Implications of central immune signaling caused by drugs of abuse: Mechanisms, mediators and new therapeutic approaches for prediction and treatment of drug dependence

Janet K. Collera,⁎ Mark R. Hutchinson

Discipline of Physiology, School of Medical Sciences, University of Adelaide, South Australia, 5005, Australia
Discipline of Pharmacology, School of Medical Sciences, University of Adelaide, South Australia, 5005, Australia

A R T I C L E   I N F O

Keywords:
Microglia
Astrocyte
Cytokine
Chemokine
Toll-like receptor
Drug addiction

A B S T R A C T

In the past two decades a trickle of manuscripts examining the non-neuronal central nervous system immune consequences of the drugs of abuse has now swollen to a significant body of work. Initially, these studies reported associative evidence of central nervous system proinflammation resulting from exposure to the drugs of abuse demonstrating key implications for neurotoxicity and disease progression associated with, for example, HIV infection. However, more recently this drug-induced activation of central immune signaling is now understood to contribute substantially to the pharmacodynamic actions of the drugs of abuse, by enhancing the engagement of classical mesolimbic dopamine reward pathways and withdrawal centers. This review will highlight the key in vivo animal, human, biological and molecular evidence of these central immune signaling actions of opioids, alcohol, cocainemethamphetamine, and 3,4-methylenedioxymethamphetamine (MDMA). Excitingly, this new appreciation of central immune signaling activity of drugs of abuse provides novel therapeutic interventions and opportunities to identify ‘at risk’ individuals through the use of immunogenetics. Discussion will also cover the evidence of modulation of this signaling by existing clinical and pre-clinical drug candidates, and novel pharmacological targets. Finally, following examination of the breadth of central immune signaling actions of the drugs of abuse highlighted here, the current known common immune signaling components will be outlined and their impact on established addiction neurocircuity discussed, thereby synthesizing a common neuroimmune hypothesis of addiction.

© 2012 Elsevier Inc. All rights reserved.
1. Introduction

Drug addiction reduces the quality of life of individuals, leads to increased crime and requires significant funds to treat and control (Manchikanti, 2006; United Nations Office on Drugs & Crime, 2010). Abused drugs range from legally available recreational drugs such as alcohol and legally prescribed opioids such as morphine, to illicit “street” and “party” drugs such as heroin, cocaine, methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) (United Nations Office on Drugs & Crime, 2010). There is significant physicochemical and structural diversity in each of these examples of drugs of abuse. As such, their hypothesized neuronal sites of action are just as diverse (Nestler, 2005). However, all drugs of abuse share the common trait that they are rewarding, which in many cases is accompanied by a state of addiction and dependence to the drug after repeated exposure (Büttner, 2011). The molecular mechanisms of reward produced by drugs of abuse are varied, but summate neurally to produce elevated signaling of the mesolimbic reward pathway that behaviorally presents as a rewarding and reinforcing drive (United Nations Office on Drugs & Crime, 2010). Legal and illicit drugs of abuse are often used chronically, leading to extensive exposure of the central nervous system (CNS) to the parent drug and its metabolites with a variety of consequences for neuronal adaptation and toxicity (Cappon et al., 1998; Weber et al., 2006; Salazar et al., 2008a; Tilleux & Hermans, 2007; Fantegrossi et al., 2008; Büttner, 2011). In contrast, the presentation of dependence, and relapse to drugs of abuse is varied across these abuse agents demonstrating some drug specificity in behavioral response when these drugs are absent or their neuronal actions are pharmacologically blocked (Hyman et al., 2006).

Despite several decades of research, the non-neuronal CNS effects of the drugs of abuse have only come to light in at most the past 2 decades. In addition, it has only recently been acknowledged that these non-neuronal actions of the drugs of abuse at CNS immunocompetent cells and central immune signaling pathways may play a significant role in contributing to the behavioral outcomes following drug exposure (Narita et al., 2006; Hutchinson et al., 2007; Watkins et al., 2009). Consequently, the mechanisms of drugs of abuse-induced activation of central immune signaling pathways and the molecular consequences of this activation need to be understood in order to broadly appreciate the full neuronal mechanisms and behavioral consequences of exposure to the drugs of abuse. As such, the aim of this review is to introduce and review the non-neuronal targets of drugs of abuse, highlighting the common mediators and mechanisms and the exciting opportunities these new targets have in identifying ‘at risk’ individuals and novel therapeutic opportunities. A unifying proinflammatory hypothesis of dependence will be synthesized based on the literature reviewed outlining the common central immune signaling pathways modulated by the drugs of abuse and their role in the establishment and maintenance of drug abuse and dependence. However, it is critical to appreciate that the activation of these central immune signaling pathways induced by drug of abuse exposure can only complement and work in conjunction with the established neuronal neurocircuity of reward and dependence, as engaging these central immune signaling pathways alone will not produce a relevant behavioral outcome.

The drugs of abuse to be discussed in this review are opioids (predominantly morphine), alcohol (ethanol), cocaine, methamphetamine, and 3,4-methylenedioxymethamphetamine (MDMA; ‘Ecstasy’), including discussion of the role of their respective metabolites where possible. These 5 agents were selected owing to their prominence in the central immune signaling literature and prevalence of clinical and/or social burden. For all of the drugs of abuse to be discussed, there is evidence that they elicit responses not only via classical neuronal pathways, but also via activation of central immune signaling. In the following sections we will review the evidence supporting the central immune signaling consequences of exposure to each drug of abuse, ranging from in vitro and in vivo human and rodent studies, to the impact of pharmacological targeting of central immune signaling and immunogenetics, and how all these relate to behavioral consequences of the drugs.

Owing to the focus of the existing literature the majority of the central immune signaling and non-neuronal modifications will be focused on microglia and astrocytes, and their related traditional signaling systems, such as cytokines and chemokines. A complicated issue that faces the gial research field is the use of the phrase “glial activation” as it has become apparent within the immunology field that immune activation can be both proinflammatory and anti-inflammatory in nature. As such we have attempted to provide information as to the direction of this response. Moreover, simple quantification of “proinflammatory markers of glial activation” such as microglial CD11b or Iba1 and the astrocyte marker GFAP, does not necessarily ensure a proinflammatory phenotype of these populations at the time of tissue analysis (Watkins et al., 2007a; Ransohoff & Perry, 2009). Therefore, we have also assembled information on inflammatory mediator release and central immune signaling status induced by the drugs of abuse. For general information on the immunocompetent cells of the CNS, their receptors and signaling molecules please refer to some of the many excellent recent reviews published by leaders in the central immune signaling field such as Rivest et al. (Rivest, 2003; Glezer et al., 2007; Rivest, 2009; Rivest, 2010) or Perry et al. (Perry et al., 2003; Ransohoff & Perry, 2009).

2. Neuronal actions of drugs of abuse and classical reward pathways

Whilst this review focuses on drugs of abuse-induced changes in central immune signaling and subsequent behavioral events, these are obviously not the only actions of the drugs. There are very well
described neuronal actions of these compounds. One of the major outcomes of the neuronal action of these agents is the activation of the mesolimbic dopamine reward neurocircuitry and the elevated release of dopamine in the nucleus accumbens (Joseph et al., 2003). This elevation of dopamine in the nucleus accumbens is important for mood/reward and motor functioning, with neural connections linking the prefrontal and frontal cortices, via the nucleus accumbens, to the thalamus and finally to the premotor cortex (Deutch, 1993). Beyond the dopaminergic projection from the ventral tegmental area, the nucleus accumbens receives projections from the hippocampus, frontal and prefrontal cortex, amygdala, and thalamus (Deutch, 1993). Most inputs release excitatory amino acids such as glutamate (Meredith et al., 1993). Whilst dopamine has received most attention in drug addiction, glutamate is also important in animal models of drug addiction (Kauer, 2004). Glutamate receptors and glutamatergic transmission are modified during drug-associated learning (Jones & Bonci, 2005). NMDA receptor antagonists reduce cocaine self-administration and conditioned place preference (CPP) to cocaine, morphine and methamphetamine (Tszenschtke, 2007). Similarly, AMPA receptor antagonists reduce cocaine, amphetamine and alcohol administration, and impair amphetamine- and morphine-induced CPP (Jackson et al., 2000). The global effect of increased glutamate extracellular levels and subsequent signaling by the drugs of abuse may be to increase long-term potentiation and synaptic plasticity in neural reward circuits (Jones & Bonci, 2005). Collectively, engagement of dopamine and glutamate systems are important players in the rewarding properties of drugs of abuse.

3. Introduction of the drugs of abuse: classical neuronal actions

As outlined above, this review will cover the implications of changes in central immune signaling associated with a series of drugs of abuse — specifically, opioids, alcohol, cocaine, methamphetamine, and MDMA. Morphine, derived from the opium poppy (Papaver somniferum), is in the 4,5-epoxymorphinan class. Whilst opiates (4,5-epoxymorphans found in the opium poppy) were once the predominant class of opioids prescribed and abused, this is not the case anymore (Manchikanti, 2006), as many structural modifications of morphine have been created producing semi-synthetic and fully synthetic opioid compounds with varied, but similar, pharmacology, that now represent the bulk of the prescribed and abused substances (Manchikanti, 2006). Therefore, in this review the term “opiod” will be used, and the research field should adjust their terminology in the future away from just references to opiates.

Alcohol or ethanol is widely available in western countries and best known for its consumption in alcoholic beverages. For the purposes of this review we will use the term alcohol. In contrast to opioids and alcohol, whose distribution for medical and personal use, respectively, has been controlled and legalized, the use of, and access to, cocaine, methamphetamine and MDMA is illicit. Cocaine is the principal alkaloid of Erythroxylum coca (Johanson & Fischman, 1989) and despite its production and use being criminalized, its use remains widespread worldwide. Methamphetamine and MDMA are amphetamine-like stimulants. Methamphetamine has been structurally modified via the addition of a methyl group that increases its lipid solubility, allowing it to more readily cross the blood–brain barrier (Barr et al., 2006). In contrast, MDMA is structurally similar to both the amphetamine-type stimulants and mescaline-type hallucinogens despite its pharmacology being distinct from these drug classes.

All of these drugs of abuse, despite their diverse structures and pharmacological properties, share the common characteristic of activation of the mesolimbic dopamine reward pathway, thus eliciting behavioral reward. Opioid reward is hypothesized to be created in part via inhibition of tonic GABAergic suppression of the mesolimbic dopaminergic reward pathway from the ventral tegmental area to the nucleus accumbens, the end result being increased dopamine release in the nucleus accumbens. Enhanced dopamine release may not fully explain morphine reward, however, as lesioning of the mesolimbic reward system does not disrupt heroin self-administration (Pettit et al., 1984), and numerous receptors and intracellular effectors are now implicated in morphine reward (Popik & Danyasz, 1997; Contet et al., 2004).

Alcohol on the other hand is less clear, and how alcohol affects the mesolimbic dopamine reward pathway remains a matter of great debate. Nonetheless, it has been characterized that alcohol increases the firing of dopaminergic ventral tegmental neurons (Gessa et al., 1985; Brodie et al., 1999) through direct excitatory cellular activation (Brodie et al., 1999) to increase extracellular dopamine in the nucleus accumbens (Tupala & Tilhonen, 2004). Alcohol also has GABA agonist activity that contributes to reward (Chester & Cunningham, 2002).

Cocaine acts to inhibit transporters for dopamine, serotonin and norepinephrine (Hall et al., 2004). Thus, the reinforcing properties of cocaine are mediated at least in part by the ventral tegmental area projection to the nucleus accumbens, resulting in increased dopamine signaling (Koob & Bloom, 1988; Koob & Hubner, 1988).

Methamphetamine increases dopamine (Kokoshka et al., 1998b) and serotonin (Kokoshka et al., 1998a; b) release. It causes increased dopamine levels via several mechanisms, mainly via inhibition of the dopamine transporter (Giros et al., 1996), but also via displacement of storage vesicles and inhibition of dopamine degradation by monoamine oxidase (Zaczek et al., 1991). Methamphetamine also increases the reverse transport of dopamine via its transporter thereby further increasing synaptic dopamine levels (Khoshbouei et al., 2003). The specific anatomical locations of methamphetamine’s action focus in the striatum where it elevates dopamine (Loonam et al., 2003; Zhang et al., 2006a), while it appears that glutamate in the nucleus accumbens also contributes significantly to the behavioral reward produced by methamphetamine (Fujiyo et al., 2005).

Finally MDMA possesses the ability to increase dopamine levels (Fantegrossi, 2008) and increase serotonin receptor activation, as it is able to release serotonin and dopamine (Nichols et al., 1982), and blocks its reuptake (Nichols, 1986; Steele et al., 1987). Microdialysis studies demonstrate that these changes in dopamine release occur in the striatum (Yamamoto & Spanos, 1988), nucleus accumbens (Cadoni et al., 2005) and prefrontal cortex (Nair & Gudelsky, 2004), but that the simultaneous release of serotonin modulates this dopamine activity (Gudelsky & Nash, 1996). In addition to dopamine and serotonin changes, acetylcholine release is increased in the striatum, nucleus accumbens and prefrontal cortex (Gudelsky & Yamamoto, 2008).

The evidence of non-neuronal and central immune signaling consequences of drugs of abuse exposure will be summarized and discussed in individual sections below, with common themes summarized in tables where appropriate.

4. Specific pharmacological tools to examine central immune signaling

In order to investigate the effects of central immune signaling on the action of drugs of abuse it is necessary to employ both in vitro and in vivo studies and have the ability to block specific points in the central immune signaling cascade to provide sound evidence of their involvement. Several examples of such pharmacological agents will be discussed in detail below. Of particular note are three pharmacological agents all of which are attenuators of glial activation. Ibudilast (AV411/MN–166) was first used to treat asthma in Japan. However, in the last 5 years it has been investigated for its central immune modulating properties, and in particular its ability to attenuate glial activation in pre-clinical rodent models. Minocycline is marketed as a tetracycline antibiotic for treatment of acne, however, it also possesses the ability to attenuate glial activation, with potential mechanisms unclear but may involve modulation of p38 phosphorylation. Finally, propentofylline is a xanthine derivative that has been studied.
as a treatment for Alzheimer’s disease and vascular dementia, and also possesses the ability to attenuate glial activation.

