Medical Research Archives

ESMED
European Society of Medicine

OPEN ACCESS

Published: August 31, 2022

Citation: Toloff K and Woodcock EA, 2022. Is the Neuroimmune System a Therapeutic Target for Opioid Use Disorder? A Systematic Review, Medical Research Archives, [online] 10(8). https://doi.org/10.18103/mra.v10i8.2955

DOI
https://doi.org/10.18103/mra.v10i8.2955

ISSN: 2375-1924

REVIEW ARTICLE

Is the Neuroimmune System a Therapeutic Target for Opioid Use Disorder? A Systematic Review

Katelyn Toloff, MS[1] & Eric A. Woodcock, PhD*[1]

[1]Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI, USA

* eric.woodcock@wayne.edu

ABSTRACT

Opioid use disorder (OUD) is an epidemic in the United States. In the past 12 months alone, there have been 75,000+ deaths attributed to opioid overdose: more than any other year in American history. Current pharmacotherapies for the treatment of OUD effectively suppress opioid withdrawal symptoms, but long-term relapse rates remain unacceptably high. Novel treatments for OUD are desperately needed to curb this epidemic. One target that has received considerable recent interest is the neuroimmune system. The neuroimmune system is anchored by glial cells, i.e., microglia and astrocytes, but neuroimmune signaling is known to influence neurons, including altering neurotransmission, synapse formation, and ultimately, brain function. Preclinical studies have shown that experimental attenuation of pro-inflammatory neuroimmune signaling modulates opioid addiction processes, including opioid reward, tolerance, and withdrawal symptoms, which suggests potential therapeutic benefit in patients. Whereas the peripheral immune system in OUD patients has been studied for decades and is well-understood, little is known about the *neuro*immune system in OUD patients or its viability as a treatment target. Herein, we review the literature describing relationships between opioid administration and the neuroimmune system, the influence of neuroimmune signaling on opioid addiction processes, and the therapeutic potential for targeting the neuroimmune system in OUD subjects using glial modulator medications.

Keywords: neuroinflammation, neuroimmune signaling, microglia, opioid use disorder, heroin, fentanyl

Opioid use disorder

Opioid use disorder (OUD) is a complex, chronic relapsing disorder that is shaped by the pharmacological effects of opioid use as well as the psychological and neurobiological adaptations that occur after repeated opioid use[1,2]. The Diagnostic and Statistical Manual of Mental Disorders 5 (DSM-5) characterizes OUD in terms of impaired control over opioid use and the persistence of opioid use despite negative consequences[3,4]. Dr. George Koob and colleagues have proposed a 3-stage model for conceptualizing drug addiction: preoccupation/anticipation, binge/intoxication, and withdrawal/negative affect[5,6]. Initial drug-taking experiences ('binge/intoxication') are often euphoric and positively reinforcing which can motivate repeated use. Over time, as tolerance develops, motivation for continued drug use shifts from positive reinforcement (pursuit of euphoria and 'high') to negative reinforcement (avoidance of 'withdrawal/negative affect')[3]. Avoidance of withdrawal contributes to drug craving ('preoccupation/anticipation') and motivates continued drug use despite social, economic, legal, and health consequences. It is through the lens of the 3-stage model of addiction that we will review the literature linking neuroimmune signaling and opioid addiction processes.

Novel treatments for opioid use disorder are needed

OUD has reached epidemic proportions in the United States. Between April 2020 and April 2021, an estimated 75,673 Americans died from opioid overdose: more than any other year in American history[7]. U.S. Food and Drug Administration (FDA) approved medications for the treatment of OUD include methadone, buprenorphine, and naltrexone. Methadone is a full μ opioid receptor (MOR) agonist, and an antagonist at N-methyl-D-aspartate (NMDA) receptors[8]. As a full MOR agonist, methadone more effectively suppresses opioid withdrawal symptoms than buprenorphine (a *partial* MOR agonist), though methadone has greater abuse liability[9]. Methadone overdose can cause fatal respiratory depression, especially if used in conjunction with other opioids[8]. Buprenorphine is a partial MOR agonist, and an antagonist at κ and δ opioid receptors[10]. Buprenorphine exhibits higher affinity at the MOR than other opioids, e.g., morphine, fentanyl, and oxycodone, and thus, can competitively block other opioids from binding the MOR[11,12], which can reduce the risk of opioid overdose. Further, buprenorphine can displace opioids at the MOR, e.g., heroin/morphine, which can trigger opioid withdrawal symptoms among patients actively using opioids[13]. Buprenorphine's partial MOR agonism is associated with a milder agonist side effect profile compared to methadone. In contrast with methadone and buprenorphine, naltrexone is a MOR *antagonist*, and a weak antagonist of κ and δ opioid receptors[14]. As an antagonist, OUD patients undergo opioid detoxification prior to initiating naltrexone, which can lead to treatment dropout, but once initiated, naltrexone has an excellent safety profile (though patient retention in treatment can be a challenge)[14]. Long-acting injectable formulations of naltrexone and buprenorphine may enhance treatment retention and thus, clinical outcomes[15].

Numerous clinical trials and meta-analyses have evaluated the relative effectiveness of these medications, and results vary by dose scheme. For flexible-dosing schemes, i.e., medication dose is individualized to patient need/comfort (perhaps the most clinically-relevant design), methadone is more effective than buprenorphine for retaining patients in treatment, however, among those who remain in treatment, each medication suppresses recreational opioid use with similar effectiveness[16]. Though, more recent studies suggest buprenorphine, especially at higher doses ($\geq$16mg/day), may have a slight advantage over methadone for suppression of recreational opioid use and overdose mortality[15,17]. However, it has become clear that these medications are inadequate for many OUD patients to maintain long-term abstinence[16,18]. Large clinical trials indicate that up to ~50% of OUD patients drop out of treatment within the first 6 months and among those who remained in treatment, recreational opioid use was detected in >30% urine samples tested[17]. Thus, whereas existing pharmacotherapies are effective for some patients, most OUD patients lapse (or relapse) within 6 months of treatment initiation. Novel treatments for OUD are desperately needed. Indeed, the Director of the National Institute on Drug Abuse (NIDA), Dr. Nora Volkow, has recently advocated that a poly-pharmacy approach may be needed to treat OUD, i.e., one medication to target opioid craving/withdrawal and a second (or third) medication to target other biological systems that are perturbed by chronic opioid misuse[19,20]. One biological system that has received considerable recent interest as a potential adjunctive therapeutic target is the neuroimmune system.

