, the studies conducted in our laboratory demonstrate that exposure to an acute stressor shifts the neuroimmune microenvironment toward a microglial activation state that predisposes the CNS to a heightened pro-inflammatory response if exposed to a subsequent pro-inflammatory challenge. However, delayed stress-induced activation of the neuroimmune microenvironment *does not* include tonic induction of increased levels of pro-inflammatory cytokines; rather, increased pro-inflammatory responses are not observed *until* a subsequent challenge occurs. Of note, GC levels have returned to basal levels 24 h post-stress. In fact, we have found that serum GC levels rise rapidly during the initial stress and return to baseline within 2–6 h after the stressor used in the above studies (Fleshner et al., 1995). Consistent with the results summarized above, other studies have also shown that exposure to stressors increases the immune activation state of the CNS. For example, Nair has shown that chronic restraint stress increases microglia proliferation as well as microglia activation (Nair and Bonneau, 2006). Further, blockade of the GC receptor with the GC receptor antagonist RU486 abolished stress-induced microglia activation, and exogenous GC administration mimicked the effect of stress on microglia proliferation. Likewise, a regimen of chronic stressors administered for 9 days resulted in microglia activation (as reflected by increased MHCII expression) and potentiated the neuroinflammatory response (IL-1β, tumor necrosis factor (TNF)α, nitric oxide synthase (NOS), inducible (i)NOS and p38 MAPK) to intra-hippocampal LPS (Espinosa-Oliva et al., 2011). Similar to our findings (Frank et al., 2007), chronic stress *did not* by itself tonically increase pro-inflammatory cytokine levels, although the activation state of microglia had been shifted toward a *preferential* pro-inflammatory immunophenotype. Consistent with the results of Nair and Bonneau, (2006), blockade of the GC receptor with the GC receptor antagonist RU486 abolished stress-induced potentiation of the microglia activation (reflected by MHCII) and the pro-inflammatory response to LPS. Similarly, Munhoz subjected rats to a 14-day chronic mild stress regimen (Munhoz et al., 2006). Twenty-four hours after the last stressor, LPS was administered peripherally and, 2 h later, various measures of inflammation were examined in brain. Prior chronic stress potentiated the LPS-induced hippocampal and prefrontal cortex increase in IL-1β, TNFα, and iNOS mRNA, as well as NF-κB activity. Importantly, this potentiation was shown to be GC mediated (i.e. blocked by RU486). In a similar study, de Pablos exposed mice to a 9-day regimen of chronic variable stress, with LPS injected

intracortically 24 h after the last restraint. The stressor regimen facilitated LPS-induced microglial activation (MHCII expression) and cytokine expression; such effects were blocked by daily RU486 (de Pablos et al., 2006). However, in these chronic stress experiments, the number of days of stress was not manipulated, so it is possible that a single session of stress (followed by a 24 h delay before LPS) would have produced the same outcomes. It is important to note that although our laboratory uses a single session of *acute* stress (1.5 h of unpredictable shock) to sensitize the neuroimmune microenvironment, the results obtained from our laboratory (summarized above) are remarkably similar to the results obtained by other laboratories using *chronic* stressors.

3.2. Glucocorticoids

There has been tremendous interest in the effects of stress and GCs on inflammation since the pioneering reports by Selye (1950) that “stress” produces thymic involution and subsequent findings that “stress” seemed to suppress various aspects of immune function (Selye, 1955). The subsequent discoveries of the GC hormones, and their role in mediating many of the effects of stressors, led naturally to the view that GCs are immunosuppressive in general and anti-inflammatory in particular (Boumpas et al., 1993). An enormous literature has examined the effects of GCs on inflammatory responses. The general findings have shown that elevated levels of GCs suppress a variety of functions (cytokine, chemokine, NO, PG, ROS production) of innate immune cells as well as inflammatory responses to infection and injury. Although a variety of mechanisms have been implicated, it is agreed that GCs inhibit both NF-κB and AP-1 mediated gene transcription (Smoak and Cidlowski, 2004). Multiple mechanisms are involved. For example, activated glucocorticoid receptors (GRs) induce IkBalpha expression, preventing NF-κB translocation to the nucleus (Aljada et al., 1999) and also block NF-κB binding activity via interactions with the NF-κB p65 subunit (McKay and Cidlowski, 2000). Of course, these transcription factors, particularly NF-κB, are the key regulators of inflammatory genes (Solt and May, 2008).

It should be noted that a categorically anti-inflammatory effect for GCs is somewhat counter-intuitive, and does not fit with other GC actions. It is unclear why it would be adaptive for an innate immune function to be reduced during fight/flight emergencies, as these are periods of enhanced probabilities of infection. There is little reproductive value to an injured animal that escapes a predator only to die of infection. Because the idea that immune function would be reduced during an acute stressor is so obviously non-intuitive, several explanations have been offered to support why this might be rational (Munck et al., 1984). However, the basic idea that GCs are always, canonically, immunosuppressive has rarely been challenged.

Over the years, a number of papers have reported either no effect or even pro-inflammatory effects of GCs (Sapolsky, 1999), but these have largely been ignored. However, this issue has recently been re-examined in a review by Sorrells and Sapolsky (2007), with the authors concluding that GCs are indeed sometimes either without effect or strongly pro-inflammatory. Their review has elicited a great deal of controversy (e.g. see comment, Miller, 2007) because the conclusion that GCs are anti-inflammatory is strongly entrenched, and the issue is of great importance both theoretically and clinically. Clearly, if GCs and

stress are not always anti-inflammatory, then the critical issues are (a) the conditions that determine when GCs are anti- and when pro-inflammatory, and (b) the mechanisms involved. Sorrels and Sapolsky concluded that pro-inflammatory effects have been most often observed when neuroinflammatory responses, rather than peripheral innate immune responses have been examined, with dose, duration of GC increase, and timing being important variables.

We recently conducted an investigation into whether the timing of GC administration relative to a pro-inflammatory challenge was an important factor in GC-induced sensitization of neuroinflammatory responses (Frank et al., 2010). A single dose of GC was administered 24 h before, 2 h before, and 1 h after an ip injection of LPS. Of note, the dose of GCs used results in blood levels of GCs similar to those observed during and after a severe acute stressor (Fleshner et al., 1995). Several mediators of neuroinflammation were measured 4 h after LPS. GCs given 1 h *after* LPS reduced the neuroinflammatory response. However, GCs given either 2 h or 24 h *before* LPS potentiated the neuroinflammatory response to LPS. The potentiation at 2 h occurred despite the fact that blood levels of GCs were still elevated by the injection at this time. Importantly, exogenous GCs increased central expression of TLR2 and potentiated the LPS induction of TLR4 24 h post-GC administration. These effects of GCs on TLR2 and TLR4 are consistent with a small literature showing that GCs up-regulate TLRs on various peripheral cell types (Galon et al., 2002; Hermoso et al., 2004; Rozkova et al., 2006; Sakai et al., 2004; Shibata et al., 2009).