5. Opioids and central immune signaling

5.1. Animal behavioral immune signaling evidence of a critical role for opioid-induced central immune signaling

The first crucial evidence that there may be a link between central immune signaling and the primary human response to continual exposure to drugs of abuse, i.e. reward and development of dependence, has arisen from preclinical animal behavioral models. Numerous in vivo animal behavioral studies have demonstrated that central immune changes impact morphine responses manifested as altered locomotor activity, behavioral reward (CPP) and withdrawal (naloxone-precipitated), and levels of opioid self-administration. Table 1 provides a summary of these study observations, which will be discussed briefly below.

Central immune signaling modulation of morphine behavior was first implicated by studies which revealed that direct injection of astrocyte-conditioned medium (that contained important soluble co-factors such as cytokines and chemokines) into the nucleus accumbens heightened morphine CPP (Narita et al., 2006). This study, and others to follow further highlighted the role of central immune signaling following observations that proinflammatory glial activation attenuators could: block morphine CPP (Narita et al., 2006; Hutchinson et al., 2008b, 2009a); block morphine and oxycodone spontaneous and precipitated withdrawal, and simultaneously prevent brain increases or caused brain decreases of cytokines and chemokines in various brain regions shown to mediate withdrawal (e.g. in the ventral tegmental area and in the nucleus accumbens (Hutchinson et al., 2009a); and block morphine-induced elevations of dopamine in the nucleus accumbens (Bland et al., 2009)). Further studies have shown other immune mediators to also play a role via alteration in downstream events to initial glial activation. For example, TLR4 involvement is implicated as (+)-naloxone, a functional TLR4 antagonist with no opioid receptor activity, significantly attenuated morphine withdrawal (Hutchinson et al., 2010c), while (+)-morphine (opioid receptor inactive, TLR4 receptor active) blocked (−)-morphine CPP (Terashvili et al., 2008). More indirectly, aquaporin expression (strongly expressed by astrocytes and involved in regulation of cerebral GLT-1 expression) is related to

<table>
<thead>
<tr>
<th>Behavior type</th>
<th>Drug of abuse</th>
<th>Intervention</th>
<th>Influence of Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Withdrawal</td>
<td>Opioid</td>
<td>(+)-naloxone</td>
<td>↑ via NMDA receptor phosphorylation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>metalloproteinas-9</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exogenous IFN-α</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exogenous IL-2</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exogenous IL-1(α)</td>
<td>↓</td>
</tr>
<tr>
<td>Craving</td>
<td>Cocaine</td>
<td>Exogenous GDNF</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Methamphetamine</td>
<td>mGlur5 antagonist</td>
<td>↑</td>
</tr>
<tr>
<td>Conditioned place preference (CPP)</td>
<td>Cocaine</td>
<td>CD81 knockout</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Methamphetamine</td>
<td>Astrocyte-conditioned media</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Jak/STAT inhibition</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GDNF</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Microsomal epoxide hydrolase knockout</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exogenous TNF-α</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TNF-α knockout</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Propentofylline</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>Morphine</td>
<td>Astrocyte-conditioned media</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(+)-Morphine</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Minocycline</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Propentofylline</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Budilast</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exogenous TNF-α</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exogenous Leu-ile</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exogenous INF-α</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exogenous IL-2</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TNF-knockout</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GDNF heterozygous (+/−)</td>
<td>↑</td>
</tr>
<tr>
<td>Locomotor sensitization</td>
<td>Cocaine</td>
<td>CXCRR4 antagonist</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Methamphetamine</td>
<td>LPS challenge</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exogenous TNF-α</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TNF-α knockout</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>Morphine</td>
<td>Fibroblast growth factor-1 (FGF-1)</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exogenous Leu-ile</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Exogenous TNF-α</td>
<td>↑</td>
</tr>
<tr>
<td>Self-administration</td>
<td>Alcohol</td>
<td>CCL2, CCR2, CCL3 knockout</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td>Cocaine</td>
<td>GDNF</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Methamphetamine</td>
<td>GDNF deficiency</td>
<td>↑</td>
</tr>
<tr>
<td>Sedation</td>
<td>Alcohol</td>
<td>IL-1Ra</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td>Methamphetamine</td>
<td>GDNF deficiency</td>
<td>↑</td>
</tr>
<tr>
<td>Motor impairment</td>
<td>Alcohol</td>
<td>Minocycline</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>or ⇔</td>
<td>↓</td>
</tr>
</tbody>
</table>

↑ indicates an increase, ↓ indicates a decrease, and ⇔ indicates no change in the behavior.

Withdrawal was assessed by a multi-factorial behavioral score that included symptoms such as jumping, wet dog shakes, tremor, ptosis, posture, diarrhea, chewing, exploratory behavior and teeth chatters.

Withdrawal was assessed by scoring of jumping behavior only.
physical dependence and withdrawal (Wu et al., 2008); and blockade of spinal matrix metalloproteinase-9 (MMP-9, which is expressed in neurons and glia and modulates inflammatory responses via direct cleavage of proteins including cytokines) reduced morphine withdrawal (Liu et al., 2010). Interestingly, a novel opioid agonist, PTh-609, developed by Pain Therapeutics (Burns & Wang, 2010), that combines filamin A inhibitor functions and is also capable of reduced TLR4 proinflammation (i.e. decreased TLR4 signaling) has been found in animal models to have limited CPP reward, whilst maintaining opioid analgesia. Collectively, these are key data demonstrating that the beneficial analgesic actions of opioids can be separated from the unwanted rewarding properties, by either co-administration of glial targeted activation inhibitors or rational drug design to avoid activation of central immune signaling.

Morphine exposure causes a time-dependent increase in locomotor activity, termed locomotor sensitization as repeated administration over time causes an enhancement of the motor activating effect of morphine. Several immune factors have been implicated in modulating the establishment of sensitization: fibroblast growth factor-1 (FGF-1) whose receptor is expressed not only on neurons but also on astrocytes in the ventral tegmental area (Flores et al., 2010); PEA-15 (phosphoprotein in astrocytes needed for FADD-ERK binding) (Ramos-Miguel et al., 2010); TNF-α (Niwa et al., 2007b); and CDNF (Niwa et al., 2007a).

Given the increasing volume of evidence indicating a central immune role in modulation of animal behavioral responses, specifically reward and dependence, of opioids, it is pivotal to examine the underlying biological and molecular mechanisms (see relevant section below) and how these then alter the neuronal consequences and subsequently the behavior.

5.2. Indirect human evidence of possible opioid-induced immune signaling

In order to extrapolate conclusions regarding the role of central immune signaling from animal behavioral studies in response to opioids discussed above to the in vivo human state of dependence, it is necessary to investigate if similar central immune changes are evident in human samples. Obviously, the most comprehensive and conclusive evidence can be observed via immunohistochemical analysis of post-mortem brains of opioid users (classified after death according to the presence of heroin’s metabolite monoacetyl-morphine, morphine or methadone in blood and hair samples) versus nonusers. However, other supporting evidence has been reported by studies that have utilized ex vivo peripheral blood mononuclear cell (PBMC) challenge responses in heroin addicts in comparison to treatment with either methadone or buprenorphine, perhaps indicating specificity of response to some opioids (Sacerdote et al., 2008). In summary, the human post-mortem and peripheral immune cell studies showed similar observations to the data of in vivo animal behavioral models, indicating that proinflammatory central immune events do occur following opioid administration and abuse in humans. The clinical importance of this conclusion is supported by the fact that a Phase II trial is currently underway to determine the clinical use of ibudilast (AV411) as a novel treatment for opioid dependence (US NIH Clinicaltrials.gov database).

5.3. Human genetic evidence of a possible role of central immune signaling in opioid abuse

Given that up to 60% of opioid dependence is heritable (Tsuang et al., 1998), if central immune signaling is indeed a major contributor to the development of opioid dependence then another crucial step is to determine whether or not genetic variability in immune modulators (e.g. proteins and receptors in immune signaling pathways) is associated with dependence. To date, only two studies investigating this association with opioid dependence have positively linked the following genes with risk of dependence (Table 3): IL-1β (−511C/T and −31T/C) (Liu et al., 2009); and prodynorphin (PDYN) in females only (rs1997794 in the promoter and rs1022563 in the 3’ untranslated region, UTR) (Clarke et al., 2009). With regard to the how these genes agree with the central hypothesis of proinflammation linked to risk of dependence, the IL-1β wild-type sequence at −511 and −31 is associated with increased IL-1β release, hence it is hypothesized that carriers of the wild-type have greater central immune signaling and proinflammatory responses following opioid exposure (Liu et al., 2009) and hence greater risk of dependence. In contrast, although the SNPs in the PDYN gene are known to lie in the regulatory regions of the genes and therefore likely to influence expression of prodynorphin, the functional impact of these SNPs, i.e. either increased or decreased expression, are yet to be characterized. Interestingly, the central immune signaling consequence of prodynorphin expression and subsequent conversion to dynorphin A may lie in the recent discovery that the opioid inactive degradative product dynorphin A 2−17 causes p38 MAPK signaling in microglia and is responsible for dynorphin A-induced elevations in prostaglandin E2 (PGE2) (Svensson et al., 2005).

In summary, the research conducted to date using in vivo animal behavioral models and human studies have established that central immune signaling is an important contributor to the development of dependence following continued exposure to opioids. Given the complexity of immune signaling, it is necessary to review the biological and molecular studies in order to fully appreciate the specific pathways involved.

5.4. Biological and molecular evidence for central immune signaling induced by opioids

Numerous in vivo animal studies and in vitro cell culture studies have revealed that opioids, specifically morphine, can cause microglia and astrocytes to assume a proinflammatory phenotype. In the context of this review this is indicated by the presence and/or elevation of specific glial activation markers: glial fibrillary acidic protein, GFAP for astrocytes; and integrin alpha M, CD11b for microglia. Importantly, this proinflammatory phenotype induced by morphine has been observed to be brain region specific and in some regions glial cell type specific. For example, astrocytes are activated following chronic morphine administration in the locus ceruleus and nucleus of the solitary tract (Alonso et al., 2007), lateral septal nucleus (Lazriev et al., 2001), ventral tegmental area, nucleus accumbens, dentate gyrus, Cornu ammonis of the hippocampus, dorsal periaqueductal gray, medial prefrontal cortex, ventral periaqueductal gray, caudate putamen, rostral ventromedial medulla and trigeminal nucleus, but not in the dorsal raphe nucleus, caudate nucleus and the substantia nigra (Lazriev et al., 2001; Hutchinson et al., 2009a). In contrast, microglia are only activated by chronic systemic morphine in the ventral tegmental area, dentate gyrus, dorsal periaqueductal gray, ventral periaqueductal gray, caudate putamen, rostral ventromedial medulla and the trigeminal nucleus, but not in the nucleus accumbens, C. ammonis of the hippocampus, substantia nigra, medial prefrontal cortex and dorsal raphe nucleus (Hutchinson et al., 2009a). Definitive evidence that this is indeed glial cell activation comes from observations that the activation is sensitive to the administration of glial attenuators such as ibudilast (Hutchinson et al., 2009a) and propentofylline (Narita et al., 2006). Therefore, increases in glial activation, and hence proinflammatory central immune phenotype has been demonstrated in specific brain regions of importance in opioid withdrawal, i.e. ventral tegmental area, nucleus accumbens, dentate gyrus,
Table 2
Changes in immune/glial cell markers observed following drugs of abuse exposure in post-mortem studies (post-mort) from drug dependent individuals and in ex vivo peripheral blood mononuclear cell (PBMC) studies from drug dependent individuals following immune challenge. Direction of change: ⇔ = no change; ↑ = increase; and ⇓ = decrease. Type of sample: post-mort = post-mortem; and PBMC = peripheral blood mononuclear cell. Glial cell type: MG = only in microglia; and AS = only in astrocytes. Brain region involved: Am = amygdala; NAcc = nucleus accumbens; CaPut = caudate putamen; FrCor = frontal cortex; LoCer = locus ceruleus; HP = hippocampus; SN = substantia nigra; and VTA = ventral tegmental area.

<table>
<thead>
<tr>
<th>Immune/glial cell marker</th>
<th>Drug of abuse</th>
<th>Opioid</th>
<th>Alcohol</th>
<th>Cocaine</th>
<th>MDMA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytokine expression</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>post-mort 1: ↑ TNF-α (LoCer)</td>
<td></td>
<td>PBMC, spontaneous 2: ○ IL-1β, IL-6, TNF-α, IFN-γ</td>
<td>PBMC after cocaine 7: ○ IL-10, ○ IFN-γ</td>
<td>PBMC 10, 11: ○ IL-2, IL-6, TNF-α, IFN-γ</td>
<td>PBMC 13: ○ IL-4, IL-10, TGF-β</td>
</tr>
<tr>
<td>PBMC 2, 3: ○ TNF-α, IL-4, IFN-γ</td>
<td>⇔ IL-2</td>
<td>PBMC challenge 5: ○ TNF-α</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBMC gene expression 4:</td>
<td>↑ TNF signaling cascade, IL-2 signaling cascade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>○ IL-1β</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytokine receptor expression</td>
<td>PBMC gene expression 4:</td>
<td>○ TGFβR3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemokine expression</td>
<td>PBMC gene expression 4:</td>
<td>○ CXCL3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monomeric p-FADD expression</td>
<td>post-mort 14: ○ (FrCor)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total FADD expression</td>
<td>post-mort 14: ○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-ERK1/2 expression</td>
<td>post-mort 14: ○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-JNK1/2 expression</td>
<td>post-mort 14: ○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Akt expression</td>
<td>post-mort 14: ○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEA-15 expression</td>
<td>post-mort 14: ○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-38-MAPK expression</td>
<td>post-mort 14: ⇔</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iNOS expression</td>
<td>PBMC gene expression 4: ○ MAPK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOS 1/3</td>
<td>post-mort 1: ↑ (LoCer)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superoxide anion formation</td>
<td>PBMC gene expression 4: ○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD68 expression</td>
<td>post-mort 15: ○ (HP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFAP expression</td>
<td>post-mort 16: ○ (HP)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1 expression</td>
<td>PBMC 17: ○</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apoptosis pathway</td>
<td>PBMC gene expression 4:</td>
<td>○ apoptosis inhibitor 5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References: 1Dyuizen and Lamash (2009); 2Sacerdote et al. (2008); 3Peterson et al. (1987); 4Laso et al. (2007); 5Mao et al. (1996); 6Gan et al. (1998); 7Irwin et al. (2007); 8Halpern et al. (2003); 9Paci fi ci et al. (2001); 10Paci fi ci et al. (2004); 11He and Crews (2008); 12Nair et al. (2000); 13Ramos-Miguel et al. (2009); 14Chao et al. (1991); 15Oehmichen et al. (1996); and 16Song et al. (2002).
hippocampus, and the dorsal periaqueductal gray. An additional brain region specific effect is morphine-induced proliferation of astrocytes: morphine has been shown to inhibit astrocyte proliferation from the cortex, hippocampus and striatum (Stiene-Martin et al., 1991; Stiene-Martin & Hauser, 1993), but appears to have no effect on proliferation in the nucleus accumbens, lateral septal nucleus or caudate nucleus (Lazrie et al., 2001).