The neuroimmune system

The neuroimmune system is principally anchored by microglia and astrocytes.[21-23] Microglia are the resident macrophages in the brain, and initiate and maintain neuroinflammatory processes in the brain[24,25]. Microglia constantly surveil their local environment for irritants, pathogens, and cellular debris[26]. Upon detection of pathogen- or damage-associated molecular patterns (PAMPs and DAMPs, respectively), microglia can become 'classically activated' ('M1'-type state), release proinflammatory cytokines/chemokines (e.g., tumor necrosis factor α or TNF-α) and other proinflammatory mediators (e.g., nitric oxide or NO), and transform to an 'amoeboid' shape to phagocytose the irritant[27,28]. Astrocytes also respond to irritants and can perpetuate or amplify signals from microglia[29,30]. Glia activation results in increased expression of cell surface markers cluster of differentiation 11b (CD11b) for microglia, and glial fibrillary acid protein (GFAP) for astrocytes[21,31], which are often employed as biomarkers of glial activation in preclinical studies. Glia activation also results in increased expression of inflammatory mediators, such as interleukin-1β (IL-1β), IL-6, and TNF-α, monocyte chemoattractant protein-1 (MCP-1), and inducible nitric oxide synthase[21,32]. In addition to their primary role as neuroimmune signaling molecules, cytokines and chemokines influence brain function, mediate glia-neuron communication[33], interact with neuroendocrine and neuropeptide systems, and modulate central nervous system (CNS) development[34-36]. Immune responses also influence stress reactivity via the hypothalamic-pituitary-adrenal (HPA) axis and neurotransmitter systems, e.g., serotonin and dopamine[37,38]. As such, neuroinflammatory signals can modulate behavior and influence neuroplasticity and neurogenesis[39,40].

Neuroimmune responses are both context- and insult-specific, and occur along a graded and tightly-regulated continuum[21]. Whereas acute neuroinflammatory responses are normative and promote cellular survival, chronic neuroinflammation (often demarcated as lasting longer than 6 weeks) can be pathological and result in both neuronal and glia cell death[43]. In one study, administration of 0.8 ng/kg *Salmonella* endotoxin, a low-dose neuroinflammatory agent, did not cause individuals to report feeling sick, but significantly impaired declarative and working memory[41]. In another study, *Salmonella abortus equi* endotoxin (0.8 ng/kg) transiently increased anxiety and depressed mood among 20 healthy individuals, and peripheral cytokine levels were correlated with changes in anxiety and mood[42]. These studies suggest that even 'mild' perturbations of neuroimmune state can significantly impair cognitive functions and alter mood state.

Opioid-induced neuroimmune activation

Preclinical and cellular research suggests that opioid administration can activate glia, as measured by increased expression of GFAP (astrocyte marker), or CD11b and Iba1 (microglia markers)[21], pro-inflammatory cytokines[44], and morphological transformation to a pro-inflammatory glial phenotype[45]. Opioid-induced neuroinflammation has been characterized at both molecular- and cellular-levels. At the molecular level, *in vitro* studies have shown that morphine administration increases the expression of chemokines CCL2, CCL5, and IFNγ in the brain[46]. *In vivo* opioid administration in mice increased expression of IL-1, TNF-α, and IL-6 in the prefrontal cortex and hippocampus[47], neuroanatomic areas relevant to addiction[46,48,49]. Another study showed that subcutaneous implantation of a morphine pellet (50 mg/kg) for 6 consecutive days upregulated TNF-α, IL-1β, and IL-6 in the nucleus accumbens in mice, a region associated with drug reward[50]. Finally, morphine exposure for 6 consecutive days increased IL-1β levels in the spinal cord[51]. At the cellular-level, opioids have been shown to increase macrophage density in the brain and induce morphological transformations indicative of microglia activation (amoeboid shape; 'M1'-biased phenotype)[52]. Opioid administration upregulated brain and spinal astrocyte (GFAP) and microglia (CD11b) markers[30]. Five days of systemic morphine administration increased GFAP in the ventral tegmental area (VTA); a region, along with the nucleus accumbens, that forms a 'final common pathway' in addiction which is thought to mediate drug reward[53]. Finally, administration of glial modulators (e.g., ibudilast), which attenuate pro-inflammatory neuroimmune responses, have been shown to significantly reduce opioid-induced increases in astrocyte (GFAP) and microglia (CD11b) markers in areas relevant to addiction, e.g., periaqueductal gray and amygdala[21]. In sum, opioid administration has been shown to increase pro-inflammatory cytokine and chemokine mRNA levels in the brain, induce morphological changes in microglia consistent with a 'classically activated' 'M1' state, and increase glial cell density in the brain – hallmarks of a pro-inflammatory neuroimmune state.

Although the specific mechanisms by which opioids activate glia are not yet fully understood,

recent evidence suggests toll-like receptor 4 (TLR4) binding may be involved (see Figure 1)[30]. TLR4 is a pattern-recognition receptor that detects DAMPs and PAMPs[54]. Upon activation of TLR4, two pathways can mediate downstream effects; one that activates the MyD88-independent pathway, leading to release of type-1 interferons, and the other resulting in the induction of transcription factor nuclear factor-kappa B (NF-κB), which leads to the release of pro-inflammatory cytokines such as IL-6 and TNF-α[54]. TLR4 stimulation, e.g. via lipopolysaccharide (LPS; endotoxin) binding, is known to mediate 'classical activation' of microglia[55]. Recent findings suggest that opioids may evoke neuroinflammation via TLR4 stimulation, i.e., in a manner parallel to endotoxin[56]. Opioids may stimulate TLR4 directly and/or indirectly. *Direct* opioid stimulation of TLR4 has been demonstrated *in vivo*[56,57], *in vitro*[56], and *in silico*[46], but these findings are controversial[58]. Conversely, opioid administration may stimulate TLR4 *indirectly* via MOR binding which can weaken tight junctions in the gut allowing gut bacteria to leak into systemic circulation, where it can bind and activate TLR4[51,59,60]. Whereas the specific molecular pathways remain unclear and controversial, evidence to date suggests that opioid administration can be neuroinflammatory.