To further determine whether microglia mediated, at least in part, the GC-induced sensitization effects observed *in vivo,* rats were administered GCs *in vivo* and 24 h later hippocampal microglia were isolated and exposed to varying concentrations of LPS *in vitro. In vivo* GC treatment potentiated the PIC (IL-1β, TNFα) response to LPS *ex vivo* in a dose dependent manner. This experiment provided compelling evidence that GCs sensitize microglia to a pro-inflammatory challenge. A recent study also showed that chronic administration of GCs potentiated LPS-induced pro-inflammatory signaling in the CNS (Munhoz et al., 2010). In this study, exogenous GCs were administered to elevate GC to levels observed with mild, moderate, and major stressors. Prior exposure to GCs potentiated the central PIC response to LPS as well as intracellular signaling cascades (NF-κB, MAP kinases).

As a summary, these experiments show that stress and stress-induced GCs sensitize CNS innate immune responses to pro-inflammatory stimuli. In light of evidence that drugs of abuse exhibit pro-inflammatory properties and activate TLRs (see below), stress- and GC-induced sensitization of neuroinflammatory responses to drugs of abuse may be involved in driving the neuroadaptations underlying drug abuse liability. It is important to note that stress- and GC-induced sensitization of the neuroinflammatory response to drugs of abuse has not been investigated.

4. Drugs of abuse and neuroinflammation

An emerging body of evidence suggests that activated glia and glia-derived neuroinflammatory mediators play an important role in drug reward, dependence, tolerance and analgesic properties (Hutchinson et al., 2007).

4.1. Morphine

Several lines of evidence indicate that drugs of abuse activate glial cells. Song and Zhao found that chronic morphine activated glia in both the spinal cord and brain (Song and Zhao, 2001). Interestingly, inhibition of glial activation facilitated the analgesic efficacy of morphine. Similarly, chronic morphine activated glia and up-regulated the expression of pro-inflammatory cytokines (i.e. IL-1β, IL-6, and TNFα) (Raghavendra et al., 2004). Inhibition of pro-inflammatory cytokines reversed the development of morphine tolerance and withdrawal-induced hyperalgesia. Consistent with these findings, glial inhibition potentiated morphine analgesia and attenuated morphine tolerance (Mika et al., 2009). Studies from our laboratory have also demonstrated that chronic morphine induces pro-inflammatory cytokines. In addition, blockade of morphine-induced IL-1β signaling potentiated morphine analgesia and prevented the development of hyperalgesia and morphine tolerance (Johnston et al., 2004). Further, we found that chronic morphine as well as methadone increased the expression of TLR4 (see below for discussion of TLRs) and increased the expression of numerous pro-inflammatory cytokines and chemokines (Hutchinson et al., 2008). Inhibition of morphine-induced pro-inflammatory cytokines also increased morphine analgesia and attenuated the induction of morphine tolerance (Hutchinson et al., 2008). Importantly, we have also shown that morphine and other clinically relevant opioids (oxycodone and methadone) function as TLR4 agonists using TLR4 transfected human embryonic kidney (HEK-293) cells (Hutchinson et al., 2007). Moreover, a small molecule glial inhibitor (Ibudilast) that readily crosses the blood–brain barrier attenuated morphine-induced pro-inflammatory cytokine and chemokine expression as well as glial activation markers (Hutchinson et al., 2009), and was later found by our group to be a TLR4 antagonist as well (Hutchinson et al., 2010b). Ibudilast also reduced morphine withdrawal behaviors while potentiating morphine's analgesic properties (Hutchinson et al., 2009) Importantly, in line with such observations, we demonstrated that TLR4 knock out mice exhibit enhanced morphine analgesia and attenuated development of morphine tolerance (Hutchinson et al., 2010b).

An intriguing, and potentially clinically relevant, extension of the work with morphine arises when morphine metabolites are considered. From our work with in silico (computer) modeling of opioid interactions with the TLR4–MD2 co-receptor pair (Hutchinson et al., 2010a), we discovered that one of the two morphine metabolites had strong attraction to the same binding pocket on MD2 as does morphine. This dissociation of metabolite characteristics was very interesting as the metabolite that had no attraction to TLR4–MD2 was morphine-6-glucuronide (M6G), which binds opioid receptors avidly and produces potent analgesia (Abbott and Palmour, 1988). In contrast, M3G has been quite a mysterious metabolite, as it is a very long-lived metabolite known to fail to bind classical opioid receptors and to produce potent pain enhancement by as-yet-to-be-discovered mechanisms

(Bartlett et al., 1994). This profile of long-lived presence plus potent pain enhancement has gained focus on M3G as an attenuator of the pain-relieving effects of its parent compound, morphine, and an important contributor to the development of morphine tolerance (Mazoit et al., 2007). What our group has discovered is that the strong attraction of M3G to the key TLR4–MD2 binding pocket is paralleled by: (a) activation of TLR4 receptor signaling, blocked by LPS-RS (LPS derived from *Rhodobacter sphaeroides*), a classic TLR4 antagonist, (b) stimulation by M3G of IL-1β production in microglial cells, and (c) amplification of pain which is blocked by the TLR4 antagonist (+)-naloxone, the microglial inhibitor minocycline, and IL-1 receptor antagonist (Lewis et al., 2010). Taken together, these data suggest that metabolites of drugs of abuse bear careful consideration, in addition to the parent molecules, for exploring how glial activation may contribute to the effects observed.