However, transition of glial cells to a proinflammatory phenotype is simply the first step in the complicated network of pathways that determines the central inflammatory environment, without which, there would be no impact on neuronal response to opioids. This additional evidence of an increased proinflammatory central environment stems from the occurrence of a plethora of downstream events to the initial upregulation of glial activation markers, including an increase in the synthesis and release of proinflammatory cytokines and chemokines (see Table 4 for summary of these events and individual study citations). Importantly, this evidence has been characterized in both in vivo animal models and in vitro primary glial (astrocyte and microglia) cell culture models using multiple complimentary methods (e.g. Western blot, ELISA, RT-PCR and immunohistochemistry/immunofluorescence). Further, these opioid-induced changes can collectively be viewed as proinflammatory and were observed following both acute and chronic exposure, and were time-, dose- and brain region-dependent, which has resulted in some discrepancies between individual study conclusions. For example, an in vitro study demonstrated 24 h morphine exposure of human microglial cells did not alter TNF-α release (El Ghazi et al., 2010), while others have clearly shown increases in TNF-α expression in mouse microglia from various regions following longer (9 days) in vivo exposure (Niwa et al., 2007b). These observations may therefore be explained by species differences, time-dependency or type of exposure (in vitro versus in vivo) required to elicit the effect. An additional explanation may be found in the lack of biotransformation in in vitro systems, thereby omitting the possible role metabolites may play in initiating central immune signaling cascades. This may be key based on the data from Lewis et al. that has shown morphine-3-glucuronide is an active TLR4 agonist to alter IL-1β expression (Lewis et al., 2010) (see section below). Consequently, it will be crucial to address specific rodent to human species discrepancies and the role of key metabolites to ascertain the relevance of the wealth of data being generated in rodent models and the potential future requirement to validate these data in human tissue analogs.

The receptors involved in modulating proinflammatory glial activation in response to morphine have been implicated by animal models employing either pharmacological or genetic knockout blockade design and are summarized in Table 5. In brief these include: α2-adrenoceptor, opioid, chemokine, P2X4 (ionotropic purinergic receptor), Toll-like receptor 4 (TLR4 and myeloid differentiation factor-2 (MD-2)), TLR2 (Zhang et al., 2011) and TLR9 (He et al., 2011). In addition, TLR4 receptor activation has been implicated in the non-stereoselective activity of opioid inactive (+)-isomers and opioid active (−)-isomers of methadone and morphine-induced microglial activation (evidenced by increased IL-1β expression) in the spinal cord (Hutchinson et al., 2010a, c). Moreover, these responses can be non-stereoselectively blocked by naltrexone and naltrexone (Hutchinson et al., 2010a, c). This TLR4 activity of (+)-naltrexone (Hutchinson et al., 2008c) extends to the classical ligands of TLR4 with LPS-induced glial activation also blocked by (+)-naltrexone (Liu et al., 2000b). Further, blockade of the opioid receptors by (−)-naltrexone dose-dependently altered in glial activation in response to chronic morphine administration, with ultra-low doses (5 ng) increasing activation in the spinal cord (Mattioli et al., 2010), but larger doses (1 μM, 60 μg) blocking activation in vitro and in vivo (Takayama & Ueda, 2005a; Hutchinson et al., 2010a).

In summary, numerous biological and molecular events occur following opioid exposure that are mediated by a variety of receptors including Toll-like receptors to alter central immune signaling towards a proinflammatory phenotype. It is these changes that then impact on the glial–neuronal cross-talk to modulate neuronal responses including reward and dependence, and thereby are the underlying mechanisms by which central immune signaling is hypothesized to alter behavioral responses.

### 5.5. Indirect opioid-induced responses that impact central immune signaling

Aside from the direct evidence that opioids directly induce central immune signaling, other indirect effects are of equal importance in altering the inflammatory environment. For example, morphine-induced in vitro microglial migration occurs following chronic administration and is mediated in part by opioid and P2X4 receptors, via

<table>
<thead>
<tr>
<th>Drug of abuse</th>
<th>Gene</th>
<th>SNP</th>
<th>Functional change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opioid</td>
<td>IL-1β</td>
<td>−511, −31</td>
<td>Wild-type: † expression</td>
</tr>
<tr>
<td></td>
<td>PDYN</td>
<td>rs1997794 promoter</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1022563</td>
<td>Unknown</td>
</tr>
<tr>
<td>Alcohol</td>
<td>IL-1β</td>
<td>−511, −31</td>
<td>Wild-type: † expression</td>
</tr>
<tr>
<td></td>
<td>IL-1RN</td>
<td>VNTR in intron 2</td>
<td>Allele 1: † expression</td>
</tr>
<tr>
<td></td>
<td>IL-10</td>
<td>−592</td>
<td>A variant: † expression</td>
</tr>
<tr>
<td></td>
<td>NKA2</td>
<td>rs2072450</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs9924016</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>NPsB</td>
<td>8 SNPs spanning 5′-UTR, Introns 3–4, 8, 15, 22–23</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>NKIR</td>
<td>3 SNP haplotype</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>TACR3</td>
<td>1 SNP in intron 3</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 SNP in 3′-UTR</td>
<td>Unknown</td>
</tr>
<tr>
<td>Cocaine</td>
<td>PDYN</td>
<td>3 SNP haplotype in 3′-UTR</td>
<td>Haplotype: lower mRNA prodynorphin</td>
</tr>
<tr>
<td></td>
<td>NPY2R</td>
<td>5 SNPs spanning 5′UTR, promoter and 3′UTR</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td>TACR3</td>
<td>1 SNP in intron 1</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 SNPs in 3′-UTR</td>
<td>Unknown</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>BDNF</td>
<td>Val86Met</td>
<td>Val: optimal BDNF secretion</td>
</tr>
<tr>
<td></td>
<td>PDYN</td>
<td>VNTR promoter</td>
<td>3–4 repeat: † promoter activity and expression</td>
</tr>
<tr>
<td></td>
<td>GLYT1</td>
<td>IVS3 + 411</td>
<td>Unknown</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1056</td>
<td>Unknown</td>
</tr>
</tbody>
</table>
Table 4
Summary of changes in glial (astrocyte or microglia) cellular events observed following exposure to drugs of abuse that alters central immune signaling from in vitro cell culture and in vivo rodent studies. Direction of change: ↔ = no change; ↑ = increase; and ↓ = decrease. Glial cell type: MG = only in microglia; and AS = only in astrocytes. Brain region involved: NAcc = nucleus accumbens; CaPut = caudate putamen; CCor = cerebral cortex; FrCor = frontal cortex; HP = hippocampus; HyP = hypothalamus; St = striatum; and StNig = substantia nigra. Impact of pharmacological glial attenuators: MINO = sensitive to minocycline; and PROPEN = sensitive to propentofylline.

<table>
<thead>
<tr>
<th>Cellular event</th>
<th>Drug of abuse</th>
<th>Opioid</th>
<th>Cocaine</th>
<th>Methamphetamine</th>
<th>MDMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine synthesis and release</td>
<td>↑ TNF-α (NAcc, CaPut)</td>
<td>↑ TNF-α, IL-1β, IL-6 (AS)</td>
<td>↑ TNF-α, IL-1β, IL-6, IFN-γ (MG; HP, St, FrCor)</td>
<td>↑ TNF-α, IL-1β</td>
<td>↓ IL-1β (MG; FrCor; MINO)</td>
</tr>
<tr>
<td>Cytokine receptor expression</td>
<td>↑ IL-1R (AS)</td>
<td>↑ TNFR1 (MG)</td>
<td>↑ TNFR1 (MG)</td>
<td>↑ TNFR1 (MG)</td>
<td>↑ TNFR1 (MG)</td>
</tr>
<tr>
<td>Chemokine synthesis and release</td>
<td>↑ CCL5, MCP-1</td>
<td>↑ CXCL10 (AS)</td>
<td>↑ CCL2/MCP-1, MCP-1 (MG)</td>
<td>↑ CB2 (MG)</td>
<td></td>
</tr>
<tr>
<td>Chemokine receptor expression</td>
<td>↑ CCR3, CCR5</td>
<td>↑ CCL2/MCP-1 (HP)</td>
<td>↑ CCL2/MCP-1 (MG; HP, St, FrCor)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracellular calcium</td>
<td>↑ creatinine, taurine, thymidine</td>
<td>↑ creatinine, taurine, glutamate (AS)</td>
<td>↑ creatinine, taurine, glutamate (AS)</td>
<td>↑ creatinine, taurine, glutamate (AS)</td>
<td></td>
</tr>
<tr>
<td>Superoxide anion formation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free radical formation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive nitrogen species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid peroxidation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitric oxide</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostaglandin E2 (PGE2) synthesis and release</td>
<td>↑ (MG)</td>
<td>↑ (AS)</td>
<td>↑ (AS)</td>
<td>↑ (MG; HP, CCor)</td>
<td>↑ (AS)</td>
</tr>
<tr>
<td>Pro-apoptosis pathway: caspase-3 expression/activity</td>
<td>↑ (MG); ↓ during withdrawal (HP, Cor)</td>
<td>↑ (AS)</td>
<td>↑ (AS)</td>
<td>↑ (MG; HP, CCor)</td>
<td>↑ (AS)</td>
</tr>
<tr>
<td>Category</td>
<td>Expression</td>
<td>References</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>------------</td>
<td>------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclooxygenase (COX-1, COX-2) expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microsomal epoxide hydrolase expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heme oxygenase 1 expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opioid receptor expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRAK expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Akt expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Src expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-COX-3p (glucocerebrosyl synthase kinase 3-beta)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-38 MAPK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p70 S6 activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERK/MAPK signaling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein kinase C activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CREB activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RhoE GTPase signaling</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF-kB activation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NF-kB DNA binding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP-1 DNA binding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepro-nociceptin/orphanin FQ</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoskeletal proteins and associated chaperones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biosynthesis proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineral binding/channel proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Signal transduction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transport proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell cycle proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other proteins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell viability</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References: 1Niwa et al. (2007b); 2Sawaya et al. (2009); 3Valles et al. (2004); 4Qin et al. (2008); 5Alfonso-Loeches et al. (2010); 6Nakajima et al. (2004); 7Gonçalves et al. (2008); 8Buchanan et al. (2010); 9Gonçalves et al. (2010); 10Jung et al. (2010); 11Roachus et al. (2010); 12Siriam et al. (2006); 13Orio et al. (2010); 14El-Hage et al. (2005); 15El-Hage et al. (2006); 16Avdoshina et al. (2010); 17Davis and Syapin (2004b); 18Yao et al. (2010); 19Mahajan et al. (2005a); 20Torres et al. (2010); 21Ikeda et al. (2010); 22Fitting et al. (2010); 23González et al. (2007); 24Salazar et al. (2008b); 25El Ghazi et al. (2010); 26Smith and Navratilova (1999); 27Aschner et al. (2001b); 28Aschner et al. (2001c); 29Othman et al. (2002); 30D'Ascenzo et al. (2007); 31Colton et al. (1998); 32Gonthier et al. (1997); 33Horeni et al. (2010); 34Luo et al. (2000); 35Banno et al. (2005); 36Magazine et al. (1996); 37Wang and Sun (2001); 38Davis and Syapin (2004a); 39Blanco et al. (2005); 40Hu et al. (2002); 41Xie et al. (2010); 42Emeterio et al. (2006); 43Hutchinson et al. (2008b); 44Shin et al. (2009); 45Huang et al. (2009); 46Turcich-Chiolewe et al. (2008); 47Takayama and Ueda (2005a); 48Horvath and Delero (2009); 49Smith and Navrilova (2003); 50Hebert and O'Callaghan (2000); 51Roger et al. (2009); 52Miyatake et al. (2009); 53Narita et al. (2006); 54Reynolds et al. (2006); 55Narita et al. (2005); 56Guasch et al. (2007); 57Davis and Syapin (2004c); 58Takayama and Ueda (2005b); 59Suder et al. (2009); and 60Straiko et al. (2007).
activation of the PI3K (phosphoinositide 3-kinase)/Akt signaling pathway (Takayama & Ueda, 2005a; El-Hage et al., 2006; Horvath & DeLeo, 2009). Interestingly, P2X4 is required for activation of the NLRP3 inflammasome following a Toll-like receptor 2 (TLR2) or TLR4 signal that enables cleavage of immature pro-IL-1β to the mature form (Cassel & Sutterwala, 2010; Babelova et al., 2009). As such, the role of TLR2 or TLR4 in P2X4-dependent morphine migration deserves further investigation. However, the link between in vitro observations of microglial migration and the behavioral endpoint of opioid reward and dependence is yet to be explored.

Another interaction at microglia that is of increased clinical importance is the interaction in opioid dependent individuals who are also HIV positive, is that morphine increased HIV-Tat-induced in vitro microglial proinflammatory activation, production of reactive oxygen species (ROS) and increased intracellular calcium (El-Hage et al., 2005; Turchan-Cholewo et al., 2009). The combined effect on cytokine release however, is not consistent between studies, with reports of attenuation of the Tat-induced release of TNF-α, IL-6, MCP-1, MCP-5 and CCL5/RANTES (El-Hage et al., 2005; Turchan-Cholewo et al., 2009), and others of increased TNF-α expression (Mahajan et al., 2008; Sawaya et al., 2009). However, at some concentrations (10−10 to 10−6 M), morphine inhibits microglial migration and production of CCL5/RANTES (Hu et al., 2000). This likely reflects dose-dependent changes in the interaction, with lower morphine causing an increase, and higher morphine blocking the increase. Further, although these effects were resolved following short-term exposure (Turchan-Cholewo et al., 2009), the increase in ROS is thought to contribute to long-term neurotoxicity. Another important clinical consequence of the interaction between morphine and HIV-1-Tat is regulation of the blood–brain barrier, with decreased tight-junctions observed in an in vitro model, that consequently allows increased cellular migration (Mahajan et al., 2008). Indeed, the presence of the HIV-Tat protein was observed to potentiate morphine-induced microglial migration (El-Hage et al., 2006). Further, there is also some evidence that general peripheral immune responses are tied to dependence behavior, such that morphine withdrawal increased the lethality of LPS challenge that corresponded to elevated serum expression TNF-α, IL-6, INOS and NO and lowered IL-12 (Feng et al., 2005).