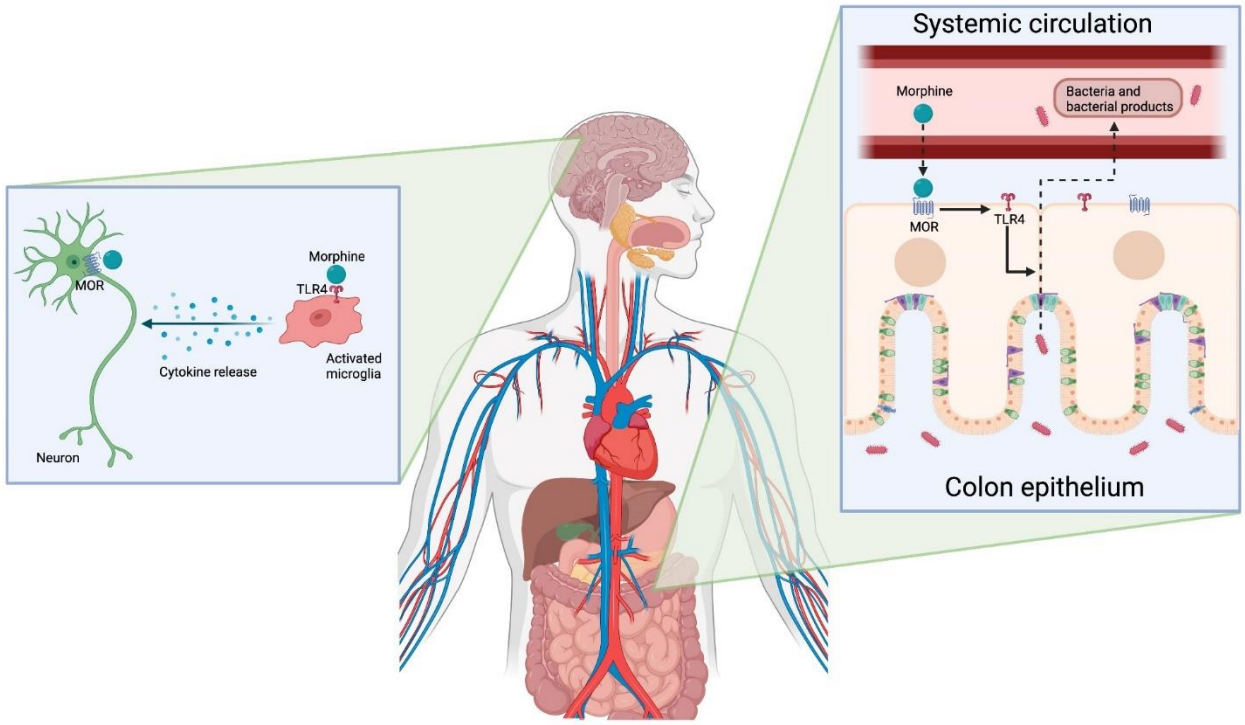

Figure 1 – Putative Opioid-Neuroimmune Mechanisms
The putative mechanisms through which opioids, e.g., morphine/heroin, may evoke neuroinflammatory signaling are depicted. *Left panel*. The 'direct' pathway is shown. In addition to binding the MOR, opioids may also bind TLR4 in the brain. Activation of TLR4 can increase secretion of pro-inflammatory cytokines/chemokines and evoke 'classical activation' ('M1'-type) of microglia. Direct opioid-TLR4 binding has been challenged and remains controversial. *Right panel*. The 'indirect' pathway is shown. Opioids bind MOR in the gut which can weaken tight junctions allowing bacteria to 'leak' into the blood stream. From there, bacteria can bind TLR4s throughout the body evoking systemic inflammation, including in the brain.

The neuroimmune system among opioid-using individuals

Much of the literature above focused on the neuroinflammatory effects of acute or short-term opioid dosing in rodents. What is known regarding chronic opioid administration in humans, i.e., OUD patients? There is postmortem evidence of neuroinflammation in deceased opioid users[61]. Moretti et al., evaluated immunohistochemical markers in sections of the frontal cortex of 40 postmortem cocaine, heroin, or polydrug users and 10 controls[62]. Findings indicated higher levels of CD3 (a T-cell marker) and intracellular adhesion molecule-1 positivity (indicating upregulated inflammatory processes), and less ZO-1 immunopositivity (reflecting integrity of tight

junctions) in drug users compared to controls[62]. Of note, astrocyte density (GFAP immunopositivity) did not differ between groups[62]. Büttner and Wies analyzed markers of glial activation in cortical and subcortical brain areas of 50 polydrug users and 30 controls[63]. Findings indicated *lower* levels of GFAP-positive astrocytes, but *higher* levels of perivascular and parenchymal microglia (HLA-DR) in white matter and subcortical regions[63]. These findings suggest that living OUD patients may exhibit neuroinflammation, but postmortem findings are somewhat mixed and glial activation may be cell specific (microglia, but not astrocyte, activation). Ultimately, while postmortem studies are insightful, findings can be difficult to interpret due to confounding factors, including cause of death, and thus, may not reflect the neurobiology of living OUD patients.

To study the neuroimmune state of living people, the most widely-used tool is positron emission tomography (PET) imaging of the 18kDa Translocator Protein (TSPO). TSPO is a mitochondrial protein that is highly expressed in glial cells, especially microglia. PET TSPO levels have been shown to scale with microglia levels, with robust increases reported after pro-inflammatory challenges[64-66], and marked decreases after pharmacological depletion of microglia[67]. Thus, TSPO is often purported to represent a 'microglial marker'. While this characterization is an oversimplification, PET TSPO imaging studies have reliably shown higher TSPO levels after neuroinflammatory challenges[64-66] and in patients with neuroinflammatory conditions compared to controls[68-70]. With regard to opioids, two acute challenge studies have been conducted to date (to our knowledge). Relative to baseline levels, Auvity et al., showed acute TSPO increases of ~30% throughout the brains of five baboons 2-hour after a single dose of morphine (1mg/kg i.m.)[71]. The clinical translation of this study showed similar findings in people. Woodcock et al., showed that a single dose of intramuscular morphine evoked a significant increase in TSPO levels by 25-32% across brain regions among 8 healthy adult volunteers[72]. Additionally, plasma concentrations of morphine were strongly positively correlated with TSPO increases suggesting a linear relationship between morphine in the blood and inflammatory response in the brain[72]. While these studies directly link opioid administration and elevated neuroimmune signaling *in vivo* (consistent with rodent studies), findings from acute challenge studies may not reflect the neuroimmune state of OUD patients who have been repeatedly administering opioids for years or decades. To date, no PET TSPO studies in OUD patients have been published to our knowledge. Thus, it remains unknown whether living OUD patients exhibit elevated neuroimmune signaling or whether treatment (and abstinence from illicit opioid use) is associated with neuroimmune recovery or 'normalization'. Future research is needed to address these critical gaps in the literature.