Opioid-induced glial activation also plays a role in morphine dependence and withdrawal behaviors (Hutchinson et al., 2007) as well as opioid reward (Bland et al., 2009; Hutchinson et al., 2008). Intriguingly, TLR4 has been implicated in both the behavioral and neurochemical sequelae of morphine reward (Northcutt et al., 2010). Similarly, Narita found that inhibition of glial activation decreased the rewarding properties of morphine (Narita et al., 2006). They also documented that astrocyte conditioned media enhanced morphine reward. Although there are a variety of ways in which glial activation and the consequent release of inflammatory mediators could play a role in drug-related processes, it may be especially important that IL-1β can down-regulate the glutamate transporter GLT-1 (Tilleux and Hermans, 2007), thereby increasing glutamatergic neurotransmission. If this were to occur in the ventral tegmental area (VTA) or nucleus accumbens (NAcc), the dopaminergic neurotransmission that is critical to drug reward would be potentiated. Indeed, Narita et al. (2006) found down-regulation of glial glutamate transporters to be implicated in the glial-induced modulation of drug reward, and we have shown that the glial inhibitor, Ibudilast and the TLR4 antagonist (+)-naloxone each attenuate morphine-induced dopamine efflux in the nucleus accumbens (Bland et al., 2009; Northcutt et al., 2010).

4.2. Methamphetamine (Meth)

A considerable number of reports show that Meth activates microglia (Bowyer and Ali, 2006; Buchanan et al., 2010; Escubedo et al., 1998; Fantegrossi et al., 2008; Goncalves et al., 2010; Guilarte et al., 2003; Ladenheim et al., 2000; LaVoie et al., 2004; Pubill et al., 2003; Sekine et al., 2008; Sharma and Kiyatkin, 2009; Sriram et al., 2006; Theodore et al., 2006; Thomas et al., 2004, 2008; Thomas and Kuhn, 2005). Of note, a study in humans demonstrated that microglial activation was elevated in several brain regions including the striatum in long-term Meth users (Sekine et al., 2008). Strikingly, microglia activation was observed at least 2 years after Meth abstinence. Further, Meth induces an array of neuroinflammatory effects including PIC, chemokines, and oxidative stress (Cadet and Krasnova, 2009; Krasnova and Cadet, 2009). Two initial reports demonstrated that acute Meth treatment increased hypothalamic IL-1β (Yamaguchi et al., 1991a,b), which reflects the hyperthermic effects of Meth (Krasnova and Cadet, 2009). Several groups have extended this finding to show that Meth induces PICs across several brain regions including brain reward pathways, and Ladenheim et al. implicated IL-6 in Meth-induced astrogliosis,

oxidative stress, and neurotoxicity in several brain regions including the striatum, frontal cortex, and hippocampus (Ladenheim et al., 2000). Here, the effects of Meth were found to be ameliorated in IL-6 KO animals. Additional studies have demonstrated that Meth indeed induces PICs in CNS. Acute Meth increased striatal and frontal cortex expression of TNF (Flora et al., 2002). Likewise, acute Meth increased TNFα and IL-1β in striatum and potentiated cytokine response to HIV TAT (Flora et al., 2003), as well as the chemokine expression to the HIV Tat protein (Theodore et al., 2006). Moreover, prior Meth administration potentiated the pro-inflammatory cytokine response to LPS across several brain regions including striatum, hypothalamus, hippocampus, cortex, and cerebellum, and prior Meth also potentiated the microglial activation induced by LPS (Buchanan et al., 2010). Consistent with a key role for microglia, acute Meth-induced increases in the expression of TNF, TNFr1, TNFr2, IL-1α, and IL-6 were blocked by pretreatment with the microglia inhibitor minocycline (Sriram et al., 2006). Similarly, indomethacin (a non-steroidal anti-inflammatory) pretreatment can prevent the neuroinflammatory effects of Meth (Goncalves et al., 2010). In rhesus monkeys, chronic Meth increased interferon α/β in frontal lobe (Coutinho et al., 2008) and striatal expression of TNF and NF-κB (Lai et al., 2009). Consistent with several studies cited above, repeated Meth treatment increased TNFα throughout the brain (Nakajima et al., 2004). Interestingly, Meth-induced behavioral hyperactivity and sensitization was potentiated in TNFα KO animals. Furthermore, pretreatment with exogenous TNFα reduced Meth-induced locomotor activity, the rewarding effects of Meth, and several indicators of neurotoxicity. This study suggests that the TNFα response to Meth plays a neuroprotective role.

Few studies have examined whether stress modulates the neurotoxic effects of Meth. A regimen of chronic unpredictable stress potentiated the hyperthermic response to Meth (Matuszewich and Yamamoto, 2004). Prior chronic stress was also found to potentiate the hyperthermic response to Meth, which was blocked by treating animals with the glucocorticoid synthesis inhibitor, metyrapone, during chronic stress exposure (Tata and Yamamoto, 2008). Lastly, treatment with the glial inhibitor, Ibudilast (a drug used extensively in our laboratory to modulate the pro-inflammatory effects of morphine; see above), attenuated stress-induced reinstatement of extinguished Meth-maintained responding (Beardsley et al., 2010).

A considerable literature has demonstrated that Meth is neurotoxic, depending on dose and time course of administration (Krasnova and Cadet, 2009). It should be noted that evidence of Meth acting through PRRs such as TLRs is lacking. Therefore, the above evidence suggests that the neuroinflammatory effects of Meth may be secondary to the neurotoxic effects of Meth. In the case of Meth exposure, neurotoxicity may result in the release of inflammatory factors (i.e. HMGB1 etc.) resulting in the activation of microglia and release of pro-inflammatory mediators.

4.3. Alcohol

Chronic exposure to alcohol *in vivo* activates microglia and astrocytes (Fernandez-Lizarbe et al., 2009) and increases brain levels of inflammatory mediators such as IL-1 (Qin et al., 2008) and activates inflammatory transcription factors such as NF-κB (Valles et al., 2004).

Indeed, microglial activation, astrogliosis, and increased expression of PICs have been observed in postmortem examination of alcoholic brains (He and Crews, 2008). The duration of exposure to alcohol that is required is unknown, but as few as three daily doses of alcohol (4 g/kg i.g. in mice, Fernandez-Lizarbe et al., 2009) have been reported to induce microglial activation in cortex. *In vitro,* even a brief exposure to alcohol at physiological concentrations (50 nM) induces inflammatory processes in astrocytes (Blanco et al., 2005) and microglia (Fernandez-Lizarbe et al., 2009).

