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## Glial modulators: a novel pharmacological approach to altering the behavioral effects of abused substances

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### Abstract

**Introduction**—Commonly abused drugs including opioids, stimulants and alcohol activate glia cells, an effect that has been identified across species. Glia, specifically astrocytes and microglia, have been shown to contribute directly to behaviors predictive of the abuse liability of these drugs. Although still in its infancy, research investigating the effects of pharmacological modulation of glial activity on these behaviors has provided encouraging findings suggesting glial cell modulators as potential pharmacotherapies for substance-use disorders.

**Areas covered**—This review first explores the evidence establishing glial-mediated modulations of behaviors associated with opioid, stimulant and alcohol exposure, with emphasis placed on the neuroanatomical substrates for these effects. Next, neurobiological and behavioral studies evaluating the ability of glial cell modulators to prevent and reverse the effects of these abused substances will be considered. Finally, the potential clinical efficacy of glial cell modulators as a novel pharmacological approach to treat substance-use disorders in relation to currently available, conventional pharmacotherapies will be discussed.

**Expert opinion**—Though the relationship between drug-induced glial activity and behaviors indicative of drug abuse and dependence is not yet fully elucidated, the evidence for the association continues to grow. The use of glial modulators as pharmacological tools to investigate this relationship has also yielded findings supporting their potential clinical efficacy for treating substance-use disorders.

### Keywords

abuse; alcohol; dependence; glia; opioids; stimulants

## 1. Introduction

Glial cells are thought to constitute over 50% of the cells in the central nervous system (CNS) [1]. Though once thought to be passive bystanders in most neuronal processes, it is now widely recognized that glia play a major role in nervous system development, neuronal transmission, disease etiology and neuronal homeostasis [2–5]. There are two types of glial cells in the CNS relevant to the current discussion: astrocytes and microglia. Astrocytes perform numerous functions: they provide structural support for nerve cells, modulate the environment around neurons, regulate the production of synapses, maintain the blood–brain barrier and release a range of neuronal growth factors [6–8]. Microglia are rapidly activated

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in response to pathological events such as trauma, ischemia, inflammation, hypoxia, neurodegeneration and viral or bacterial infection. Active microglia restore tissue homeostasis by destroying dead cells, debris or infectious agents via phagocytosis and by releasing cytotoxic factors. Microglia also aid in the repair of damaged cells by releasing neurotrophic factors [3,9]. Given the variety of roles that glia play in the nervous system, a stimulus that affects their morphology and function has widespread consequences including changes in neurotransmission, metabolism of neurotransmitters, synaptic plasticity and propagation of action potentials. This review will focus on three types of psychoactive drugs that are established as stimuli that affect glial activity. Across species, opioids, stimulants and alcohol are well known for their abuse liabilities and deleterious effects on behavior, cognition and physiology that occur as a consequence of long-term exposure. Evidence for the association between increased glial activity and these deleterious effects will be highlighted. Finally, the potential role of glial cell modulators for drug-use disorders will be explored.

## 2. Opioids

Despite public health efforts to decrease heroin use, the 2009 National Survey on Drug Use and Health (NSDUH) reported that the number of people seeking treatment for heroin dependence has increased from 2002, as did the number of people trying the drug for the first time. Strikingly, the non-medical use and abuse of prescription opioids has recently soared, with 2.2 million new initiates reported in the past year and 4.8% of young adults reporting current non-medical use of prescription pain-relievers [10], and an increase in emergency department visits involving opioid analgesics (111% increase from 2004 to 2008) [11]. This rise in prescription opioid use has resulted in 1.9 million people reporting dependence or abuse of prescription pain relievers, and 739,000 receiving treatment for their use in 2009 [10]. The epidemiological findings that opioid agonists have high addictive potential have been modeled across species in an effort to understand the environmental, pharmacological and physiological variables that contribute to their robust reinforcing effects. Two factors hypothesized to contribute to increases in opioid self-administration over time are opioid tolerance and dependence [12], the two hallmark features of opioid addiction in humans. Recent evidence has reliably demonstrated that tolerance and dependence are associated with opioid-induced increases in glial cell activity, resulting in neuroadaptations that may directly contribute to the reinforcing effects of these agonists [13,14].