Neuroimmune signaling modulates opioid addiction processes

Whereas direct *in vivo* evidence of neuroinflammation in OUD patients is lacking, there is preclinical evidence that neuroimmune signaling modulates opioid addiction processes. Using glial modulators to attenuate neuroinflammatory signals, preclinical studies have shown evidence of positive effects in each of the 3 stages of the addiction cycle: preoccupation/anticipation, binge/intoxication, and withdrawal/negative affect (see Figure 2). Glial modulators are pharmaceutical agents that attenuate pro-inflammatory signals released by activated glia and thus, are useful for indirect investigation of neuroimmune signaling. The two most widely-studied glial modulators are minocycline and ibudilast. Minocycline is a tetracycline antibiotic that exhibits anti-inflammatory and anti-oxidative effects[73]. Minocycline is able to readily cross the blood brain barrier due to its small lipophilic nature[73], and is thought to inhibit production of immune signaling molecules released by microglia, such as NO or TNF-α[74-76]. While its precise mechanisms of action are not completely understood[77], minocycline has also been shown to down-regulate the pro-inflammatory signal transduction pathway NF-Kβ[78]. Conversely, ibudilast is a nonselective phosphodiesterase inhibitor, and acts to inhibit pro-inflammatory cytokine release from macrophages[79]. Ibudilast is also an antagonist at TLR-4 and inhibits glial secretion of NO[80]. Ibudilast has been shown to attenuate markers of glial activation in rodents and decrease the neurotoxic effects of inflammatory challenges[81-84].

1. Preoccupation/Anticipation. The preoccupation stage of the 3-stage addiction model is characterized by drug craving, drug desire, and motivation to seek and take a drug[5,6]. Pretreatment with the glial modulator, minocycline, decreased morphine conditioned place preference in mice[85,86], implicating

neuroimmune signaling in morphine reward and anticipation of morphine administration.

2. Binge/Intoxication. The binge/intoxication stage is characterized by the acute reinforcing effects of drug administration[5,6]. In the brain, the reinforcing properties of acute drug administration are associated with the mesolimbic dopamine system; specifically, synaptic dopamine release in projections from the VTA to nucleus accumbens, i.e., 'final common pathway' in addiction[6,87]. Pretreatment with the glial modulator, ibudilast, attenuated morphine-induced dopamine release in the nucleus accumbens in rodents[88], suggesting that neuroimmune signaling can modulate opioid-induced mesolimbic dopaminergic signaling. Further, pretreatment with ibudilast attenuated the development of morphine tolerance[47,89,90] and enhanced the analgesic effects of opioid administration[46]. Together, these findings suggest that attenuation of pro-inflammatory neuroimmune signaling may have therapeutic benefits after opioid administration, i.e., reduction of opioid tolerance and enhancement of opioid analgesic efficacy, which may translate to 'opioid sparing' effects in patients.
3. Negative Affect/Withdrawal. The negative affect/withdrawal stage is characterized by symptoms of irritability, pain hypersensitivity, depression, anxiety, and dysphoria after prolonged periods of drug abstinence[5,6]. Pretreatment with ibudilast reduced hyperalgesia, allodynia, and withdrawal-induced pain responses in opioid-treated rodents[30,46,56,86,91-95], suggesting that neuroimmune signaling modulates opioid withdrawal symptoms.

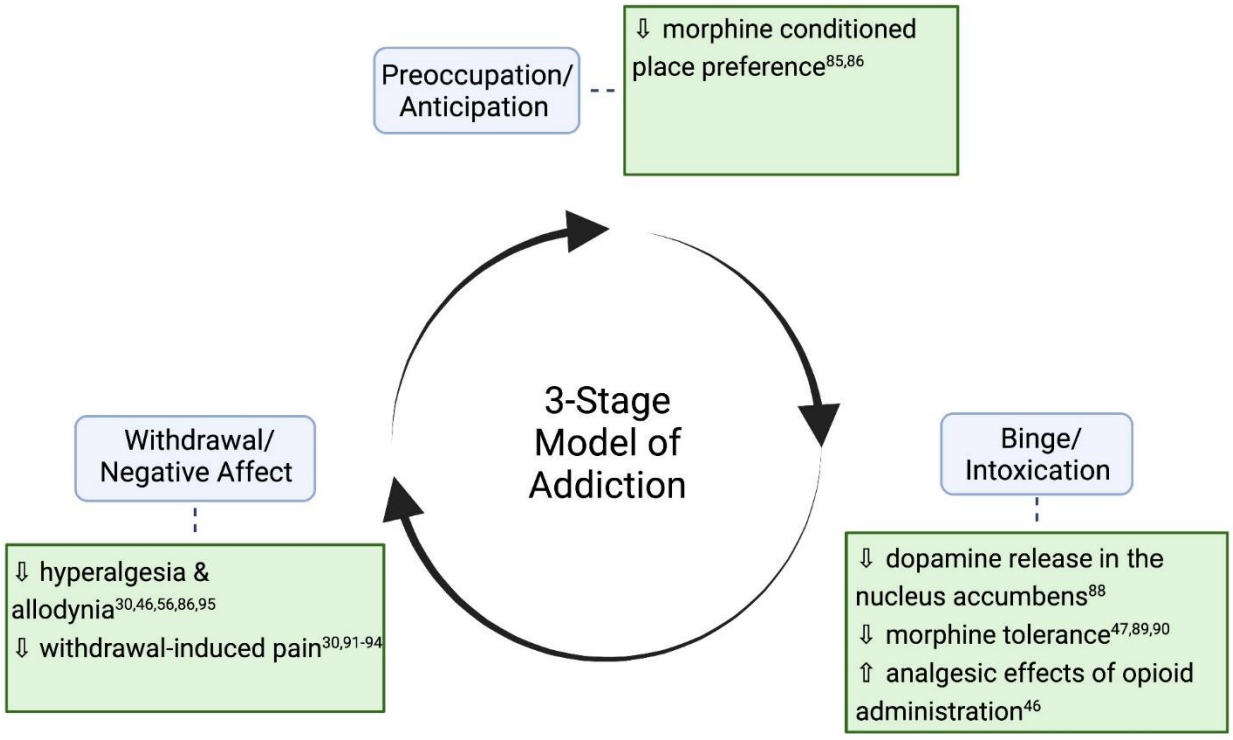

Figure 2 – Preclinical Evidence of Glial Modulator Modulation of Opioid Addiction Processes
In each stage in the 3-stage model of addiction, glial modulator administration has been shown to modulate opioid addiction processes in preclinical studies.