Although there are a variety of mechanisms by which alcohol could induce neuroinflammation, a small but consistent literature suggests action at TLR4. As noted above, alcohol rapidly activates microglia *in vitro,* up-regulating microglial activation markers and leading to NF-κB activation and the production of PICs. Fernandez-Lizarbe et al. (2009) found all of these to be absent in microglia taken from TLR4 deficient mice. Importantly, TLR4 knockout (Fernandez-Lizarbe et al., 2009) and knockdown with siRNAs (Alfonso-Loeches et al., 2010) also blocked alcohol-induced neuroinflammatory responses *in vivo.*

It is worth noting that the induction of TLR4 signaling by alcohol (and other drugs) does not imply that there is a classical ligand–receptor interaction between alcohol and TLR4. There are a number of other mechanisms by which TLR4 signaling could be produced. For example, it has been suggested (Blanco et al., 2008) that alcohol interacts with lipid rafts in such a way that TLR4 is recruited, triggering its endocytosis and interaction with co-signaling molecules such as MD2, and the consequent induction of downstream signaling. An understanding of the mechanism (s) by with alcohol and other drugs lead to TLR signaling awaits clarification.

5. Stress and GC sensitization of drug abuse behavior

Stress and GCs facilitate and sensitize the behavioral response to drugs of abuse including potentiation of drug reward, locomotor activity, self-administration, and relapse (de Jong and de Kloet, 2004; Piazza and Le Moal, 1997). This facilitation of drug reward processes by stress has been the subject of numerous reviews (Briand and Blendy, 2010; Koob, 2009; Sinha, 2008) and need not be reviewed here. Importantly, stress and GCs activate brain reward pathways, such as dopamine signaling in the nucleus accumbens, in a manner similar to drugs of abuse (Marinelli and Piazza, 2002). Further, drugs of abuse activate the hypothalamo-pituitary–adrenal (HPA) axis (Armario, 2010). Our laboratory has also found that stress as well as GCs sensitizes the rewarding properties of morphine, and that just as with stress-induced potentiation of later inflammatory responses to immune activation, adrenalectomy blocks stress-induced potentiation of drug reward (Der-Avakian et al., 2005).

6. Clinical implications

Briefly, a considerable literature has documented the high degree of comorbidity between substance abuse and psychiatric disorders (Merikangas and Kalaydjian, 2007). Particularly noteworthy is the high degree of comorbidity between major depression and alcohol abuse (Davis et al., 2008). This relationship between major depression and alcohol abuse is of

particular relevance in the context of the present review given that stress (Anisman, 2009), GCs (Pariante and Miller, 2001), and inflammatory processes (Dantzer et al., 2008) have been implicated in the etiology of major depression. Of note, pro-inflammatory cytokines administered to humans induce depression-like symptoms (Pollak and Yirmiya, 2002). Interestingly, comorbid depression and alcohol abuse result in a more extreme clinical phenotype characterized by earlier onset of major depression, longer duration of illness, increased number of depressive episodes, increased comorbid psychiatric disorders, more suicide attempts, and a greater family history of suicide (Davis et al., 2008). This raises the intriguing possibility that the pro-inflammatory effects of alcohol may potentiate the ongoing pro-inflammatory processes in major depression, thereby exacerbating the clinical course of major depression.

7. Conclusion

The evidence summarized to this point indicates that (1) stressors, possibly mediated by the GC rise that they produce, sensitize microglial pro-inflammatory responses to subsequent immune challenge, likely via up-regulation of TLRs; (2) a number of drugs of abuse activate microglia, likely via action at TLRs, with this aspect of drug action being involved in the mediation of several drug-related behaviors; and (3) stressors potentiate a variety of drug-related behaviors, with GCs playing a role. From the pattern of these findings it is natural to suggest that stressors potentiate drug-related behaviors, in part, because the drugs of abuse act at TLRs on microglia that have been up-regulated by prior stressor exposure, thereby leading to an exaggerated neuroinflammatory response to the drugs. This neuroinflammatory response then potentiates well-known drug pathways such as the VTA–NAcc dopamine system.

At the core of this model, we are proposing a new framework for understanding part of how stressors modulate reactions to drugs of abuse, in which drugs of abuse, viewed as xenobiotic agents, function as pro-inflammatory stimuli much like a microbial pathogen, which the organism “sees” as both foreign and dangerous and thereby mounts a central immune response.

References

Abbott FV, Palmour RM. Morphine-6-glucuronide: analgesic effects and receptor binding profile in rats. Life Sci. 1988; 43:1685–1695. [PubMed: 2848167]

Alfonso-Loeches S, Pascual-Lucas M, Blanco AM, Sanchez-Vera I, Guerri C. Pivotal role of TLR4 receptors in alcohol-induced neuroinflammation and brain damage. J Neurosci. 2010; 30:8285–8295. [PubMed: 20554880]

Aljada A, Ghanim H, Assian E, Mohanty P, Hamouda W, Garg R, Dandona P. Increased IkappaB expression and diminished nuclear NF-kappaB in human mononuclear cells following hydrocortisone injection. J Clin Endocrinol Metab. 1999; 84:3386–3389. [PubMed: 10487715]

Anisman H. Cascading effects of stressors and inflammatory immune system activation: implications for major depressive disorder. J Psychiatry Neurosci. 2009; 34:4–20. [PubMed: 19125209]

Aravalli RN, Peterson PK, Lokensgard JR. Toll-like receptors in defense and damage of the central nervous system. J Neuroimmune Pharmacol. 2007; 2:297–312. [PubMed: 18040848]

Armario A. Activation of the hypothalamic–pituitary–adrenal axis by addictive drugs: different pathways, common outcome. Trends Pharmacol Sci. 2010; 31:318–325. [PubMed: 20537734]

Barclay AN, Wright GJ, Brooke G, Brown MH. CD200 and membrane protein interactions in the control of myeloid cells. Trends Immunol. 2002; 23:285–290. [PubMed: 12072366]

Bartlett SE, Dodd PR, Smith MT. Pharmacology of morphine and morphine-3-glucuronide at opioid, excitatory amino acid, GABA and glycine binding sites. Pharmacol Toxicol. 1994; 75:73–81. [PubMed: 7971741]

Barton GM, Kagan JC. A cell biological view of Toll-like receptor function: regulation through compartmentalization. Nat Rev Immunol. 2009; 9:535–542. [PubMed: 19556980]

Beardsley PM, Shelton KL, Hendrick E, Johnson KW. The glial cell modulator and phosphodiesterase inhibitor, AV411 (Ibudilast), attenuates prime- and stress-induced methamphetamine relapse. Eur J Pharmacol. 2010; 637:102–108. [PubMed: 20399770]

Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. J Leukoc Biol. 2007; 81:1–5.