In summary, these indirect actions of opioid such as cell migration, production of RO5 and altered blood–brain barrier permeability linked to neurotoxicity, and general sickness responses are also likely contributors to the overall immune state, central and peripheral, and consequently, to opioid behaviors such as reward and dependence.

5.6. Effect of morphine metabolites on central immune signaling

The main metabolites of morphine, morphine-3- and morphine-6-glucuronide are yet to be fully characterized with regard to actions on central immune signaling. Recently, however, morphine-3-glucuronide was shown to activate TLR4 and cause subsequent in vivo microglial proinflammatory activation (isolated from the spinal cord) and release of IL-1β, with similar in vivo effects also observed (Lewis et al., 2010). While an earlier study reported morphine-6-glucuronide dose-dependently decreased ex vivo lymphocyte stimulated proliferation and IFN-γ production (Carrigan & Lysle, 2001). Given that both glucuronide metabolites are found in the plasma (Mazoit et al., 2007), basally and elevated following brain injury in the CSF (Morgan et al., 2008), at concentrations equal to or exceeding morphine, it is likely that these metabolites are important contributors to glial activation and associated downstream proinflammatory events induced following morphine administration.

5.7. Summary and significance of opioid-induced central immune signaling

It is apparent from the literature reviewed here on opioid-induced proinflammatory central immune signaling, that these non-neuronal pathways add significantly to the complexity of the CNS environment in which opioid-induced activation of the mesolimbic dopamine reward pathway contributes to the abuse potential of opioids. Owing to several diverse pharmacological studies demonstrating both behavioral and neurochemical modifications of opioid reward and withdrawal, opioid-induced central immune signaling clearly has a significant impact on the mesolimbic dopamine reward system. The manner and time course in which glial cells are initially engaged and the ongoing proinflammation modulates this system following opioid exposure remain to be fully characterized, but it is apparent that TLR4 and the associated MAPK signaling cascade are key new participants. The identification of TLR4 as a key initial mediator has also highlighted the importance of the opioid metabolite morphine-3-glucuronide in triggering proinflammatory central immune signaling, possibly explaining some in vivo versus in vitro discrepancies observed in the animal models. In addition, the behavioral significance of classical opioid receptors expressed by glia remains to be determined, as do other possible accessory systems such as P2X4 activation of the NLRP3 inflammasome and formation of mature IL-1β). The neuronal consequence of opioid-induced...
proinflammatory immune signaling appears to be largely dependent on proinflammation-induced down-regulation of glutamate transporter expression, which can lead to a dysregulation of extracellular glutamate (Ozawa et al., 2001; Nakagawa & Satoh, 2004; Ozawa et al., 2004; Nakagawa et al., 2005), potentially contributing at minimum to opioid withdrawal (Nakagawa & Satoh, 2004). The opioid-induced modulation of glial derived neurotrophic factors such as GDNF are also of significant interest owing to the established behavioral significance for GDNF on nucleus accumbens dopamine levels and the expression of reward behaviors (Airavaara et al., 2004; 2006). However, the significance and consequences of regional heterogeneity of opioid-induced central immune signaling are at present unclear. Fortuitously, the human relevance of these rodent, cellular and molecular studies has some support in the human post-mortem, PBMC and immunogenetic studies linking central immune responses to opioid abuse and likely the key to abuse susceptibility. The clinical and therapeutic opportunities of these and other discoveries will be discussed later in this review. We will now review the evidence of central immune signaling modulating response to the other drugs of abuse with the use of identical subheadings in order of importance to dependence as discussed above.

6. Alcohol and central immune signaling

6.1. Animal behavioral evidence of a critical role for alcohol-induced central immune signaling

Several animal studies have examined the role of central immune signaling in altering behavioral responses to alcohol, including reward and withdrawal. These studies are summarized in Table 1 and discussed briefly below.

The first studies conducted in the late 1990s linked maze performance that assessed learning, memory, motor impairment and motivation to astrocyte activation (indicated by GFAP staining) in the hippocampal regions of Wistar rats following chronic ethanol exposure for 4, 12 or 36 weeks (Franke et al., 1997). Rats which had received ethanol for 36 weeks had increased errors during the maze and also decreased neuronal cell numbers and astrocyte staining in the CA-1, CA-2 and dentate gyrus regions of the hippocampus (Franke et al., 1997). However, it was not possible to distinguish if the behavioral difference was due to neuronal or astrocytic changes. Later studies also observed decreases in the density of astrocytes in rats that were alcohol-naïve or alcohol-drinking alcohol-preferring compared to alcohol-nonpreferring and Wistar rats, and that ethanol withdrawal was associated with increased astrocyte numbers in the prelimbic cortex of alcohol-prefering rats (Miguel-Hidalgo, 2005; 2006).

More recently, a role for proinflammatory chemokines and their receptors has been implicated in both preference and level of self-administration of alcohol and motor impairment following alcohol, with CCR2, CCL2 (females) and CCL3 knockout mice displaying lower alcohol place preference and alcohol self-administration, and CCL2 and CCL3 knockouts having longer motor impairment (loss of righting reflex) following alcohol which was not observed with CCR2 and CCR5 knockout (Blednov et al., 2005). Our group has also implicated cytokines and their receptors, specifically IL-1β in mediating acute alcohol-induced sedation and motor impairment that is minocycline sensitive (Wu et al., 2011). Other recent work by Pascual and colleagues has linked TLR4 involvement with alcohol locomotor activity, memory tasks and anxiety following chronic exposure, demonstrating the genetic knockout abolished the decrease in activity and memory and increase in anxiety (Pascual et al., 2011). In addition, a role for mGluR5 that is expressed by astrocytes (Schools & Kimelberg, 1999) has been implicated in alcohol self-administration in rats genetically primed to different drinking preferences (McMillen et al., 2005; Schroder et al., 2005; Besheer et al., 2010). Finally our group has recently shown a role for microglial activation and IL-1β in acute alcohol-induced sedation, with administration of minocycline and IL-1Ra blocking the sedation (Wu et al., 2011), however, with respect to motor impairment (assessed by rotorod test) only IL-1Ra was able to block alcohol effect. Collectively, these recent studies once again highlight the significance of the central immune component in alcohol behaviors, but also suggest subtle differences in mechanisms dependent on the behavior.

Another recent study has investigated ethanol-induced in vivo gene expression changes in the nucleus accumbens in rats who prefer alcohol consumption with the following pivotal gene groups identified: anti-apoptosis, regulation of kinase activity and MAPK activity, regulation of signaling, and oncogenes (for review see Bell et al. (2009)). This data has been supported by recent work from Blednov and colleagues demonstrating that genetic knockout of immune signaling (β2-microglobulin, cathepsin S, cathepsin F, IL-1Ra, CD14 and IL-6) can reduce alcohol consumption and preference (Blednov et al., 2012), while LPS-induced of immune signaling can increase alcohol consumption in several mouse strains (Blednov et al., 2011). Further, TLR4 involvement in the LPS response was implicated as genetic knockout of the CD14 accessory protein abolished the LPS-induced increase in consumption. Minocycline, another pharmacological glial attenuator was also observed to decrease alcohol consumption in mice (Agrawal et al., 2011). However, not all alcohol behaviors were altered as there was no impact of LPS administration on alcohol CPP (Blednov et al., 2011), once again highlighting the complexity of the interaction between central immune signaling and alcohol response. Nonetheless, the collective lines of evidence point towards proinflammatory central immune signaling contributing to enhanced alcohol action.

6.2. Indirect human evidence of possible alcohol-induced central immune signaling responses

Following on from the animal behavioral studies, human studies of post-mortem brain gene expression changes have also provided evidence of a role of central immune signaling linked to alcohol dependence (Table 2). For example, post-mortem human brains from alcoholics in comparison to healthy controls revealed there are brain region specific differences (different in the frontal cortex versus the motor cortex) in genes related to immune function, cell viability and signaling and interestingly, GFAP was observed to be down-regulated in the frontal cortex (for review of individual genes see Liu et al., 2004). Further evidence of proinflammatory activation comes from elevated MCP-1 protein in the ventral tegmental area, substantia nigra, hippocampus and amygdala of brains from alcoholics compared to non-alcoholics (He & Crews, 2008).

Other studies have examined the release of immune mediators from PBMCs isolated from alcoholics with the following differences compared to PBMCs from healthy controls: increased spontaneous release of IL-1β, IL-6, TNF-α and IFN-γ (Laso et al., 2007), and decreased stimulated release of TNF-α, while PBMCs from alcoholics with cirrhosis had lower IFN-γ and higher IL-6 (Laso et al., 1997; Nair et al., 2000). It appears that these mediators may be elevated as a result of liver damage associated with alcoholism and hence, using these as biomarkers for dependence is likely to be confounded by any associated liver pathology (for review see Achur et al., 2010). It is interesting to speculate what the impact of alcohol-induced liver disease and the accompanying peripheral inflammation has on central immune signaling, as it could act in a feed-forward fashion to heighten proinflammatory central immune signaling and hence the individuals drive to consume additional alcohol.

Finally, there are a number of studies that have measured circulating levels (serum or plasma) of proinflammatory mediators in alcohol dependent individuals, and in comparison to healthy non-dependent individuals observed significant differences, including: elevated TGF-β (Kim et al., 2009); elevated IL-6 and IL-10 which decreased following withdrawal (Gonzalez-Quintela et al., 2000); and elevated IL-12 but no change in IFN-γ or IL-4 (Laso et al., 1998). Other studies have observed changes in circulating levels (serum or plasma) of...
proinflammatory mediators in healthy individuals following alcohol exposure, specifically reporting an increase in IL-8 (acute) (González-Quintela et al., 2000). While serum levels of neutrophilic factors have also been studied revealing significantly lower GDNF levels in alcohol dependent subjects compared to controls (Heberlein et al., 2010). Further, observed levels of BDNF and GDNF during early withdrawal were related to withdrawal severity and tolerance, such that high BDNF levels were related with lower withdrawal scores and high GDNF levels were related to lower alcohol tolerance (Heberlein et al., 2010).

In summary, human alcohol studies, in particular the post-mortem investigations, have similarly to opioid dependence provided the evidence to link human alcohol dependence to increased central immune signaling.

6.3. Human genetic evidence of a possible role of central immune signaling in alcohol abuse

Up to 60% of alcohol dependence is related to heritable factors, some of which may be explained by genetic variability in immune signaling. In contrast to opioid dependence there have been a number of candidate gene studies that have reported a positive association with increased risk for the following genes (Table 3): IL-1β (−511 and −31 (Pastor et al., 2005b; Liu et al., 2009)); IL-1RN (variable nucleotide tandem repeat in intron 2 (Pastor et al., 2000; 2005b; Saiz et al., 2009)); IL-10 (−592 (Marcos et al., 2008a)); NR2A (NMDA receptor subunit expressed on astrocytes (Lee et al., 2010)) rs2072450 and rs9924016 (Schumann et al., 2008)); NPB (8 single nucleotide polymorphisms (SNPs) in the 5′-UTR and introns 3–5, 8, 15, and 22–23 (Edenberg et al., 2008)); NKIR (neurokinin-1 receptor, haplotype of 3 SNPs (Seneviratne et al., 2009)); and TACR3 (neurokinin receptor 3, 1 SNP in 3′ region of the gene and 1 SNPs in intron 3 (Foroud et al., 2008)). In contrast, the following genes were not found to be associated with risk of dependence: BDNF (Val66Met (Matsushita et al., 2004; Tsai et al., 2005)); EAA1T2 (glial glutamate transporter, 603 (Sander et al., 2000; Foley et al., 2004)); IL-1A (−889 (Saiz et al., 2009)); IL-2 (−330 (Marcos et al., 2008b)); IL-4 (−33 (Marcos et al., 2009)); IL-6 (−174 (Marcos et al., 2009)); IL-8 (−251 (Marcos et al., 2009)); IL-12 (1188 (Marcos et al., 2009)); NPY2R (neuropeptide Y receptor, 3 SNPs 5′ upstream and in the promoter (Wetherill et al., 2008)); and TNFA (−238 (Pastor et al., 2005a)).

Functionally, the impact of these genetic variants largely support the hypothesis of proinflammation linked to increased risk of alcohol dependence, with specific details for the variants related to dependence as follows: i.e. IL-1β (−511 and −31 wild-type results in increased IL-1β expression (Liu et al., 2009)); IL-1RN allele 1 results in decreased IL-1Ra production (Smith & Humphries, 2009); and IL-10 (−592 A variant results in decreased IL-10 (Smith & Humphries, 2009). However, the functional impact of variants associated with dependence in the NR2A, NPB, NKIR and TACR3 genes is yet to be characterized. Following from these gene candidate studies have been a number of genome wide association studies, however, these have failed to conclusively identify and/or replicate associations between immune genetic variance and alcohol dependence (Treutlein et al., 2009; Bierut et al., 2010; Lind et al., 2010). Such issues with GWAS data have been exemplified in other studies in pathologies such as epilepsy (Kasperaviciute et al., 2010) where it was concluded that the predisposition to the pathology resulted from a myriad of related genetic targets rather than single factors. As such new approaches to analyze GWAS data that factor in multiple potential mediators is required. This is of especial need when examining immunogenetics as the signaling pathways are highly complex and controlled, translating to a myriad of sites that, if mutated, could produce the same outcome, suggesting an approach akin to the modern bioinformatic pathway or network analyzes might be required.