Given these promising findings in rodents which show positive benefits of glial modulator administration, there is considerable interest in glial modulators as therapeutic agents in OUD patients. The evidence summarized above suggests that neuroinflammatory signals modulate opioid reward, tolerance, analgesia, and withdrawal symptoms. These findings are buttressed by the preclinical and postmortem evidence which show that repeated opioid administration may lead to a persistent allostatic shift in neuroinflammatory state: one that may benefit from glial modulator treatment. While direct *in vivo* evidence of neuroinflammation in living OUD patients has yet to

be demonstrated, there is evidence that acute opioid administration evokes a robust neuroimmune response in non-human primates[71] and humans[72]. In sum, neuroinflammatory signaling may be a novel treatment target in OUD patients. In this review, we systematically evaluated the published clinical literature investigating the neuroimmune system as a therapeutic target, i.e., by administering a glial modulator, and summarize those findings below. We discuss limitations of the current research and offer suggestions for future directions.

Glial modulator studies among opioid users

To date, there have been 5 published manuscripts that investigated the effects of glial modulator administration among opioid users: 3 of which administered ibudilast and 2 administered minocycline. Below is a summary of each study presented in chronological order.

Cooper et al., 2016 assessed drug safety and tolerability of ibudilast during morphine maintenance and discontinuation among 31 non-treatment-seeking opioid-dependent adults during a 3-week inpatient study[96]. Subjects were maintained on oral morphine (30mg q.i.d.) for the first 14-days and then oral placebo (0mg q.i.d.), triggering opioid withdrawal for the final 7-days of the study prior to discharge. During days 1-7, all subjects also received placebo ibudilast capsules (0mg PO). On days 8-21, subjects were randomized to either 0mg, 20mg or 40mg ibudilast (PO, b.i.d.). Subjective and objective opioid withdrawal symptoms were significantly elevated during placebo morphine, compared to active morphine, as expected. However, there were no significant effects of ibudilast dose on opioid withdrawal symptoms during active or placebo morphine. Exploratory analyses indicated the active ibudilast groups (pooling both doses) reported lower levels of a subset of opioid withdrawal symptoms including 'anxious', 'perspiring', 'restless', and 'stomach cramps' on the Subjective Opioid Withdrawal Scale (SOWS) compared to the placebo group. Ibudilast was well-tolerated during active and placebo morphine maintenance, and no serious adverse events occurred. Thus, ibudilast may attenuate a subset of subjective opioid withdrawal symptoms.

Cooper et al., 2017 investigated the effects of ibudilast on subjective and analgesic responses to oxycodone among the subjects described above[97]. In this study, the analgesic, subjective, and physiological effects of cumulative oxycodone dosing (0, 25, 50mg/70kg; PO) were measured after cold-pressor task (CPT)-induced pain on Day 4 (in which patients received placebo ibudilast) vs. Day 11 (in which patients were randomized to receive either 20mg vs. 40mg ibudilast PO b.i.d.) during oral morphine maintenance (30mg q.i.d.). As expected, oxycodone decreased CPT-induced subjective pain ratings and increased pain threshold (latency to report pain) and pain tolerance (latency to withdraw arm from cold water bath) during placebo ibudilast. Relative to placebo ibudilast, oxycodone-elicited decreases in subjective pain ratings were enhanced by 40mg ibudilast (but not 20mg ibudilast). Also, oxycodone's analgesic effect on pain threshold was retained in both ibudilast dose conditions, whereas oxycodone failed to increase pain threshold in the placebo ibudilast condition, suggestive of opioid tolerance. Relative to placebo levels, active ibudilast did not consistently alter subjective positive responses to cumulative dosing of oxycodone. In sum, this study showed that ibudilast may enhance the analgesic efficacy of opioids and may attenuate the development of opioid tolerance to evoked thermal pain.

Metz et al., 2017 admitted non-treatment seeking, male, heroin-dependent volunteers (N=11) inpatient for a 7-day opioid detoxification assisted by sustained-release morphine (60mg b.i.d.)[98]. After detoxification, in a random cross-over design, subjects received either placebo or active ibudilast (0mg vs. 50mg b.i.d., respectively) for 5-6 days prior to 6 days of laboratory sessions and then crossed over to the other medication to repeat procedures. During the 6 days of laboratory sessions, subjects completed 'sampling' and then 'choice' sessions on consecutive days at 3 different oxycodone doses (0, 15, and 30mg/kg PO; dose order randomized). During 'sampling' sessions, subjects received $20, a dose of oxycodone, and completed subjective, behavioral, and physiological effects measures. During the 'choice' sessions, subjects completed a 10-trial progressive ratio money vs. drug choice task in which he/she could earn $1/10^{th}$ of the oxycodone dose or $1/10^{th}$ of the money sampled the day prior via computer mouse button presses. Results from the 'sampling' sessions indicated that, across oxycodone doses, subjects reported significantly less heroin craving during active ibudilast compared to placebo. Relative to placebo levels, subjective oxycodone 'liking' was significantly attenuated by ibudilast at the 15mg, but not the 30mg, oxycodone dose. Finally, relative to placebo levels, drug breakpoint values were significantly reduced by ibudilast at the 15mg, but not the 30mg, oxycodone dose (which was 'trend'-level). Together, this rigorous

within-subject inpatient study showed that ibudilast attenuated self-reported heroin craving, positive subjective response to oxycodone, and oxycodone-seeking behavior relative to placebo levels, suggesting that ibudilast may have therapeutic value for OUD patients.