Blanco AM, Perez-Arago A, Fernandez-Lizarbe S, Guerri C. Ethanol mimics ligand-mediated activation and endocytosis of IL-1RI/TLR4 receptors via lipid rafts caveolae in astroglial cells. J Neurochem. 2008; 106:625–639. [PubMed: 18419766]

Blanco AM, Valles SL, Pascual M, Guerri C. Involvement of TLR4/type I IL-1 receptor signaling in the induction of inflammatory mediators and cell death induced by ethanol in cultured astrocytes. J Immunol. 2005; 175:6893–6899. [PubMed: 16272348]

Bland ST, Hutchinson MR, Maier SF, Watkins LR, Johnson KW. The glial activation inhibitor AV411 reduces morphine-induced nucleus accumbens dopamine release. Brain Behav Immun. 2009; 23:492–497. [PubMed: 19486648]

Boumpas DT, Chrousos GP, Wilder RL, Cupps TR, Balow JE. Glucocorticoid therapy for immune-mediated diseases: basic and clinical correlates. Ann Intern Med. 1993; 119:1198–1208. [PubMed: 8239251]

Bowyer JF, Ali S. High doses of methamphetamine that cause disruption of the blood–brain barrier in limbic regions produce extensive neuronal degeneration in mouse hippocampus. Synapse. 2006; 60:521–532. [PubMed: 16952162]

Briand LA, Blendy JA. Molecular and genetic substrates linking stress and addiction. Brain Res. 2010; 1314:219–234. [PubMed: 19900417]

Buchanan JB, Sparkman NL, Johnson RW. A neurotoxic regimen of methamphetamine exacerbates the febrile and neuroinflammatory response to a subsequent peripheral immune stimulus. J Neuroinflammation. 2010; 7:82. [PubMed: 21092194]

Cadet JL, Krasnova IN. Molecular bases of methamphetamine-induced neurodegeneration. Int Rev Neurobiol. 2009; 88:101–119. [PubMed: 19897076]

Carpentier PA, Duncan DS, Miller SD. Glial Toll-like receptor signaling in central nervous system infection and autoimmunity. Brain Behav Immun. 2008; 22:140–147. [PubMed: 17920811]

Coutinho A, Flynn C, Burdo TH, Mervis RF, Fox HS. Chronic methamphetamine induces structural changes in frontal cortex neurons and upregulates type I interferons. J Neuroimmune Pharmacol. 2008; 3:241–245. [PubMed: 18594991]

Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci. 2008; 9:46–56. [PubMed: 18073775]

Davis L, Uezato A, Newell JM, Frazier E. Major depression and comorbid substance use disorders. Curr Opin Psychiatry. 2008; 21:14–18. [PubMed: 18281835]

de Jong IE, de Kloet ER. Glucocorticoids and vulnerability to psychostimulant drugs: toward substrate and mechanism. Ann N Y Acad Sci. 2004; 1018:192–198. [PubMed: 15240368]

de Pablos RM, Villaran RF, Arguelles S, Herrera AJ, Venero JL, Ayala A, Cano J, Machado A. Stress increases vulnerability to inflammation in the rat prefrontal cortex. J Neurosci. 2006; 26:5709–5719. [PubMed: 16723527]

Der-Avakian A, Will MJ, Bland ST, Deak T, Nguyen KT, Schmid MJ, Spencer RL, Watkins LR, Maier SF. Surgical and pharmacological suppression of glucocorticoids prevents the enhancement of morphine conditioned place preference by uncontrollable stress in rats. Psychopharmacology (Berl). 2005; 179:409–417. [PubMed: 15821955]

Escubedo E, Guitart L, Sureda FX, Jimenez A, Pubill D, Pallas M, Camins A, Camarasa J. Microgliosis and down-regulation of adenosine transporter induced by methamphetamine in rats. Brain Res. 1998; 814:120–126. [PubMed: 9838075]

Espinosa-Oliva AM, de Pablos RM, Villaran RF, Arguelles S, Venero JL, Machado A, Cano J. Stress is critical for LPS-induced activation of microglia and damage in the rat hippocampus. Neurobiol Aging. 2011; 32:85–102. [PubMed: 19286276]

Fantegrossi WE, Ciullo JR, Wakabayashi KT, De La Garza R 2nd, Traynor JR, Woods JH. A comparison of the physiological, behavioral, neurochemical and microglial effects of methamphetamine and 3,4-methylenedioxymethamphetamine in the mouse. Neuroscience. 2008; 151:533–543. [PubMed: 18082974]

Fernandez-Lizarbe S, Pascual M, Guerri C. Critical role of TLR4 response in the activation of microglia induced by ethanol. J Immunol. 2009; 183:4733–4744. [PubMed: 19752239]

Fleshner M, Deak T, Spencer RL, Laudenslager ML, Watkins LR, Maier SF. A long-term increase in basal levels of corticosterone and a decrease in corticosteroid-binding globulin after acute stressor exposure. Endocrinology. 1995; 136:5336–5342. [PubMed: 7588279]

Flora G, Lee YW, Nath A, Hennig B, Maragos W, Toborek M. Methamphetamine potentiates HIV-1 Tat protein-mediated activation of redox-sensitive pathways in discrete regions of the brain. Exp Neurol. 2003; 179:60–70. [PubMed: 12504868]

Flora G, Lee YW, Nath A, Maragos W, Hennig B, Toborek M. Methamphetamine-induced TNF-alpha gene expression and activation of AP-1 in discrete regions of mouse brain: potential role of reactive oxygen intermediates and lipid peroxidation. Neuromolecul Med. 2002; 2:71–85.