6.4. Biological and molecular evidence for central immune signaling by alcohol

Numerous in vivo animal studies and in vitro astrocyte cell culture studies have revealed that alcohol can proinflammatorily activate astrocytes (Blanco et al., 2005; González et al., 2007; Fernandez-Lizarbe et al., 2008; Salazar et al., 2008b). However, this is a brain region specific effect. For example, astrocytes or microglia in the molecular layer of the cerebellar cortex were not effected (Dlugos & Pentney, 2001) but astrocytes in other sub-regions of the cortex (frontal, parietal, temporal lobes and occipital) and in the striatum were activated following chronic in vivo alcohol exposure (Alfonso-Loeches et al., 2010; Udomuksorn et al., 2011). While an early study suggested that microglia were not activated by alcohol (Valles et al., 2004), more recent work has revealed a similar proinflammatory phenotype as observed with astrocytes, with an increased expression of the CD11b microglial marker in the cerebral and frontal cortex and striatum of mice treated with alcohol (Alfonso-Loeches et al., 2010). Microglial activation has also been reported in brains of alcohol dependent patients, with increased Iba-1 (another microglial activation marker) staining in the cingulate cortex and glucose transporter 5 (Glut5) staining in the cingulate cortex, ventral temporal area and midbrain (He & Crews, 2008). Further, hippocampal microglial activation (increased in MHCII or Iba-1 staining) in rats and adolescent mice exposed to alcohol in a binge-drinking regimen was also increased (Ward et al., 2009; McClain et al., 2011).

Therefore, studies have demonstrated that glial cells become activated following alcohol exposure, which as discussed above is simply the first step in the signaling process. Following this activation, a number of downstream events from initial alcohol exposure have been comprehensively characterized using in vitro and in vivo models and are summarized in Table 4. Briefly, these include increased synthesis and release of proinflammatory cytokines and chemokines, altered receptor expression and activation of the various signaling pathways such as MAP kinases. Importantly, this evidence has been gathered from studies that used multiple complimentary methods (e.g. Western blot, ELISA, RT-PCR and immunohistochemistry) and similar to opioid exposure changes, these occur with both acute and chronic exposure to alcohol and are time- and dose-dependent.

Specific receptors modulating these downstream molecular events have been identified (Table 5) with a pivotal role of not only IL-1R1 but also TLR4 as blocking either receptor prevented the multiple changes (Blanco et al., 2005), while genetic knockout of TLR4 also abolished the changes following chronic alcohol exposure seem in wild-type mice (Alfonso-Loeches et al., 2010). Further characterization of the signaling from TLR4 has revealed involvement of both MyD88 (myeloid differentiation primary response gene 88)-dependent and -independent pathways, as IRF-1 and IRF-3 expression and phosphorylation of STAT were observed (Fernandez-Lizarbe et al., 2009). The most recent evidence has completed the characterization of the TLR4 signaling pathway following chronic alcohol exposure in a genetic TLR4 knockout mouse model, with pivotal roles also demonstrated for MD-2 and CD14 accessory proteins (Alfonso-Loeches et al., 2010).

The mechanism by which alcohol activates these pathways, in particular TLR4 signaling, is thought to be by modulation of lipid rafting in the cellular membranes of astrocytes (Blanco & Guerri, 2007; Blanco et al., 2008). This appears to be dose-dependent with lower concentrations promoting lipid rafting and hence facilitating recruitment of TLR4 and subsequent activation, while higher concentrations perturb rafting to disrupt recruitment and signaling. There is also some evidence that CD14 recruitment to lipid rafts in CHO cells is altered by alcohol, with no effect on TLR2 (Dolganiu et al., 2006).
6.5. Indirect alcohol-induced responses that impact central immune signaling

The neurotoxic effects of alcohol have been attributed in part to the release of glutamate from astrocytes following alcohol exposure. This has been demonstrated in the acute setting using in vitro astrocyte cell cultures and is dose- and time-dependent, modulated by calcium concentrations and the presence of ROS, and occurs via excitotoxicity (González et al., 2007; Salazar et al., 2008b). Further, the uptake of glutamate is also inhibited following acute exposure (30 min). However, it is activated following chronic exposure (3 days) and occurs together with increase in protein kinase C- and calcium/calmodulin-dependent protein kinase-induced increase in glutamate uptake (Smith & Navratilova, 1999; Aschner et al., 2001b; Othman et al., 2002).

Altered uptake of other amino acids within astrocytes following alcohol exposure has also been observed, including taurine and aspartate, with taurine efflux elevated under withdrawal conditions and linked to the swelling of the cells (Aschner et al., 2001b; c). In addition, alcohol has also been found to alter the in vitro EAAT2 (glial glutamate transporter) regulation of neuronal potassium channels in the hippocampus (Muhlolland et al., 2009). While most recently, the importance of NF-κB signaling in modulation of neuronal induced neuronal plasticity and degeneration and the synergistic effects observed following HIV infection and alcohol has been highlighted in other reviews (Persidsky et al., 2011; Yakovleva et al., 2011).

A number of studies have also investigated how alcohol alters the proliferation of either astrocytes or microglia, with decreased proliferation reported in the following in vitro cell cultures: adult human cerebrum astrocyte cultures, with astrocytes from white matter more sensitive to higher concentrations and those from gray matter more sensitive to low to moderate concentrations (Kane et al., 1996); and primary rat cortical astrocytes following chronic exposure (Miller & Luo, 2002; Sarc & Lipnik-Stangelj, 2009). However, the effect of alcohol on glial cell proliferation may be dose- and brain region-dependent, such that intermittent exposure over 5.5 months in rats (that also experienced constant withdrawal periods over the course of the study) resulted in higher anterior cerebellar vermis compared to rats who were continuously exposed or had no exposure, while no change was seen in the posterior cerebellar vermis (Riikonen et al., 2002). There were also morphological changes in astrocytes, with chronic (6 week) in vivo ethanol treatment increasing the cell volume and the number and thickness of processes from the cell and increasing S-100β protein expression, a GDNF released following activation, in the CA-1 hippocampal region (Tagliaferro et al., 2002).

Cellular morphological studies of the human hippocampus (neuronal and molecular layer) of alcoholics compared to controls reported a significant reduction in astrocytes (42%) but not in microglia (Korbo, 1999). Other toxic effects of alcohol on glia include increased swelling (astrocytes from cerebral cortex) (Aschner et al., 2001a), and decreased fibronectin mRNA and protein expression in rat C6 glial cells following immune challenges (Ren et al., 2000). Given the role of fibronectin in cell growth and differentiation, it could be speculated that this may be a structural requirement to facilitate the morphological changes when transitioning from the quiescent to activated state or simply act as an activation signal in the integrin pathway.

Alcohol has also been shown to alter the electrophysiology of glial cells mediated through ion channel and gap junction functions (Adermark & Lovinger, 2006). These gap junction changes are primarily region specific with astrocytes from the cerebral cortex, hippocampus and brain stem affected, but olfactory bulb and hypothalamus unaffected (Adermark et al., 2004).

Other studies have suggested that alterations in oxidative stress and ROS are also involved in CNS toxicity, with alcohol increasing superoxide anion formation and decreasing NO levels in non-active hamster microglial cell cultures (Colton et al., 1998) and increasing free radical formation in rat astrocytes (Gonthier et al., 1997), decreasing cytokine-induced NO levels in immortalized astrocytes (Wang & Sun, 2001), and preventing stimulated rat microglial cell cultures from increasing superoxide anion formation (Aroor & Baker, 1998). Further, response of astrocytes to oxidative stressors such as hydrogen peroxide is significantly worse in the presence of alcohol, as evidenced by decreased viability and increased DNA damage (Gonthier et al., 2004). Protection against this altered response to oxidative stress is mediated by heat shock protein HSP70, with expression associated with less cellular damage (Russo et al., 2001).

Finally, studies have shown that alcohol could impact on CNS immunity by altering the response of rat and mouse microglia to immune-challenges, decreasing the LPS-induced release of IL-1β, iNOS expression (Syapin et al., 2001) and NO production via the NF-κB pathway (Lee et al., 2004), reducing phagocytosis in rat microglial cultures (Aroor & Baker, 1998), and increasing astrocyte sensitivity to TNF-α-induced toxicity (DeVito et al., 2000). Microglial release of TNF-α has also been shown to be neurotoxic in the hypothalamus, causing apoptosis of neurons in primary culture (Boyadjieva & Sarkar, 2010). Some of these changes are contradictory to observations previously observed, for example, it would be expected that LPS-induced release of cytokines would increase, and therefore it is important to remember that the whole impact of central immune signaling (both direct and indirect) is not simple, but determined by a highly complex system of pathways.

In conclusion, there are several mechanisms by which central immune signaling can impact on the neurotoxicity that develops following sustained alcohol exposure, i.e. during dependence, that range from altered cell proliferation to glutamate homeostasis.

6.6. Alcohol versus acetaldehyde effects on central immune signaling

Under chronic exposure conditions, such as that of dependence, the impact of not only alcohol, but also its major metabolite, acetaldehyde, needs to be considered. For example, rat astrocyte cultures have been shown to produce detectable levels of acetaldehyde following alcohol exposure, hence they have the enzymatic capability of transforming alcohol to acetaldehyde independent of any transport from outside the CNS (Eysseric et al., 1997). Further, under chronic exposure conditions (12 days), the production of acetaldehyde in rat astrocytes increased significantly, together with catalyze activity (Eysseric et al., 2000). In contrast to the plethora of studies investigating alcohol, few have considered the effect of acetaldehyde exposure on central immune signaling and its functional potential in a result of the difficulty of accurately studying due to the volatility of acetaldehyde under cell culture conditions. Hence, the most reliable evidence has been provided by studies that have utilized coculturing of rat astroglia and CHO cells which produce a constant source of acetaldehyde. These co-cultures revealed decreased proliferation and overall cell viability following 4 to 7 day exposures to acetaldehyde (concentration-dependent), and in comparison to alcohol, acetaldehyde was more toxic (Holownia et al., 1996; Sarc & Lipnik-Stangelj, 2009). With regard to specific cellular changes, acetaldehyde increases intracellular calcium and malondialdehyde concentrations, transglutaminase activity, superoxide dismutase activity, chromatin condensation and DNA fragmentation in rat astrocytes (Holownia et al., 1996; 1999; Signorini-Allibe et al., 2005). It has been shown in HepG2 cells that acetaldehyde induces IL-1β and TNF-α expression that is mediated by NF-κB (Hsiang et al., 2005). In summary, following chronic alcohol administration, proinflammatory central immune signaling activation is most likely to be a combined effect of both alcohol and acetaldehyde. As such, this urgently needs to be examined further.
6.7. Summary and significance of alcohol-induced central immune signaling

In a similar fashion to that previously discussed for opioids, alcohol-induced proinflammatory central immune signaling significantly contributes to the complexity of the CNS environment in which alcohol-induced activation of the mesolimbic dopamine reward pathway appears to contribute to alcohol's abuse potential. Whilst the behavioral data are sparser for alcohol, the cellular and molecular data reviewed share striking similarities with the opioid central immune signaling literature, including modulation of proinflammatory cytokine expression. This is highlighted by the recent characterization of TLR4-dependency of some alcohol-induced proinflammatory glial activation responses. Moreover, the human alcohol dependence immunogenetic data draws parallels with that of the opioid dependence literature, and builds upon this with a greater array of immunogenetic polymorphisms examined to date. The central immune signaling consequences of the different abuse dosing regimens employed by opioid versus alcohol dependent populations are not yet clear. This stark difference between opioids and alcohol will significantly alter the absolute exposure of the CNS to parent drugs and metabolites, again with significantly different pharmacokinetic characteristics. Nonetheless, these data also point to a common proinflammatory hypothesis of alcohol dependence.

7. Cocaine and central immune signaling

7.1. Animal behavioral evidence of a critical role for cocaine-induced central immune signaling

Animal behavioral studies have demonstrated central immune changes result in altered cocaine response manifested as altered locomotor activity, working memory, cocaine craving and learned self-administration (summarized in Table 1). These behavioral changes are not unexpected however, and are largely due to altered plasticity in the neuronal–glial synapse interaction that appears to be central to the development of drug dependence.

Injections of a chemokine CXCL12 into the lateral ventricle of the brain, which binds and activates the CXCR4 receptor on dopaminergic neurons, microglia and astrocytes, prior to cocaine administration dose-dependently altered the activity (total, ambulatory and stereotypic) response observed, with lower concentrations potentiating the cocaine-induced increases (Trecki & Unterwald, 2009). This modulation of activity was blocked with a specific antagonist to CXCR4, while the antagonist alone did not alter activity. Further, the change in activity was brain region specific, with different responses to injection into the following regions: the ventral tegmental area potentiated ambulatory but not stereotypes activity; the caudate putamen potentiated stereotypic but not ambulatory activity; the lateral accumbens shell inhibited ambulatory with no effect on stereotypic activity. Induction into the following regions: the ventral tegmental area potentiated ambulatory but not stereotypic activity; the caudate putamen potentiated stereotypic but not ambulatory activity; the lateral accumbens shell inhibited ambulatory with no effect on stereotypic activity; and the nucleus accumbens did not alter either activity. In-...
microglia and astrocytes to assume a time- and dose-dependent proinflammatory phenotype, some of which may be brain region specific. For example, no microglial or astrocyte activation was observed in the striatum of mice following single cocaine administration (Cappon et al., 1998; Thomas et al., 2004c), yet astrocytes are activated in the dentate gyrus (Fattore et al., 2002). Astrocyte activation has also been observed following cocaine withdrawal in rats, with increased expression of GFAP in the prefrontal cortex and the shell and core of the nucleus accumbens, and an increase in astrocyte cell numbers in the core of the nucleus accumbens (Bowers & Kalivas, 2003). Table 4 summarizes the downstream events characterized by studies using multiple complimentary methods (e.g. Western blot, ELISA, RT-PCR and immunohistochemistry/immunofluorescence).

Cocaine also has the potential to be cytotoxic to glia. An early study demonstrated that cocaine dose-dependently inhibited DNA and RNA synthesis in cultures of primary cortical glia and C6 glioma cells, and protein synthesis in the C6 glioma cells (Garg et al., 1993). This was manifested in overall decreased cell proliferation. More recently this has been further characterized using an in vitro astroglial cell culture study, where treatment (24 to 48 h) with cocaine dose-dependently reduced mitochondrial membrane potential, altered cell morphology and viability, and arrested the cell cycle in the G0/G1 and G2/M phases, reducing the S phase (Badisa et al., 2010).

Unlike opioids and alcohol, there is not yet any evidence linking TLRs with cocaine-induced glial proinflammation. Instead other receptors are indirectly activated, for example the mGluR5 expressed on astrocytes regulates their calcium levels to cause activation when high levels of glutamate are present, causing the release of immune mediators, including glutamate, to initiate NDMA receptor mediated neuronal responses (D’Ascenzo et al., 2007; Fellin et al., 2007) (see Table 5 for summary).