Arout et al., 2019 reported limited efficacy of minocycline among OUD patients. In this study, male (n=15) and female (n=5) OUD patients enrolled in opioid agonist therapy (either buprenorphine or methadone) were randomly assigned to either minocycline (200mg/day PO) or placebo for 15 days[99]. On days 1, 8, and 15, subjective (mood, self-reported pain, and subjective response to pain on the CPT), cognitive (Go/No-Go task and Digit Symbol Substitution task) and experimental pain (objective CPT metrics) were assayed. Serum cytokines (IL-1β, IL-6, and TNF-α) were assayed prior to, and again on day 15, of the study. Finally, subjective measures were periodically assayed throughout week 2 of the study via ecological momentary assessments with the addition of opioid craving and opioid withdrawal measures. Results indicated that minocycline did not alter any subjective effects measures, response to thermal pain, or serum levels of pro-inflammatory cytokines. OUD participants committed fewer commission errors (failure to inhibit to 'No-Go' stimuli) after minocycline, suggesting that minocycline may improve response inhibition, but no effect was observed for the Digit Symbol Substitution task. Thus, 15 days of minocycline (200mg/day) enhanced response inhibition, but did not alter mood, subjective pain, pain tolerance, opioid craving or opioid withdrawal symptoms among OUD patients enrolled in outpatient opioid agonist maintenance therapy.

Mogali et al., 2021 assessed the effects of pretreatment with minocycline on the subjective, physiological, and analgesic effects of oxycodone among 12 non-treatment seeking, non-dependent recreational opioid users[100]. This study was conducted in an outpatient setting, and utilized a within-subject, randomized, double-blind design. Five individual laboratory sessions were conducted in which subjects received either 0mg, 100mg, or 200mg PO of minocycline pretreatment and were challenged with either placebo or active oxycodone (0mg or 40mg PO, respectively). Measures included subjective effects (visual analog scale), physiological effects (respiratory rate, tidal CO_2, and cardiovascular function), pain assessments (CPT), cognitive tasks (Digit Symbol Substitution and Divided Attention task), and side effects (adverse events). Results from this study found that 100mg and 200mg minocycline were safe and well-tolerated in conjunction with the active oxycodone dose (40mg PO). Pretreatment with 200mg minocycline attenuated oxycodone positive subjective effects, e.g., 'liking' and 'good effect', compared to oxycodone alone. Conversely, minocycline did not alter subjective opioid craving, or the physiological or analgesic effects of oxycodone. Thus, a single dose of minocycline may attenuate subjective positive responses to opioid administration among non-dependent opioid users.

Table 1. Clinical studies of glial modulators among opioid users.

	Subjects	Study Design	Doses	Maintenance Rx	Key Findings	Limitations/ Null Findings
Cooper et al., 2016	Non-treatment OUD; N = 31	Inpatient, double-blind, placebo-controlled, within-subject and between-group design	0mg, 20 mg, or 40mg ibudilast, PO, b.i.d.	30mg morphine, PO, q.i.d.	Exploratory analyses pooling both ibudilast doses reported lower ratings of a subset of withdrawal symptoms, relative to placebo.	Total subjective opioid withdrawal scale scores did not differ between groups. Mostly male subjects.
Cooper et al., 2017	Non-treatment OUD; N = 31	Inpatient, double-blind, placebo-controlled, within-subject and between-group design	0mg, 20mg, or 40mg ibudilast, PO, b.i.d	30mg morphine, PO, q.i.d.	40mg of ibudilast was associated with higher pain threshold and lower subjective pain ratings, compared to placebo	Ibudilast did not consistently affect subjective drug effect ratings associated with abuse liability. Mostly male subjects.
Metz et al., 2017	Non-treatment OUD;	Inpatient, randomized, placebo-	0mg or 50 mg b.i.d.	None; 'Sampling' and 'choice'	Ibudilast decreased 'drug liking' following 15mg of oxycodone	Subjective response to oxycodone and

	N = 11	controlled, within-subject crossover design	ibudilast, PO	lab sessions for 0mg, 15mg, or 30mg/70 kg, PO oxycodone	and reduced mean drug breakpoint value for 15mg oxycodone. Heroin craving and subjective pain ratings were lower during active ibudilast.	mean drug breakpoint value was not significantly lower for 30mg oxycodone. Only male subjects.
Arout et al., 2019	Treatment-engaged OUD; N = 20	Outpatient, double-blind, randomized, placebo-controlled design	0mg or 200mg minocycline, PO	Standard-of-care opioid agonist treatment (either buprenorphine or methadone)	Minocycline increased accuracy on a Go/No-Go task	Minocycline did not change pain threshold or tolerance. Minocycline did not change severity of pain ratings, opioid craving or withdrawal, or serum cytokines
Mogali et al., 2021	Non-treatment OUD; N = 12	Outpatient, double-blind, randomized placebo-controlled within-subject design	0mg, 100mg, or 200mg minocycline, PO	None; 0mg or 40mg oxycodone lab sessions PO	Both minocycline doses were safe and well-tolerated in conjunction with oxycodone 40mg. Minocycline 200mg attenuated oxycodone subjective positive effects ('good effect', 'liking') compared to oxycodone alone.	Minocycline did not alter opioid craving, or the physiological or analgesic responses to oxycodone. Small sample size, mostly male, non-dependent opioid users were used.

In sum, synthesis of the existing clinical literature indicates that ibudilast exhibited positive effects that map onto each of the 3 stages of addiction (see Figure 3): 'preoccupation' (reduced heroin craving and oxycodone-seeking behavior)[98], 'binge/intoxication' (reduced subjective positive response to oxycodone and enhanced opioid-induced analgesia)[97,98], and 'negative affect/withdrawal' (reduction of subjective pain ratings, objective pain tolerance, and some subjective opioid withdrawal symptoms)[96,97]. These initial findings are encouraging given the rigorous experimental designs used, and suggest that ibudilast may have therapeutic potential for the treatment of OUD, implicating the neuroimmune system as viable treatment target. Conversely, minocycline was less effective. Minocycline attenuated the subjective positive responses to oxycodone in one study[100] but did not alter opioid craving, analgesia, or other physiological effects. In another study, minocycline improved response inhibition but did not improve mood state, analgesia, or reduce opioid craving/withdrawal symptoms.[99]

Viability of the neuroimmune system as a therapeutic target in OUD patients

There is tremendous urgency to identify and evaluate novel therapeutic targets for treatment of OUD. One target that has received considerable recent interest is the neuroimmune system and specifically, attenuation of pro-inflammatory neuroimmune signaling via glial modulator administration. The neuroimmune system is anchored by glial cells: principally, microglia. Microglia are the brain's resident macrophages and, upon activation undergo morphological transformation and release of pro-inflammatory cytokines/chemokines, e.g., IL-1β, IL-6, and TNF-α. Cytokines and chemokines are the principal signaling molecules of neuroinflammation, but also influence synaptic function, glia-neuron communication, and neurogenesis, and interact with the neuroendocrine, neuropeptide, and neurotransmitter systems. Neuroinflammatory signaling is associated with numerous consequences including cognitive decrements, impaired motivation, and depressed mood. In this review, we described evidence linking opioid administration to neuroinflammatory signals, both acute and

repeated opioid administration, and summarize what is known to date regarding the viability of the neuroimmune system as a therapeutic target for OUD.