Frank MG, Baratta MV, Sprunger DB, Watkins LR, Maier SF. Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses. Brain Behav Immun. 2007; 21:47–59. [PubMed: 16647243]

Frank MG, Miguel ZD, Watkins LR, Maier SF. Prior exposure to glucocorticoids sensitizes the neuroinflammatory and peripheral inflammatory responses to *E. coli* lipopolysaccharide. Brain Behav Immun. 2010; 24:19–30. [PubMed: 19647070]

Galon J, Franchimont D, Hiroi N, Frey G, Boettner A, Ehrhart-Bornstein M, O'Shea JJ, Chrousos GP, Bornstein SR. Gene profiling reveals unknown enhancing and suppressive actions of glucocorticoids on immune cells. FASEB J. 2002; 16:61–71. [PubMed: 11772937]

Goncalves J, Baptista S, Martins T, Milhazes N, Borges F, Ribeiro CF, Malva JO, Silva AP. Methamphetamine-induced neuroinflammation and neuronal dysfunction in the mice hippocampus: preventive effect of indomethacin. Eur J Neurosci. 2010; 31:315–326. [PubMed: 20074221]

Goujon E, Parnet P, Laye S, Combe C, Dantzer R. Adrenalectomy enhances pro-inflammatory cytokines gene expression, in the spleen, pituitary and brain of mice in response to lipopolysaccharide. Brain Res Mol Brain Res. 1996; 36:53–62. [PubMed: 9011765]

Guilarte TR, Nihei MK, McGlothan JL, Howard AS. Methamphetamine induced deficits of brain monoaminergic neuronal markers: distal axotomy or neuronal plasticity. Neuroscience. 2003; 122:499–513. [PubMed: 14614914]

He J, Crews FT. Increased MCP-1 and microglia in various regions of the human alcoholic brain. Exp Neurol. 2008; 210:349–358. [PubMed: 18190912]

Hennessy MB, Schiml-Webb PA, Deak T. Separation, sickness, and depression: a new perspective on an old animal model. Curr Dir Psychol Sci. 2009; 18:227–231. [PubMed: 20221300]

Hermoso MA, Matsuguchi T, Smoak K, Cidlowski JA. Glucocorticoids and tumor necrosis factor alpha cooperatively regulate Toll-like receptor 2 gene expression. Mol Cell Biol. 2004; 24:4743–4756. [PubMed: 15143169]

Hutchinson MR, Bland ST, Johnson KW, Rice KC, Maier SF, Watkins LR. Opioid-induced glial activation: mechanisms of activation and implications for opioid analgesia, dependence, and reward. Sci World J. 2007; 7:98–111.

Hutchinson MR, Coats BD, Lewis SS, Zhang Y, Sprunger DB, Rezvani N, Baker EM, Jekich BM, Wieseler JL, Somogyi AA, Martin D, Poole S, Judd CM, Maier SF, Watkins LR. Proinflammatory cytokines oppose opioid-induced acute and chronic analgesia. Brain Behav Immun. 2008; 22:1178–1189. [PubMed: 18599265]

Hutchinson MR, Lewis SS, Coats BD, Rezvani N, Zhang Y, Wieseler JL, Somogyi AA, Yin H, Maier SF, Rice KC, Watkins LR. Possible involvement of Toll-like receptor 4/myeloid differentiation factor-2 activity of opioid inactive isomers causes spinal proinflammation and related behavioral consequences. Neuroscience. 2010a; 167:880–893. [PubMed: 20178837]

Hutchinson MR, Lewis SS, Coats BD, Skyba DA, Crysdale NY, Berkelhammer DL, Brzeski A, Northcutt A, Vietz CM, Judd CM, Maier SF, Watkins LR, Johnson KW. Reduction of opioid withdrawal and potentiation of acute opioid analgesia by systemic AV411 (Ibudilast). Brain Behav Immun. 2009; 23:240–250. [PubMed: 18938237]

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, Watkins LR. Evidence that opioids may have Toll-like receptor 4 and MD-2 effects. Brain Behav Immun. 2010b; 24:83–95. [PubMed: 19679181]

Janeway CA Jr, Medzhitov R. Innate immune recognition. Annu Rev Immunol. 2002; 20:197–216. [PubMed: 11861602]

Johnson JD, Campisi J, Sharkey CM, Kennedy SL, Nickerson M, Greenwood BN, Fleshner M. Catecholamines mediate stress-induced increases in peripheral and central inflammatory cytokines. Neuroscience. 2005; 135:1295–1307. [PubMed: 16165282]

Johnson JD, O'Connor KA, Hansen MK, Watkins LR, Maier SF. Effects of prior stress on LPS-induced cytokine and sickness responses. Am J Physiol Regul Integr Comp Physiol. 2003; 284:R422–R432. [PubMed: 12399247]

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, 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. [PubMed: 15317861]

Kawai T, Akira S. Signaling to NF-kappaB by Toll-like receptors. Trends Mol Med. 2007; 13:460–469. [PubMed: 18029230]

Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010; 11:373–384. [PubMed: 20404851]

Koning N, Swaab DF, Hoek RM, Huitinga I. Distribution of the immune inhibitory molecules CD200 and CD200R in the normal central nervous system and multiple sclerosis lesions suggests neuron–glia and glia–glia interactions. J Neuropathol Exp Neurol. 2009; 68:159–167. [PubMed: 19151626]

Koob GF. Brain stress systems in the amygdala and addiction. Brain Res. 2009; 1293:61–75. [PubMed: 19332030]

Krasnova IN, Cadet JL. Methamphetamine toxicity and messengers of death. Brain Res Rev. 2009; 60:379–407. [PubMed: 19328213]

Ladenheim B, Krasnova IN, Deng X, Oyler JM, Polettini A, Moran TH, Huestis MA, Cadet JL. Methamphetamine-induced neurotoxicity is attenuated in transgenic mice with a null mutation for interleukin-6. Mol Pharmacol. 2000; 58:1247–1256. [PubMed: 11093760]

Lai YT, Tsai YP, Cherng CG, Ke JJ, Ho MC, Tsai CW, Yu L. Lipopolysaccharide mitagates methamphetamine-induced striatal dopamine depletion via modulating local TNF-alpha and dopamine transporter expression. J Neural Transm. 2009; 116:405–415. [PubMed: 19271121]

LaVoie MJ, Card JP, Hastings TG. Microglial activation precedes dopamine terminal pathology in methamphetamine-induced neurotoxicity. Exp Neurol. 2004; 187:47–57. [PubMed: 15081587]

Lehnardt S, Massillon L, Follett P, Jensen FE, Ratan R, Volpe JJ, Vartanian T. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. Proc Natl Acad Sci USA. 2003; 100:8514–8519. [PubMed: 12824464]

Lewis SS, Hutchinson MR, Rezvani N, Loram LC, Zhang Y, Maier SF, Rice KC, Watkins LR. Evidence that intrathecal morphine-3-glucuronide may cause pain enhancement via Toll-like receptor 4/MD-2 and interleukin-1beta. Neuroscience. 2010; 165:569–583. [PubMed: 19833175]

Maier SF. Bi-directional immune–brain communication: implications for understanding stress, pain, and cognition. Brain Behav Immun. 2003; 17:69–85. [PubMed: 12676570]