7.5. Indirect cocaine-induced responses that impact central immune signaling

The ability of cocaine to induce lymphocyte/monocyte migration across cerebral vessels has been documented using both in vitro and in vivo models and is thought to be pivotal in the potentiation and susceptibility of cocaine users to neuroinflammation and stroke. The first study to report this used an in vitro blood–brain model and not only reported increased adhesion and migration of monocytes, but also increased secretion of IL-6 and TNF-α (Gan et al., 1999). The second study matched results from in vitro and in vivo models and importantly identified that activation of microglia and subsequent increased expression of MCP-1 following cocaine administration is pivotal to the resultant monocyte transmigration across the blood–brain barrier in both in vitro and in vivo models, which is absent in MCP-1/CCL2 knockout mice hence demonstrating mediation by MCP-1 (Yao et al., 2010). In vivo these cells are observed in perivascular cuffs and in the parenchymal regions of the brain and this migration can result in further neuroinflammation and toxicity. Therefore, for long-term abusers of cocaine, in addition to contributing to the development of dependence, central immune signaling can potentially significantly contribute to, and possibly exacerbate, cocaine's neurotoxicity.

7.6. Cocaine metabolites effects on central immune signaling

There is currently no evidence in the literature describing the effect of the metabolites of cocaine, benzoylcegonine and ecgonine methyl ester, on glia. However, given the key role established for opioid and alcohol metabolites in modifying central immune signaling future work should examine these as possible candidates in mediating activation of cocaine's proinflammatory central immune signaling.

7.7. Summary and significance of cocaine-induced central immune signaling

Once again, the evidence in the literature for cocaine mirrors the general theme presented for opioids and alcohol, that cocaine also causes proinflammatory central immune signaling that will significantly add to the complexity of the CNS environment in which cocaine-induced activation of the mesolimbic dopamine reward pathway operates and appears to contribute to its abuse potential. Moreover, whilst sparser than for opioid and alcohol, a possible link between TLR4 is also drawn owing to the sensitivity of cocaine behaviors to (+)-naloxone – a TLR4 specific antagonist. This also agrees with similar proinflammatory cellular and molecular consequences observed with cocaine. Again, the neuronal consequences of cocaine-induced central immune signaling appear to be indelibly linked to inflammation-induced alterations in glutamate homeostasis, resulting from reduced glial glutamate transporter expression. Interestingly, GDNF once again plays a key role in the expression of cocaine reward behaviors.

8. Methamphetamine and central immune signaling

8.1. Animal behavioral evidence of a critical role for methamphetamine-induced central immune signaling

Animal behavioral studies have demonstrated CNS immune changes result in altered methamphetamine response manifested as altered locomotor activity, reward (CPP), methamphetamine relapse, craving and learned self-administration (summarized in Table 1). Chronic methamphetamine has also been shown to alter gene expression in the midbrain of mice with methamphetamine-induced behavioral responses of sensitization and CPP (Funada et al., 2004).

Methamphetamine administration causes a time-dependent increase in locomotor activity, however the nature and degree of increase is dose-dependent, i.e. repeated 5 mg/kg doses increased locomotor activity, while 10 mg/kg decreased ambulatory activity but increased stereotypy of activity (Fantegrossi et al., 2008). In addition, some studies have shown that altered locomotor activity escalated over repeated administrations, or sensitization (mediated in part by protein kinase C activation (Narita et al., 2005)), that was modulated by LPS challenge (Buchanan et al., 2010).

Administration of LPS directly into the striatum of rats reduced locomotor activity, which was further reduced by co-administration of methamphetamine, with similar reductions observed in rotorod performance (Jung et al., 2010). These behavioral changes were directly related to reduced dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanilic acid (HVA) levels, and increased dopamine turnover in the striatum (Jung et al., 2010).

Further evidence of the central immune signaling role in modulation of methamphetamine-induced behavioral responses includes: injection of astrocyte-conditioned medium into the nucleus accumbens heightened CPP, while pharmacological glial attenuation with propentofylline and inhibition of the Jak/STAT pathway blocked CPP (Narita et al., 2006); GDNF blocked CPP and altered sensitivity (Niwa et al., 2007c) while reduction of GDNF (genetic heterozygous GDNF +/- mouse model) potentiated self-administration, seeking and susceptibility to reinstatement (Yan et al., 2007); microsomal epoxide hydrolase (expressed in activated astrocytes) knockout mice had increased CPP, and sensitization increased microsomal epoxide hydrolase expression (Shin et al., 2009); GLT-1 expression in the nucleus accumbens (via gene transfer) inhibited CPP (Fujio et al., 2005); exogenous administration of TNF-α blocked CPP, discrimination and hyper-locomotor activity and prevented dopamine depletion, while TNF-α knockout mice had increased CPP and locomotor sensitization (Nakajima et al., 2004).

Studies have also investigated the effect of pharmacological glial attenuators on methamphetamine behavioral responses. An early
study using propentofylline established that glial attenuation blocked methamphetamine reward in a CPP model (Narita et al., 2006), while more recently ibudilast attenuated methamphetamine relapse in rats under prime- and stress-induced models (Beardsley et al., 2010). Similar to cocaine, a role for mGluR5 in mediating the glial behavioral response to methamphetamine has been postulated as co-administration of an mGluR5 antagonist with methamphetamine blocked both reinforcement and seeking behaviors in rats (Gass et al., 2009).

In summary, similar to the previously discussed drugs of abuse, there is substantial evidence that has linked central immune signaling to methamphetamine behavioral responses including reward.

8.2. Indirect human evidence of possible methamphetamine-induced central immune signaling response

Immunohistochemical analysis of post-mortem brains of chronic methamphetamine users (determined after death by presence of methamphetamine in hair samples) versus non-users had no differences with regard to microglia or astrocytes proinflammation in the nucleus accumbens, caudate nucleus and putamen brain regions (Kitamura et al., 2010). However, increased microglial cell numbers, but not proinflammatory microglia, has been observed in the post-mortem striatum of chronic methamphetamine users (Kitamura, 2009).

In contrast, studies employing different in vivo imaging techniques have observed microglial activation in current cocaine addicts. For example, an early MRI study of current methamphetamine users reported loss of gray matter in the cingulate brain regions, hypertrophy of white matter and decreased hippocampus volume that likely results in glosis (Thompson et al., 2004). While more recently, PET imaging in humans reported increased microglial activation (assessed by a specific radiotracer, 11C]-PK11195) in the midbrain, striatum, thalamus, orbito-frontal cortex and insular cortex regions of former chronic methamphetamine users (who had used for more than 6 years and been abstinent for at least 2 years) in comparison to healthy non-users (Sekine et al., 2008). Further, although there was no correlation between length of methamphetamine use or craving scores and activation, the length of abstinence was negatively correlated with microglial activation in the midbrain, striatum and thalamus (Sekine et al., 2008). Consequently, prolonged microglial proinflammation following chronic methamphetamine use can resolve over time, as demonstrated in the animal studies discussed above.

There has also been some cell culture studies supporting the role of inflammation in methamphetamine response as human monocyte (THP-1) cells have also revealed methamphetamine exposure dose-dependently decreased cell viability and potentiated LPS-induced IL-1β release (Tipton et al., 2010). However, perhaps the most compelling evidence to date of a role of proinflammatory response mediating dependence is the recent case report which detailed that IL-1β (THP-1) cells have also revealed methamphetamine exposure dose-dependently decreased cell viability and potentiated LPS-induced IL-1β release (Tipton et al., 2010).

In conclusion, human studies and in particular the more recent use of PET scanning in current methamphetamine addicts (see Table 2 for summary), supports the wealth of animal evidence to demonstrate a significant role of central immune signaling in methamphetamine dependence.

8.3. Human genetic evidence of a possible role of central immune signaling in methamphetamine abuse

Of the studies to investigate association of genetic variants in immune mediators and methamphetamine dependence (Table 3), positive associations with risk have been reported for BDNF (Val66Met) (Cheng et al., 2005; Bousman et al., 2009), GLYT1 glycine transporter expressed on glia (Morita et al., 2008; Bousman et al., 2009), and prodynorphin (PDYN, promoter repeat alleles) (Nomura et al., 2006; Bousman et al., 2009). However, TNFA (−308G>A and −857C>T) and its receptor TNFR-SF1A (36A>G) genetic variants (Nomura et al., 2006a) and tag SNPs of GDNF (Yoshimura et al., 2011) were not significantly associated with methamphetamine dependence.

Functionally, the impact of these genetic variants largely support the hypothesis of proinflammation linked to increased risk of dependence, with specific details for the variant related to dependence as follows: BDNF Val wild-type results in optimal secretion of BDNF (Chen et al., 2004); and 3 or 4-repeat in promoter of PDYN gene results in increased promoter activity in a construct assay (Zimprich et al., 2000) and hence is predicted to result in increased in vivo prodynorphin expression, which may impact dynorphin A 2−17 induced microglial p38 activation. In contrast, the functional impact of the GLYT1 SNPs is yet to be determined.

8.4. Biological and molecular evidence for activation of central immune signaling by methamphetamine

Numerous in vivo animal studies and in vitro cell culture studies have revealed that methamphetamine can direct microglia and astrocytes to assume a proinflammatory phenotype, some of which may be brain region specific.

Methamphetamine caused microglial activation in the hippocampus, cerebral cortex, substantia nigra, striatum caudate-putamen, mid-striatum, central gray, thalamus, parietal and piriform cortices and in the ventromedial column of the periaqueductal gray, however, was unable to modify microglial activity in the nucleus accumbens and ventral tegmental area (Guijarte et al., 2003; Pubill et al., 2003; Asanuma et al., 2004; LaVoie et al., 2004; Thomas et al., 2004c; Kawasaki et al., 2006; Bowyer et al., 2008; Thomas et al., 2008b; Boger et al., 2009; Huang et al., 2009; Thomas et al., 2009; Gonçalves et al., 2010). However, proinflammation in some brain regions (e.g. cortex, hippocampus and substantia nigra) has not been consistently observed across studies (Pubill et al., 2003; Fantegrossi et al., 2008). These discrepancies are most likely due to differences in time of tissue collection following last methamphetamine exposure, as methamphetamine-induced microglial activation has been shown to be time-dependent after the last dose, with maximum activation at 24 h followed by decline over time, with studies showing either partial or complete resolution after 3−7 days (Guijarte et al., 2003; LaVoie et al., 2004; Thomas et al., 2004c; Kawasaki et al., 2006; Fantegrossi et al., 2008; Gonçalves et al., 2010). Minocycline administration attenuated methamphetamine-induced proinflammation (Boger et al., 2009), while administration of a single dose of methamphetamine at higher room temperatures (29 versus 23 °C) potentiated activation in the cortex, hippocampus, thalamus and hypothalamus (Sharma & Kiyatkin, 2009).

In contrast, some studies have reported that while methamphetamine alone has failed to produce microglial activation, it has shown to potentiate inflammation mediated by LPS challenge in the striatum and substantia nigra. However, there was no effect in the hippocampus, dorsal raphe nuclei or locus ceruleus (Jung et al., 2010). Further, in vivo methamphetamine sensitization attenuated LPS-induced microglial activation and increases in mRNA expression of IL-1β (hippocampus, hypothalamus and striatum), IL-6 (hippocampus, hypothalamus and striatum), and TNF-α (hypothalamus and striatum), with no impact on IL-10 mRNA expression (Buchanan et al., 2010). Finally, it has been shown that methamphetamine co-administered with agents that increase cytoplasmic dopamine (clorgyline, L-DOPA and reserpine) resulted in extensive microglial activation in the nucleus accumbens (Thomas et al., 2009). These data implicate dopamine as a possible secondary signal required for methamphetamine-induced central immune signaling, which may contribute to the regional heterogeneity of its central immune actions.

A number of studies have reported methamphetamine-induced astrocyte activation in several brain regions, including striatum, hippocampus, nucleus accumbens, cingulate cortex, and cerebral cortex
(Hebert & O’Callaghan, 2000; Guiart et al., 2003; Pubill et al., 2003; Narita et al., 2005; Kawasaki et al., 2006; Siriram et al., 2006; Straiko et al., 2007; Huang et al., 2009; Gonçalves et al., 2010). This activation follows a time course following acute dosing, with early studies showing that proinflammation in the striatum is minor in the first 24 h and increases substantially by 3 days after the last dose (Kawasaki et al., 2006; Siriram et al., 2006). More recently, a single dose was shown to result in maximum proinflammation in the hippocampus at 1 day that returned to baseline by 3 days after the last dose (Gonçalves et al., 2010). In addition, rat oligodendrocyte cell cultures are also modulated by methamphetamine in a time- and dose-dependent manner, and displayed decreased cell viability and increased expression of pro-apoptotic genes (Genc et al., 2003).

The downstream events from methamphetamine exposure have been characterized with in vitro and in vivo studies using multiple complimentary methods (e.g. Western blot, ELISA, RT-PCR and immunohistochemistry/immunofluorescence) and are summarized in Table 4. Changes were observed with acute exposure to methamphetamine (also in combination with LPS) and were time-, dose- and brain region-dependent. In addition, an early study investigated gene expression in the striatum of mice following a single in vivo methamphetamine dose reported that of the 152 genes with changed expression up to 3 days post-dose, BDNF, GAFP, HSP70, Oncostatin M receptor (Osrm), and suppressor of cytokine signaling-3 (SOCS-3) all increased over 3 days, and chemokine receptor 6 initially decreased (24 h) but then increased over the 3 day period post-dose (Thomas et al., 2004b).

GDNF once again also plays a role in modulation of microglial reactivity by methamphetamine, as mice with a genetic deficiency in GDNF (heterozygous +/− GDNF) had increased reactivity in the substantia nigra compared to wild-type mice (Boger et al., 2007; 2009). The role of free radicals in glial proinflammation has also been investigated, with administration of edaravone (a radical scavenger) preventing astrocyte, but not microglial proinflammatory phenotypes in the striatum in the 3 day period following the last dose (Kawasaki et al., 2006). However, cell culture studies have shown edaravone blocked stimulated in vitro microglial production of ROS, NO and iNOS, but had little effect on the production of proinflammatory cytokines with the exception of potentiation of IL-6 and TNF-α production at a high concentration (Banno et al., 2005).