Opioid administration has been shown to evoke neuroinflammatory signals at the molecular level, e.g., morphine administration increasing the expression of chemokines CCL2, CCL5, and IFNγ in the brain[46], and at the cellular level, e.g., opioid-induced proliferation of microglia[21] in neuroanatomic brain regions relevant to addiction[46,48,49]. Using PET TSPO imaging, acute challenge studies have shown the opioid administration robustly increases *in vivo* TSPO levels, indicative of elevated neuroimmune signaling, in non-human primates[71] and healthy adult volunteers[72]. The effects of repeated or chronic opioid administration are less well-understood. Preclinical and postmortem evidence suggests that repeated/chronic opioid use may evoke a neuroinflammatory state[61-63]. However, to date, no PET TSPO studies in OUD patients have been published and thus, the *in vivo* neuroimmune state of OUD patients remains unknown. Further, it remains unknown whether neuroinflammation in OUD patients, should it be present, will resolve with prolonged opioid abstinence/treatment or with glial modulator administration. While direct *in vivo* evidence of neuroinflammatory state is lacking, indirect evidence has been shown via the beneficial effects of glial modulators, especially ibudilast, in preclinical and clinical studies.

Glial modulators are pharmaceutical agents that suppress pro-inflammatory signaling. The two most widely studied glial modulators are ibudilast and minocycline. Though mechanisms of action differ (and are not completely understood), both ibudilast and minocycline are thought to act on glial cells to suppress pro-inflammatory cytokine and chemokine secretion, i.e., attenuate neuroinflammatory signaling[73-76,79,81-84]. In preclinical studies, pretreatment with glial modulators reduced opioid-induced dopamine release in the nucleus accumbens[88] and morphine conditioned placement preference[85,86], suggesting that glial modulators may suppress opioid reward (Figure 2). Further, ibudilast attenuated the development of morphine tolerance[47,89,90] and enhanced opioid analgesia[46], suggesting that glial modulators may reduce opioid demand in patients, i.e., 'opioid-sparing' effects. Finally, ibudilast reduced hyperalgesia, allodynia, and withdrawal-induced pain responses in opioid-treated rodents[30,46,56,86,91-95], suggesting possible beneficial effects during opioid withdrawal. Together, these preclinical findings highlight the therapeutic potential of glial modulator medications and motivated our review of the clinical literature.

Our literature searches revealed 5 published clinical studies that evaluated ibudilast or minocycline among opioid users. Ibudilast showed positive findings in 3 small, inpatient clinical studies among non-treatment-seeking OUD subjects. First, Cooper et al., found that 20mg and 40mg ibudilast reduced a subset of opioid withdrawal symptoms among OUD subjects and doses were well-tolerated[96]. Second, Cooper et al., found that, relative to placebo, opioid-induced decreases in subjective pain ratings were enhanced by 40mg ibudilast (but not 20mg ibudilast).[96] Further, oxycodone's analgesic effect on pain threshold was retained in both 20mg and 40mg ibudilast conditions, but not the 0mg ibudilast condition, suggestive that ibudilast may reduce development of opioid tolerance[97]. Third, Metz et al., found that 50mg ibudilast attenuated self-reported heroin craving, as well as positive subjective response to oxycodone and oxycodone-seeking behavior at the 15mg, but not the 30mg, oxycodone dose, relative to placebo levels[98]. Together, these studies show that ibudilast exhibited therapeutic effects in each of the 3 stages of drug addiction (Figure 3). Given the scale of the ongoing opioid epidemic and tremendous mortality associated, there is great urgency to investigate novel therapeutic targets for OUD, such as the neuroimmune system. To date, the experimental literature indicates that further investigation of ibudilast as an adjunctive medication for OUD is warranted. However, excitement must be tempered as these were relatively small studies among *non-treatment* OUD patients and many effects were non-significant (see Limitations below).