Marinelli M, Piazza PV. Interaction between glucocorticoid hormones, stress and psychostimulant drugs. Eur J Neurosci. 2002; 16:387–394. [PubMed: 12193179]

Matuszewich L, Yamamoto BK. Chronic stress augments the long-term and acute effects of methamphetamine. Neuroscience. 2004; 124:637–646. [PubMed: 14980734]

Mazoit JX, Butscher K, Samii K. Morphine in postoperative patients: pharmacokinetics and pharmacodynamics of metabolites. Anesth Analg. 2007; 105:70–78. [PubMed: 17578959]

McKay LI, Cidlowski JA. CBP (CREB binding protein) integrates NF-kappaB (nuclear factor-kappaB) and glucocorticoid receptor physical interactions and antagonism. Mol Endocrinol. 2000; 14:1222–1234. [PubMed: 10935546]

Merikangas KR, Kalaydjian A. Magnitude and impact of comorbidity of mental disorders from epidemiologic surveys. Curr Opin Psychiatry. 2007; 20:353–358. [PubMed: 17551350]

Mika J, Wawrzczak-Bargiela A, Osikowicz M, Makuch W, Przewlocka B. Attenuation of morphine tolerance by minocycline and pentoxifylline in naive and neuropathic mice. Brain Behav Immun. 2009; 23:75–84. [PubMed: 18684397]

Miller AH. Letter to the Editor Re: “An inflammatory review of glucocorticoids in the CNS” by Sorrells et al. Brain Behavior and Immunity. 2007; 21:259–272. 2007. Brain Behav. Immun. 21, 988–989. author reply 990.

Minami M, Kuraishi Y, Yamaguchi T, Nakai S, Hirai Y, Satoh M. Immobilization stress induces interleukin-1 beta mRNA in the rat hypothalamus. Neurosci Lett. 1991; 123:254–256. [PubMed: 2027540]

Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol. 2008; 8:958–969. [PubMed: 19029990]

Munck A, Guyre PM, Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr Rev. 1984; 5:25–44. [PubMed: 6368214]

Munhoz CD, Lepsch LB, Kawamoto EM, Malta MB, Lima Lde S, Avellar MC, Sapolsky RM, Scavone C. Chronic unpredictable stress exacerbates lipopolysaccharide-induced activation of nuclear factor-kappaB in the frontal cortex and hippocampus via glucocorticoid secretion. J Neurosci. 2006; 26:3813–3820. [PubMed: 16597735]

Munhoz CD, Sorrells SF, Caso JR, Scavone C, Sapolsky RM. Glucocorticoids exacerbate lipopolysaccharide-induced signaling in the frontal cortex and hippocampus in a dose-dependent manner. J Neurosci. 2010; 30:13690–13698. [PubMed: 20943909]

Nair A, Bonneau RH. Stress-induced elevation of glucocorticoids increases microglia proliferation through NMDA receptor activation. J Neuroimmunol. 2006; 171:72–85. [PubMed: 16278020]

Nakajima A, Yamada K, Nagai T, Uchiyama T, Miyamoto Y, Mamiya T, He J, Nitta A, Mizuno M, Tran MH, Seto A, Yoshimura M, Kitaichi K, Hasegawa T, Saito K, Yamada Y, Seishima M, Sekikawa K, Kim HC, Nabeshima T. Role of tumor necrosis factor-alpha in methamphetamine-induced drug dependence and neurotoxicity. J Neurosci. 2004; 24:2212–2225. [PubMed: 14999072]

Narita M, Miyatake M, Shibasaki M, Shindo K, Nakamura A, Kuzumaki N, Nagumo Y, Suzuki T. Direct evidence of astrocytic modulation in the development of rewarding effects induced by drugs of abuse. Neuropsychopharmacology. 2006; 31:2476–2488. [PubMed: 16407899]

Nguyen KT, Deak T, Owens SM, Kohno T, Fleshner M, Watkins LR, Maier SF. Exposure to acute stress induces brain interleukin-1beta protein in the rat. J Neurosci. 1998; 18:2239–2246. [PubMed: 9482808]

Nguyen KT, Deak T, Will MJ, Hansen MK, Hunsaker BN, Fleshner M, Watkins LR, Maier SF. Time course and corticosterone sensitivity of the brain, pituitary, and serum interleukin-1beta protein response to acute stress. Brain Res. 2000; 859:193–201. [PubMed: 10719064]

Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science. 2005; 308:1314–1318. [PubMed: 15831717]

Northcutt AL, Sfregola C, Miles NE, Taylor FR, Loeffler MF, Bland ST, Amat J, Hutchison MR, Rozeske RR, Maier SF, Rice KC, Watkins LR. Novel Toll-like receptor (TLR4) involvement in drug reward. Proc Soc Neurosci. 2010

O'Connor KA, Johnson JD, Hansen MK, Wieseler-Frank JL, Maksimova E, Watkins LR, Maier SF. Peripheral and central proinflammatory cytokine response to a severe acute stressor. Brain Res. 2003; 991:123–132. [PubMed: 14575884]

Pariante CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biol Psychiatry. 2001; 49:391–404. [PubMed: 11274650]

Perry VH. The influence of systemic inflammation on inflammation in the brain: implications for chronic neurodegenerative disease. Brain Behav Immun. 2004; 18:407–413. [PubMed: 15265532]

Piazza PV, Le Moal M. Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. Brain Res Brain Res Rev. 1997; 25:359–372. [PubMed: 9495563]

Pollak Y, Yirmiya R. Cytokine-induced changes in mood and behaviour: implications for 'depression due to a general medical condition', immunotherapy and antidepressive treatment. Int J Neuropsychopharmacol. 2002; 5:389–399. [PubMed: 12466037]

Pubill D, Canudas AM, Pallas M, Camins A, Camarasa J, Escubedo E. Different glial response to methamphetamine- and methylenedioxy-methamphetamine- induced neurotoxicity. Naunyn Schmiedebergs Arch Pharmacol. 2003; 367:490–499. [PubMed: 12684743]

Qin L, He J, Hanes RN, Pluzarev O, Hong JS, Crews FT. Increased systemic and brain cytokine production and neuroinflammation by endotoxin following ethanol treatment. J Neuroinflammation. 2008; 5:10. [PubMed: 18348728]