Further evidence of the role of inflammation in response to methamphetamine comes from studies that have shown alteration in inflammation status: co-administration of methamphetamine and indomethacin (a non-selective inhibitor of COX-1 and COX-2), a) blocked microglial and astrocyte proinflammation in the hippocampus and the associated increase in TNF-α and TNFR1 (Gonçalves et al., 2010) and b) blocked microglial proinflammation in the striatum (Asanuma et al., 2004); co-administration of ketoprofen (a non-steroidal anti-inflammatory drug) blocked microglial proinflammation in the striatum (Asanuma et al., 2003); and MK-801 and dextromethorphan (both NMDA antagonists) blocked microglial stimulated activation in cell culture and methamphetamine-induced in vivo microglial proinflammation in the striatum (Thomas & Kuhn, 2005b). Further, minocycline blocked microglial proinflammation in the substantia nigra, via attenuation of activation of p38-MAPK signaling (Boger et al., 2009). Similarly, propentofylline blocked mouse cortical astrocyte proinflammation in culture (Narita et al., 2006). Conversely, while minocycline did not change methamphetamine-induced striatal astrocyte proinflammatory activation 12 or 72 h post-dose it did attenuate production of proinflammatory cytokines (IL-1β, IL-6, TNF-α and CCL2) 12 h post-dose (Sriram et al., 2006).

The receptors involved in modulating glial proinflammation to methamphetamine (either attenuating or enhancing) have been implicated by animal models employing either pharmacological or genetic knockout blockade design (Table 5), and include the mGlUR5 (Gass et al., 2009) and opioid peptide nociceptin/orphanin FQ (N/OFQ) receptor for astrocytes only (Sakoori & Murphy, 2010). Similarly, other receptors have been ruled out as modulating methamphetamine-induced glial proinflammation such as the fractalkine receptor (CX3CR1) (Thomas et al., 2008a).

8.5. Indirect methamphetamine-induced responses that impact central immune signaling

Methamphetamine-induced neurotoxicity significantly contributes to clinical symptomatology following chronic exposure to methamphetamine, and is thought to be largely due to altered levels of dopamine within the tripartite synapse. The direct relationship between dopamine, methamphetamine neurotoxicity and striatal microglia proinflammation has been investigated in mice, with reversal of methamphetamine-induced dopamine depletion mirrored by decreased proinflammation (that was blocked by treatment with i-DOPA), and potentiation of methamphetamine-induced dopamine depletion or inhibition of dopamine metabolism mirrored by increased proinflammation (Thomas et al., 2008b). In addition, studies have shown that repeated low level methamphetamine dosing in mice followed by a neurotoxic dose attenuates not only methamphetamine-induced microglial proinflammation, but also dopamine release (Thomas & Kuhn, 2005a). Finally, IL-6 knockout mice were protected from methamphetamine-induced glial proinflammation, depletion of dopamine and neurotoxicity in the caudate putamen and frontal cortex (Ladenheim et al., 2000). Also of note is an early report detailing that methamphetamine alters dopamine metabolism in cultured rat astrocytes (Kita et al., 1998), highlighting another mechanism by which dopamine levels and astrocyte activity in response to methamphetamine is linked.

Further evidence supporting the importance of dopamine levels in microglial proinflammation are observations that murine BV-2 microglial cultures exposed to dopamine-quinones (formed by activation of reactive oxygen and nitrogen species with dopamine) display time-dependent increased proinflammation and altered expression of 101 genes — 73 increased and 28 decreased (Kuhn et al., 2006). The genes with increased expression were generally classified as having a role in signal transduction and molecular functioning and included: cytokines, chemokines, receptors, heat shock protein, COX-2 and NOS. While those with decreased expression were also involved in molecular functioning and included: caverolin 2, purinergic receptor and TLR4. Further, comparison between genes induced by dopamine quinones and methamphetamine in vivo revealed some commonality and included: chemokine ligand 12, IL-1α, IL-1RA, and heat shock protein 1A (for review see Kuhn et al., 2006).

Methamphetamine also induces a dose- and time-dependent hyperthermic response (Fantegrossi et al., 2008), which was independent of microglial activation in the cortex but perhaps related to activation in the striatum, with some discrepancies across studies (Thomas et al., 2004c; Fantegrossi et al., 2008). In contrast, there has been some suggestion that a hyperthermic response and disruption to the blood–brain barrier are pivotal events required prior to microglial and astrocyte proinflammatory activation in specific brain regions such as the caudate putamen, cingulate-parietal cortex, and hippocampus (Bowyer & Ali, 2006; Kiyatkin et al., 2007; Bowyer et al., 2008). In addition, methamphetamine administration at higher ambient temperatures was observed to heighten the hyperthermic response, which then was directly correlated with exacerbated disruption to the blood–brain barrier and astrocyte proinflammation (Kiyatkin et al., 2007). Systemic and intracerebral administration of IFN-γ protected against methamphetamine-induced neurotoxicity, and systemic administration prevented methamphetamine-induced hyperthermia (Hozumi et al., 2008). Similarly, MK-801 and dextromethorphan (both NMDA antagonists) prevented methamphetamine-induced hyperthermia and striatal microglial proinflammation (Thomas & Kuhn, 2005b).
8.6. Effect of methamphetamine metabolites on central immune signaling

There is currently no evidence in the literature describing the effect of one of the metabolites of methamphetamine, para hydroxy-methamphetamine (pOH-MA) on glia. However, amphetamine, another metabolite of methamphetamine, has been extensively characterized with regard to glial activation. Specifically, amphetamine dose- and time-dependently caused microglial activation (HAPEL cell culture (Tocharus et al., 2008)), but did not alter activation of microglia in the striatum and midbrain of rats (Jakab & Bowyer, 2002). Chronic amphetamine also dose-dependently increased astrocyte activation in the hippocampus but not the cortex (Frey et al., 2006), with an early study demonstrating more widespread activation in the striatum, midbrain, hippocampus and thalamus (Jakab & Bowyer, 2002). Some of the downstream events have been characterized and include: increased basic fibroblast growth factor (bFGF) in astrocytes of the ventral tegmental area and substantia nigra when given acutely, and also in the nucleus accumbens following longer term administration (2 weeks), with declining expression with chronic administration (5 weeks), while expression in the striatum and prefrontal/occipital cortex was maximal at 2 weeks (Flores et al., 1998; Flores & Stewart, 2000); and iNOS mRNA expression (Tocharus et al., 2008). Amphetamine in an astrocytoma cell line (human U373 MG) culture increased production of ROS and 4-hydroxyxenonenal adducts, c-Jun, c-Fos, FosB protein expression, and AP-1 DNA binding, and decreased JunB, JunD and FRA-2 protein expression (Malaplate-Armand et al., 2005).

In addition to altering inflammatory responses of glia, amphetamine-induced increases in the glutamine (astrocytes)/glutamate (neurons) ratio in various brain regions (striatum, caudate putamen, hippocampus, frontal cortex) is likely to be an important modulator of neuronal–glial crosstalk in response to amphetamine, with these changes being acute and transient (observed 4 h after a single dose and resolving by 24 h) (Pereira et al., 2008). Not surprisingly, this study also observed increases in the GABA (whose precursor is glutamine)/glutamate ratio in the striatum at 24 h and hippocampus at 4 h (Pereira et al., 2008). In addition, increased amphetamine-induced bFGF expression in astrocytes was blocked by a glutamate receptor antagonist (Flores et al., 1998).

Behaviorally, amphetamine also induces locomotor activity and rearing which was independent from astrocyte proinflammation in the cerebral cortex but was observed at the same time as astrocyte proinflammation in the hippocampus at specific chronic dosing levels (Frey et al., 2006). In addition, roles for immune mediators and receptors linking sensitization with astrocyte proinflammation have been detailed: astrocyte proinflammation in striatum and hippocampus was observed in mice with amphetamine-induced sensitization, and correlated with increased expression of P2Y1 (purinergic) receptor on both astrocytes and neurons (Franke et al., 2003); bFGF expression by astrocytes in the ventral tegmental area and nucleus accumbens in mice correlated with the degree of amphetamine-induced sensitization, and blockade of bFGF astrocyte expression in ventral tegmental area prevented this sensitization (Flores et al., 2000); exogenous systemic IL-6 potentiated amphetamine-induced sensitization (Zalcman et al., 1999); and exogenous systemic IL-2 potentiated amphetamine-induced discrimination, and was blocked by co-administration of naltrexone with IL-2 (Ho et al., 1994). Most recently, co-administration of amphetamine with minocycline to humans revealed that glial attenuation blocked the reward, i.e., the “high” and “good” effects experienced following amphetamine (Sofuoglu et al., 2011). Collectively, these data reveal that amphetamine and methamphetamine have similar effects on glial activation and are likely to be equally important in mediating the reward and dependence experienced following chronic exposure. This hypothesis is supported by the fact that a Phase I safety interaction trial is currently underway to determine the possible clinical use of ibudilast as a novel treatment for methamphetamine dependence (US NIH Clinicaltrials.gov database).

8.7. Summary and significance of methamphetamine-induced central immune signaling

Methamphetamine and its active metabolite amphetamine both contribute significantly to alterations in proinflammatory central immune signaling. As previously discussed for the other drugs of abuse this will add significantly to the complexity of the CNS environment in which methamphetamine and amphetamine-induced activation of the mesolimbic dopamine reward pathway operates and appears to contribute to their abuse potential. The behavioral reward pharmacological data share striking similarities with morphine in that both morphine and methamphetamine display behavioral sensitivity to both ibudilast and propentofylline. This pharmacological evidence is extended into the human realm with evidence that minocycline treatment beneficially modifies methamphetamine and amphetamine behavior, also agreeing with similar proinflammatory cellular and molecular consequences observed with methamphetamine. Again, the neuronal consequences of methamphetamine-induced central immune signaling appear to be indelibly linked to inflammation-induced alterations in glutamate homeostasis, resulting from reduced glial glutamate transporter expression. Interestingly, GDNF once again plays a key role in the expression of methamphetamine reward behaviors.

9. MDMA and central immune signaling

9.1. Animal behavioral evidence of a critical role of MDMA-induced central immune signaling

There are currently no reports detailing the impact of glial activation on MDMA behavioral effects in animal models. However, given the similarity between MDMA and methamphetamine with regard to glial activation and the resultant central proinflammatory downstream events described below, and the similar overall behavioral effects (i.e. locomotor activation, reward), it is hypothesized that MDMA glial activation is likely to be an important modulator of MDMA’s behavioral effects and should be explored with appropriate studies in the near future.

9.2. Indirect human evidence of possible MDMA-induced central immune signaling responses

Evidence from PBMC inflammatory challenge studies in MDMA users have revealed altered levels of immune mediators in response to ex vivo immune challenge following in vivo MDMA exposure (Table 2). Specifically, IL-2, IL-6, IFN-γ and TNF-α release was reduced and IL-4, IL-10 and TGF-β release was increased from stimulated PBMCs from MDMA users (Pacifi ci et al., 2001; 2004). Thus, although there is limited human evidence to implicate central immune signaling in MDMA responses, the reported peripheral immune changes are similar to the previously discussed drugs of abuse and therefore may be indicative of similar central immune changes following exposure to MDMA. Hence, future studies similar to the post-mortem brain investigations or PET scanning of current addicts are urgently required to address this knowledge gap.

9.3. Human genetic evidence of a possible role of central immune signaling in MDMA abuse

No specific studies have been published relating genetic variants in any immune mediators mentioned above with the risk of MDMA.
dependence. However, similar to the animal behavioral evidence, it would be expected that gene candidates related to methamphetamine dependence risk would be common with MDMA dependence risk. Similar to the human studies discussed above, there is an urgent need for future research to address this knowledge gap through investigation of genetic loci pivotal in modulating central immune signaling.

9.4. Biological and molecular evidence for activation of central immune signaling by MDMA

Numerous in vivo animal studies and in vitro cell culture studies have revealed that MDMA can produce microglia and astrocyte proinflammation, some of which may be brain region specific. The downstream events are summarized in Table 4, and are time- and dose-dependent in response to acute and chronic exposure as demonstrated by studies that have used multiple complimentary methods (e.g. Western blot, ELISA, RT-PCR and immunohistochemistry/immunofluorescence).

Microglia in the frontal cortex, hippocampus, hypothalamus, striatum and substantia nigra undergo proinflammatory activation following MDMA exposure (Orio et al., 2004; Thomas et al., 2004a; Zhang et al., 2006b; Khairnar et al., 2010; Orio et al., 2010; Torres et al., 2010; Touriño et al., 2010). However, some studies have failed to replicate this proinflammation in the hippocampus, caudate, striatum and frontal cortex (Pubill et al., 2003; Wang et al., 2004; Zhang et al., 2006b; Fantegrossi et al., 2008). At first it appeared that differences in observed activation may have been as a result of species differences, i.e. mouse versus rat models. However, this can be ruled out as both animals have displayed increases in proinflammation following exposure. A more likely explanation appears to be differences in the dosage regimen used and the time of brain harvest for analysis, as this response has been shown to be time-dependent in both the hypothalamus and cortex of rats (Orio et al., 2004). Nonetheless, the evidence that minocycline was able to attenuate this response supports the overall conclusion that microglia are activated by MDMA (Zhang et al., 2006b; Orio et al., 2010).

Early evidence for MDMA-induced astrocyte activation reported concentration-dependent increases in glycogen phosphorylase activity in primary rat cell cultures following acute exposure (Poblete & Azmitia, 1995). Since then, a number of studies have reported MDMA-induced astrocyte activation, with some brain region specificity, e.g. astrocytes in striatum, cortex, hippocampus and cerebellum are activated (Adori et al., 2006; Sharma & Ali, 2008; Khairnar et al., 2010; Touriño et al., 2010), but those in substantia nigra are not (Khairnar et al., 2010). While similar to the microglial activation, some studies have failed to observe astrocyte activation in the frontal cortex and striatum (Pubill et al., 2003; Orio et al., 2004), most probably as a result of the time course examined (Adori et al., 2006). Finally, one study has observed a time- and dose-dependent increase in astrocytes expressing HSP27 in the CA-1 region of the hippocampus (Adori et al., 2006), which may indicate an increase in ROS in these cells as a downstream event to initial activation.