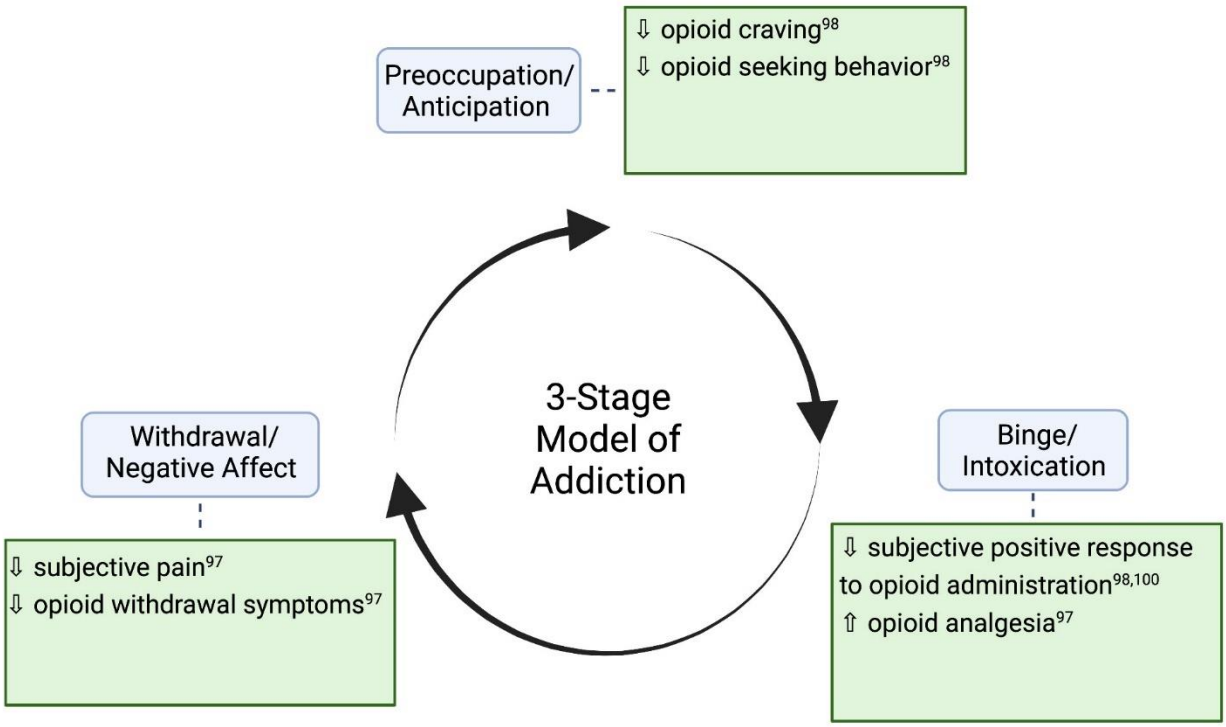

Figure 3 – Clinical Evidence of Glial Modulator Modulation of Opioid Addiction Processes
In each stage in the 3-stage model of addiction, glial modulator administration has been shown to modulate opioid addiction processes in clinical studies among opioid users. These findings suggest that glial modulator administration may augment treatment outcomes among OUD patients undergoing gold-standard medication assisted therapy.

Conversely, minocycline, which acts via multiple mechanisms (that differ from ibudilast), was not effective in OUD patients undergoing opioid-agonist treatment. Arout et al., found that minocycline (200mg/day PO) did not significantly alter subjective pain, experimental pain, opioid withdrawal or craving, or serum cytokine levels, compared to placebo levels[99]. In a separate study, Mogali et al., found that minocycline (200mg PO) pretreatment attenuated subjective positive response ("liking" and "good effect") to oxycodone self-administration (40mg) compared to placebo levels, among non-dependent opioid users[100]. However, in that study, minocycline did not improve mood, analgesia, or reduce opioid craving[100]. Minocycline at the 200mg dose (PO) may offer some clinical benefit, but findings thus far are less encouraging than ibudilast. Finally, there is some evidence that minocycline may exhibit some cognitive- and mood-enhancing properties, especially with repeated/daily dosing[99,101,102], and thus, therapeutic benefits may be indirect and may not manifest immediately.

Limitations

The investigation of the neuroimmune system in OUD patients is in its proverbial infancy. The extant clinical literature is limited to 5 studies and numerous limitations must be highlighted. First, the 3 studies that investigated the effects of ibudilast were conducted among non-treatment-seeking, opioid users[96-98]. Thus, it remains unknown whether these positive effects will translate to treatment-motivated individuals. Second, those studies were effectively limited to male subjects. A total of 4 females were included across the 3 ibudilast studies[96-98]. Thus, it remains unknown whether females will exhibit similar responses to ibudilast as male subjects. Third, all 3 ibudilast studies were conducted inpatient and thus, findings may not generalize to outpatient settings[96-98]. Fourth, none of the ibudilast studies were conducted in combination with a gold standard opioid maintenance medication, i.e., buprenorphine or methadone, and thus, it remains unknown whether similar positive effects will be observed among OUD patients enrolled in medication assisted therapy[96-98]. Fifth, as noted above, in each of the 5 studies published to date, positive findings were sporadic and many planned comparisons yielded non-significant findings, especially for the 2 minocycline studies[96-100]. This could be due to limited statistical power in these relatively small studies or it may reflect the modest and/or isolated

therapeutic effects of glial modulators. Sixth, 4 of the 5 studies published to date were conducted by the Columbia University group[96-98,100]. While the Columbia University group conducts exemplary human behavioral pharmacology research, studies conducted by other groups are needed.

Future directions

As this is a nascent field, numerous relationships remain to be evaluated and many future studies are needed (more than will be suggested here) to evaluate the viability of the neuroimmune system as a therapeutic target in OUD. However, a few studies we hope to see conducted are as follows. Future neuroimaging studies are needed to determine whether OUD patients exhibit a neuroinflammatory phenotype *in vivo*. Longitudinal neuroimaging studies are needed to determine whether opioid maintenance therapy (and abstinence from recreational opioid use) or glial modulator administration can reduce/suppress neuroimmune signaling in OUD patients and whether those brain changes correspond with positive clinical effects. Dose-finding studies are needed to optimize the therapeutic dose range of glial modulators which may vary by severity of OUD, primary opioid abused, preferred route of administration, presence of co-occurring disorders, patient age, and/or biological sex. Large and diverse clinical samples of OUD patients are needed to investigate demographic and patient-level factors that may influence or predict therapeutic benefit from glial modulator treatment. And, finally, large-scale multi-site clinical trials that combine gold-standard medication assisted therapy plus adjunctive glial modulator medications are needed to determine whether glial modulators augment treatment outcomes in OUD patients.

Conclusion

In this review, we summarized the literature linking opioid administration and neuroinflammation, and the potential viability of the neuroimmune system as a therapeutic target in OUD patients. There is evidence that opioid administration can activate glia, evoking stereotyped morphological transformations in microglia, expression of cell surface markers, and secretion of pro-inflammatory cytokines and chemokines. Further, there is a growing literature that neuroimmune signals alter mood, impair cognition, and amplify addiction processes, including opioid craving and opioid-seeking behavior. Whereas preclinical and postmortem findings suggest that OUD patients may exhibit a neuroinflammatory phenotype, direct *in vivo* evidence is lacking: at present, no PET TSPO studies of OUD patients have been published. However, review of the clinical literature showed that attenuation of neuroinflammatory signals via ibudilast administration reduced opioid craving, opioid withdrawal symptoms, opioid-seeking behavior, and subjective positive response to opioid administration among opioid users. These positive clinical findings are indirect evidence suggestive that OUD patients may exhibit a neuroinflammatory phenotype and that targeting neuroinflammation with ibudilast may have therapeutic benefits. Future studies are needed to investigate the *in vivo* neuroimmune state of OUD patients, to confirm that glial modulator medications can modulate *in vivo* neuroimmune state in OUD patients, and to evaluate the effectiveness of ibudilast as an adjunctive medication to supplement opioid agonist therapies, e.g., buprenorphine or methadone, among OUD patients. These studies are urgently needed to advance our understanding of the neuroimmune system in OUD and its potential as a therapeutic target for enhancing treatment adherence and preventing relapse.

Funding: This study was supported by a grant from the National Institute on Drug Abuse (R00 DA048125) (awarded to EAW) and by institutional support from Wayne State University School of Medicine.

Acknowledgements: The authors thank Drs. Mark Greenwald and Kelly Bosse for their feedback, insight, and suggestions.

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