Raghavendra V, Tanga FY, DeLeo JA. Attenuation of morphine tolerance, withdrawal-induced hyperalgesia, and associated spinal inflammatory immune responses by propentofylline in rats. Neuropsychopharmacology. 2004; 29:327–334. [PubMed: 14532913]

Ransohoff RM, Cardona AE. The myeloid cells of the central nervous system parenchyma. Nature. 2010; 468:253–262. [PubMed: 21068834]

Ransohoff RM, Perry VH. Microglial physiology: unique stimuli, specialized responses. Annu Rev Immunol. 2009; 27:119–145. [PubMed: 19302036]

Rozkova D, Horvath R, Bartunkova J, Spisek R. Glucocorticoids severely impair differentiation and antigen presenting function of dendritic cells despite upregulation of Toll-like receptors. Clin Immunol. 2006; 120:260–271. [PubMed: 16765091]

Sakai A, Han J, Cato AC, Akira S, Li JD. Glucocorticoids synergize with IL-1beta to induce TLR2 expression via MAP kinase phosphatase-1-dependent dual inhibition of MAPK JNK and p38 in epithelial cells. BMC Mol Biol. 2004; 5:2. [PubMed: 15125785]

Salminen A, Huuskonen J, Ojala J, Kaarniranta K, Suuronen T. Activation of innate immunity system during aging: NF-jB signaling is the molecular culprit of inflamm-aging. Ageing Res Rev. 2008; 7:83–105. [PubMed: 17964225]

Sapolsky RM. Glucocorticoids, stress, and their adverse neurological effects: relevance to aging. Exp Gerontol. 1999; 34:721–732. [PubMed: 10579633]

Schiltz JC, Sawchenko PE. Signaling the brain in systemic inflammation: the role of perivascular cells. Front Biosci. 2003; 8:s1321–s1329. [PubMed: 12957837]

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, Cadet JL. Methamphetamine causes microglial activation in the brains of human abusers. J Neurosci. 2008; 28:5756–5761. [PubMed: 18509037]

Selye H. Stress and the general adaptation syndrome. Br Med J. 1950; 1:1383–1392. [PubMed: 15426759]

Selye H. "Critical Period" for inhibition of inflammation by a primarily neurogenic stress-situation. Psychosom Med. 1955; 17:124–127. [PubMed: 14371907]

Sharma HS, Kiyatkin EA. Rapid morphological brain abnormalities during acute methamphetamine intoxication in the rat: an experimental study using light and electron microscopy. J Chem Neuroanat. 2009; 37:18–32. [PubMed: 18773954]

Shibata M, Katsuyama M, Onodera T, Ehama R, Hosoi J, Tagami H. Glucocorticoids enhance Toll-like receptor 2 expression in human keratinocytes stimulated with Propionibacterium acnes or proinflammatory cytokines. J Invest Dermatol. 2009; 129:375–382. [PubMed: 18704103]

Shintani F, Nakaki T, Kanba S, Sato K, Yagi G, Shiozawa M, Aiso S, Kato R, Asai M. Involvement of interleukin-1 in immobilization stress-induced increase in plasma adrenocorticotropic hormone and in release of hypothalamic monoamines in the rat. J Neurosci. 1995; 15:1961–1970. [PubMed: 7891145]

Sinha R. Chronic stress, drug use, and vulnerability to addiction. Ann N Y Acad Sci. 2008; 1141:105–130. [PubMed: 18991954]

Smoak KA, Cidlowski JA. Mechanisms of glucocorticoid receptor signaling during inflammation. Mech Ageing Dev. 2004; 125:697–706. [PubMed: 15541765]

Solt LA, May MJ. The IkappaB kinase complex: master regulator of NF-kappaB signaling. Immunol Res. 2008; 42:3–18. [PubMed: 18626576]

Song P, Zhao ZQ. The involvement of glial cells in the development of morphine tolerance. Neurosci Res. 2001; 39:281–286. [PubMed: 11248367]

Sorrells SF, Sapolsky RM. An inflammatory review of glucocorticoid actions in the CNS. Brain Behav Immun. 2007; 21:259–272. [PubMed: 17194565]

Sriram K, Miller DB, O'Callaghan JP. Minocycline attenuates microglial activation but fails to mitigate striatal dopaminergic neurotoxicity: role of tumor necrosis factor-alpha. J Neurochem. 2006; 96:706–718. [PubMed: 16405514]

Tata DA, Yamamoto BK. Chronic stress enhances methamphetamine-induced extracellular glutamate and excitotoxicity in the rat striatum. Synapse. 2008; 62:325–336. [PubMed: 18288648]

Theodore S, Stolberg S, Cass WA, Maragos WF. Human immunodeficiency virus-1 protein tat and methamphetamine interactions. Ann N Y Acad Sci. 2006; 1074:178–190. [PubMed: 17105916]

Thomas DM, Dowgiert J, Geddes TJ, Francescutti-Verbeem D, Liu X, Kuhn DM. Microglial activation is a pharmacologically specific marker for the neurotoxic amphetamines. Neurosci Lett. 2004; 367:349–354. [PubMed: 15337264]

Thomas DM, Francescutti-Verbeem DM, Kuhn DM. Methamphetamine-induced neurotoxicity and microglial activation are not mediated by fractalkine receptor signaling. J Neurochem. 2008; 106:696–705. [PubMed: 18410508]

Thomas DM, Kuhn DM. Attenuated microglial activation mediates tolerance to the neurotoxic effects of methamphetamine. J Neurochem. 2005; 92:790–797. [PubMed: 15686480]

Tilleux S, Hermans E. Neuroinflammation and regulation of glial glutamate uptake in neurological disorders. J Neurosci Res. 2007; 85:2059–2070. [PubMed: 17497670]

Valles SL, Blanco AM, Pascual M, Guerri C. Chronic ethanol treatment enhances inflammatory mediators and cell death in the brain and in astrocytes. Brain Pathol. 2004; 14:365–371. [PubMed: 15605983]

Yamaguchi T, Kuraishi Y, Minami M, Nakai S, Hirai Y, Satoh M. Methamphetamine-induced expression of interleukin-1 beta mRNA in the rat hypothalamus. Neurosci Lett. 1991a; 128:90–92. [PubMed: 1922954]

Yamaguchi T, Kuraishi Y, Yabuuchi K, Minami M, Satoh M. In situ hybridization analysis of the induction of interleukin-1beta mRNA by methamphetamine in the rat hypothalamus. Mol Cell Neurosci. 1991b; 2:259–265. [PubMed: 19912807]