With regard to specific receptor involvement, the cannabinoid receptors (CB1/CB2) have been shown to modulate both microglial and astrocyte activation to provide a protective role against MDMA neurotoxicity (Table 5). For example, in vivo administration of a CB2 receptor agonist before MDMA blocked microglial activation and IL-1β release, while a CB2 receptor antagonist reversed the effect of the agonist–MDMA interaction in the hypothalamus and frontal cortex (Torres et al., 2010). Further, Δ 9-tetrahydrocannabinol prevented microglial and astrocyte in vivo activation, but only when functional CB1/CB2 receptors were present (Touriño et al., 2010).

9.5. Indirect MDMA-induced responses that impact central immune signaling

Animal studies have also demonstrated that CNS immune changes result in altered MDMA temperature response. For example, MDMA-induced hyperthermia is modulated in part by CB1 receptors, which as discussed above are expressed on both microglia and astrocytes and modulate activation, such that activation of CB1 receptors by Δ 9-tetrahydrocannabinol prevented hyperthermia (Touriño et al., 2010). Interestingly, however, minocycline was unable to reverse MDMA-induced hyperthermia (Zhang et al., 2006b; Orio et al., 2010). Further, coadministration of MDMA and IL-1Rα did not alter the MDMA-induced hyperthermia (Orio et al., 2004). Hence this response appears to be partially independent to MDMA-induced activation of central immune signaling.

9.6. MDMA metabolite effects on central immune signaling

There is currently no evidence in the literature describing the effect of the metabolites of MDMA, 4-hydroxy-3-methoxymethamphetamine (HMMA), 3,4-dihydroxy-methamphetamine (HMA), 3,4-methylene-dioxymethamphetamine (MDA), 4-hydroxy-3-methoxymethamphetamine (HMA) and 3,4-dihydroxyamphetamine (HHA), on glia. However, one study did compare the immunosuppressive activity of MDMA and MDA in rats, reporting the following: both MDMA and MDA reduced the levels of circulating lymphocytes; MDA caused greater reduction in IL-10 and INF-γ stimulated release compared to MDMA; MDMA caused greater increase in IL-2 stimulated release compared to MDA; and both caused a similar decrease in TNF-α stimulated release, with no effect on IL-1β (Connor et al., 2000). Hence, it can be speculated that some of the central immune action of MDMA may be mediated by MDA, especially following chronic exposure in dependent individuals.

9.7. Summary and significance of MDMA-induced central immune signaling

Whilst it is clear MDMA exposure creates a proinflammatory central immune signal from in vitro and in vivo studies, of the drugs of abuse reviewed here, there is the least translational evidence of MDMA-induced central immune signaling having profound in vivo behavioral consequences for MDMA abuse liability behaviors. Nonetheless, when viewed in light of existing data reviewed for opioids, alcohol, cocaine and methamphetamine demonstrating profound pharmacodynamic impacts of their respective central immune signaling activities, it is apparent that the a priori hypothesis that MDMA abuse liability will also be indelibly linked to its central immune signaling.

10. Common central immune signaling consequences of drugs of abuse and their behavioral significance

From the literature reviewed on each of the drugs of abuse several common cellular and molecular themes are clear (summarized in Tables 1–5). Firstly, all the drugs of abuse discussed clearly cause profound proinflammatory central immune signaling within disparate brain regions, albeit in a heterogeneous fashion. The proinflammatory mediators are derived from various systems such as proinflammatory cytokines, to chemokines, ROS, and NO. Given the breadth of proinflammation resulting from exposure to drugs of abuse it is unclear which mediators are directly upregulated as a result of exposure, and which are engaged following the first inflammatory signaling response. This proinflammation is also associated with the upregulation of glial activation markers thus collectively providing an indication, but still an incomplete picture of the complex microenvironment in which the mesolimbic dopamine reward system and dependence neurocircuitry are engaged following drug exposure.

Prior to this broad proinflammatory response, key activation steps must have occurred that unleash this central immune signaling. Evidence from opioids, alcohol and cocaine provide a link to the possible role of TLR4 in initiating the proinflammatory central immune signaling
and associated drug of abuse pharmacodynamic behavior. Either (+)-naloxone sensitivity or TLR4 genetic knockout models have been used to implicate this fascinating candidate receptor, although the activation mechanisms of TLR4 signaling activation by these agents remain unclear. At this stage the greatest evidence is available for opioids, and suggests that TLR4 signaling is directly activated following opioid binding to the LP5 binding cleft of the accessory protein MD-2. Interestingly, this action of MD-2 also opens a possible role for TL2 in this activation process (Dziarski et al., 2001) owing to the MD-2 dependent signaling events via TL2 in some situations. In comparison, alcohol has been hypothesized to facilitate TLR4 signaling via disruption of lipid rafts. Alternatively, TL4 may be engaged by a drug of abuse-induced endogenous danger signal that subsequently activates TL2 and/or TL4 signaling. Nonetheless, the implications of TL4 in this role as a direct or indirect drug of abuse “sensor” is significant. This provides a common point at which drugs of abuse may converge to produce proinflammatory response within the CNS. Downstream signaling of TL4 is compatible with the activation of MAPK signaling, gene transcription and translation previously reported following exposure to drug of abuse. Moreover, the glial-targeted pharmacological agents all have demonstrated ability to reduce TL4 proinflammatory responses via mechanisms at downstream sites. Critically, the established neuronal sites of action of each drug of abuse discussed herein are essential for the complete complex pharmacodynamic response of each drug of abuse, but we hypothesize that TL4 is essential for the central immune signaling component of this multifaceted system. Clearly, other receptors at non-neuronal sites may also be directly modulated by the drugs of abuse, but this may be in a drug specific fashion rather than the more general nature of the response by TL4.

The neuronal consequences of the myriad of central immune signaling mediators released and cellular adaptations are complex. However, two key systems stand out across the drugs of abuse examined here: regulation of glutamate homeostasis and GDNF. Proinflammatory glial activation results in the dysregulation of glial glutamate transporter activity and expression thereby providing a link to the hyper-activity of neuronal NMDA systems in drug abuse models. Moreover, the ability of proinflammatory mediators such as IL-1β and metalloproteases to cause NDMA receptor subunit phosphorylation also supports this hypothesis. The role of GDNF in drug abuse pharmacodynamics are complex, but given it is associated with the pharmacodynamics of each of the drugs examined here it clearly has a pivotal action. Since proinflammatory glial activation decreases its expression and GDNF supplementation is able to decrease this response, underlyng proinflammatory central immune signaling may work in parallel with this mediator to contribute to the neurocircuity of drug abuse.

Finally, the behavioral significance of this drug of abuse-induced central immune signaling is highlighted in Table 1 that summarizes the behavioral consequences of central immune signaling. Importantly, agreeing with the breadth of neuronal centers found to engage central immune signaling, the breadth of complex drugs of abuse-induced behaviors that are altered by proinflammatory immune factors are evident. At this stage the complete molecular and cellular mechanisms of these interventions remain elusive, but these validate the importance of central immune signaling in the pharmacodynamics of drugs of abuse.

11. The contribution of immunogenetics to predisposition to drugs of abuse addiction

Based on the data reviewed a broader examination of immunogenetic factors that predispose individuals to drug abuse is warranted. As outlined in Table 3 there is already significant evidence that SNPs that are associated with proinflammation in other diseases are also associated with increased prevalence in drug dependent populations. It remains to be determined the extent of this proinflammatory immunogenetic relationship with drug abuse. Nonetheless, in the absence of approved pharamcotherapies, immunogenetics appears to be an excellent means of demonstrating new indications for central immune signaling targeted therapies.

As with any genetic predisposition discovery, great responsibility comes with the personal implications of defining an individual or even a population as ‘at risk’ of drug abuse. If such genetic tests eventuate, significant efforts should be made in drug education and counseling provided. Fortunately, the development of applicable pharmacotherapies has paralleled these discoveries, facilitating prophylactic treatment of ‘at risk’ individuals if their environment warrants it. Such pharmacological approaches will be discussed below.

12. Implications of cross sensitization to drug abuse by preexisting or concomitant proinflammatory central immune signaling

Similar to immunogenetic predisposition to drug abuse via central immune signaling mechanisms, environmental factors may also produce similar CNS proinflammatory effects. Infection is the clearest example, where even peripheral inflammation results in CNS proinflammation (Rivet, 2009). However, more complex situations such as alcohol-induced liver toxicity may also produce similar proinflammatory cytokine profiles. Clearly, central immune signaling alone will not engage the mesolimbic dopamine reward neurocircuitry in a fashion that will drive reinforcement. Instead, if this central immune signaling were to occur in the correct locations and preempts or coincides with exposure to a drug of abuse, augmentation of the rewarding drive may occur. This is an interesting hypothesis as chronic infection and inflammatory organ damage is prevalent in drug dependent populations, and viral infection is especially evident in injecting drug users (United Nations Office on Drugs & Crime, 2010). It would be difficult to establish that infections or inflammatory events in these “at risk” populations led to their addiction, but perhaps aggressive treatment of both infection/inflammatory disease and their drug addiction may be required to remove the central immune signaling component of their drug abuse.

Beyond classical infection and immunity mechanisms, there is a growing literature that various environmental factors and psychological stressors can significantly impact central immune signaling (Woodruff et al., 2008; Goshen & Yirmiya, 2009; Racke & Drew, 2009; Frank et al., 2010). Whilst speculative, it would follow that such non-immunological insults may cross-sensitize specific brain nuclei to subsequent drug abuse-induced central immune signaling. A hypothesis such as this may contribute to the existing hypotheses of increased rates of drug abuse in patients with post-traumatic stress disorder, depression, and schizophrenia.

13. Novel pharmacological intervention opportunities by targeting drugs of abuse central immune signaling

As we have discussed (Watkins et al., 2005; Hutchinson et al., 2007; Watkins et al., 2007a, 2007b; 2009) and demonstrated previously for the beneficial modification of morphine pharmacodynamics (Hutchinson et al., 2008a, b; Bland et al., 2009; Hutchinson et al., 2009a; 2009b, 2010a, 2010b,2010c; Lewis et al., 2010) there are several points in the signaling cascade that can be pharmacologically targeted with existing pharmacological technologies (see Hutchinson et al. (2007) for review). Given the wealth of data reviewed here, it appears that similar direct techniques may also be employed for other drugs of abuse to beneficially modify their abuse liabilities. As outlined, there are several putative receptors involved in the engagement of proinflammatory central immune signaling by the drugs of abuse. One that appears to be the key is TL4R. The use of centrally acting TL4R functional signaling inhibitors such as (+)-naloxone one or (+)-naltrexone may therefore prove beneficial in decreasing drug abuse-induced proinflammation and associated behavioral enhancement. Several novel TL4R inhibitors are being developed for
peripheral innate immune applications, if these were demonstrated to cross the blood–brain barrier they too may have efficacy in this indication.

It therefore follows that targeting any of the associated downstream consequences of TLR4 activation may also prove efficacious, but there is considerable risk for non-specificity of action as pharmacological manipulation occurs further down the signaling cascade. Nonetheless, this less specific method of central immune signaling proinflammatory control using agents such as ibudistat, propentofylline or minocyline may prove beneficial in some cases where the sources of proinflammation are of diverse and are of non-TLR4 origins. Arguments can be made that one therapeutic strategy is better than the other, however the safety issues of attenuating proinflammatory central immune signaling responses in both cases need to be acknowledged. Any pharmacological agent of known or unknown mechanistic etiology that decreases, for example, LPS-induced TLR4 proinflammatory signaling in the CNS has this potential safety concern. Having said this, several of the pharmacological tools discussed here such as minocyline and ibudistat have excellent, well-established clinical safety records.

An exciting rationale drug development opportunity for the development of new opioid agonists for the treatment of acute and chronic pain is to combine opioid agonist activity with the ability to block TLR4, a component of its signaling cascade or glial activation generally in one small molecule. As such, an agent with this specific pharmacology would have profound opioid analgesia but reduced abuse liability owing to its limited central immune signaling capacity. Such an agent appears to have been fortuitously developed, PTI-609 (Burns & Wang, 2010), which has excellent analgesic action with limited rewarding behavioral consequences in the data published to date. Such rationale drug design strategies may prove extremely beneficial in the future to have powerful but safe analgescics.

Indirect methods of pharmacological modification of this drug of abuse-induced central immune signaling and its ensuing downstream consequences are also possible. For example, elevation of nucleus accumbens GDNF expression, upregulation of glial glutamate transporters by cetrixalone (Ramos et al., 2010), or the institution of an anti-inflammatory environment using IL-10 therapies may also be worthy future candidates.

Owing to the existing literature, several clinical trials have been conducted and are ongoing employing pharmacological agents hypothesized a priori to beneficially modify the recovery from or ameliorate the actions of drugs of abuse. If successful, the ongoing studies will be further proof-of-concept that future treatments focusing on central immune signaling may be a viable adjunct or alternative approaches for the treatment of drug abuse.

14. Conclusions

This review has highlighted that the drugs of abuse, that is, opioids (predominantly morphine), alcohol (ethanol), cocaine, methamphetamine, and 3,4-methylenedioxymethamphetamine (MDMA; ‘Ecstasy’), and their metabolites, clearly have both neuronal and central immune signaling modulation properties that combine to create the rewarding and dependence behaviors associated with repeated exposure. It is also evident that with regard to central immune properties, priming or shifting of the CNS to a proinflammatory state is connected with this modulation. As studies in this burgeoning field continue, the clinical utilization of this relatively new property of the drugs of abuse will allow ‘at risk’ individuals (identified by genetic testing to be augmented/deficient in a particular immune signaling pathway) to be treated with novel anti-dependence agents that specifically target CNS proinflammation. Importantly, this paradigm shift in understanding may hold the key to treating drug dependence in individuals who are currently non-responsive to classical pharmacological therapies that target neuronal pathways of dependence.

Acknowledgments

Dr. Janet K. Coller is a FIT Fricker Research Fellow (University of Adelaide Medical Endowment Funds) and Dr. Mark R. Hutchinson is an Australian Research Council Research Fellow (DP110100297) at the University of Adelaide.

References


Horvat, R. J., Romero-Sandoval, E. A., & De Leo, J. A. (2010). Inhibition of microglial P2X4 receptors attenuates morphine tolerance, Iba1, GFAP and mu opioid receptor protein expression while enhancing perivascular microglial ED2. Pain 150, 401–413.


mitogen activated protein kinase, phosphoprotein enriched in astrocytes of 15 kDa, and Akt signaling pathways involved in neuroplasticity. Neurosciencia 161, 23–38.