### 2.1 Opioid exposure alters glial cell activity and behavior

Morphine administration induces region-specific upregulation of glial fibrillary acidic protein (GFAP), a protein constituent found specifically in astrocytes [15], suggestive of increases in proliferation of astrocytes (astrogliosis), the area of individual astrocytes and/or astrocytic migration in rodents [16–18]. Chronic morphine exposure, achieved by surgically implanted morphine pellets and previously verified to produce opioid tolerance and dependence [19], increased GFAP immunoreactivity in the ventral tegmental area (VTA) [16]. This effect was not observed when morphine tolerance and dependence was blocked by concomitant administration of the mu-opioid antagonist, naltrexone [16]. A later study reported that daily morphine administration, using a dosing regimen that produced tolerance and dependence (10 mg/kg, i.p., twice daily for 13 days), increased GFAP immunostaining in the VTA, nucleus accumbens (NAcc) and the frontal cortex, thus replicating and extending the earlier findings [17]. Repeated morphine exposure has also been reported to increase the GFAP mRNA and protein levels in the striatum, with corresponding increases in protein immunoreactivity verifying the functional impact of morphine-induced changes of mRNA levels for this gene [20]. The relationship between opioid-induced tolerance and GFAP expression was also demonstrated by increases in GFAP immunostaining in the

spinal cord, hippocampus and posterior cingulate cortex of rats rendered tolerant to morphine-induced antinociception by daily systemic morphine administration (50 mg/kg, once per day for 9 days) [18]. Daily intrathecal morphine administration also produced tolerance to the drug's antinociceptive effects and was associated with increased GFAP immunostaining in the spinal cord [18]. Both of these effects were attenuated when the glial metabolic inhibitor, fluorocitrate, was administered in conjunction with morphine [18], strengthening the relationship between these behavioral and neurobiological changes in response to repeated morphine administration.

As discussed above, chronic morphine exposure increases GFAP levels in the VTA, NAcc, striatum and frontal cortex, areas hypothesized to contribute to the positive reinforcing effects of stimuli [21]. Therefore, later studies sought to investigate whether changes in glial regulation were also associated with the conditioning effects of opioid-associated cues. In mice, astrocyte-conditioned medium (ACM) was injected specifically in the NAcc and the intracingle cortex, areas associated with learning and reward, thereby selectively increasing glial activity in these two regions [22]. Mice treated with ACM showed significantly greater preference for morphine-associated cues relative to those treated with vehicle, supporting the hypothesis that region-specific increases in astrocyte-released factors seem to be capable of modifying neuronal and glial activity in a manner that may alter the rewarding effects of opioids [22]. These findings demonstrate that glial activation, in part, may contribute to some of the behavioral effects associated with opioid dependence and abuse liability.

While opioid agonists are some of the most effective tools for chronic pain management [23], opioid-induced hyperalgesia is a unique phenomenon whereby increases in pain perception result from long-term use of opioid analgesics [24]. In order to maintain adequate analgesia, escalating the dose and frequency of opioid administration is sometimes necessary, resulting in increased opioid dependence. Thus, hyperalgesia can decrease the therapeutic potential and increase the abuse liability of opioids [13]. The neurobiological mechanism of this effect is thought to be a result of glial-induced increases in the production of immune factors known as cytokines, which carry signals locally between cells and act to mediate/regulate immunity, inflammation and hematopoiesis [25,26]. Numerous studies have found evidence suggestive of enhanced glial activation following chronic morphine administration. Their findings include morphine-induced glial production of nitric oxide, fractalkine and proinflammatory cytokines. More specifically, morphine has been found to induce chemotaxis and activation of extracellular signal-regulated kinase in microglia (ERK) 1/2 [27–30]. Additionally, compared with saline, intrathecal morphine and methadone increased spinal glial activation and the consequent production of immune factors (chemokines and cytokines) as measured by multiplex analysis of cerebrospinal fluid and dorsal spinal cord tissue [31]. The hypothesis that opioid administration causes proinflammatory cytokine-mediated actions that oppose their analgesic effects is supported by earlier findings demonstrating that the development of hyperalgesia and spinal analgesic tolerance to morphine were temporally correlated with glial activation and cytokine release as determined using real-time reverse transcription polymerase chain reaction (RT-PCR), RNase protection assay (RPA) and ELISA of cytokine RNA [32]. Furthermore, these inflammatory and behavioral responses were attenuated by the co-administration of propentofylline, a drug that depresses the activation of microglia and astrocytes [33].

## **2.2 Glial cell modulators attenuate opioid tolerance, dependence and conditioned place preference**

Recently, studies have investigated whether pharmacological modulation of opioid-agonist induced effects on astrocytes and microglia alter opioid dependence, tolerance and behaviors associated with abuse liability. Ibudilast, minocycline and propentofylline are three glial cell

modulators that have been demonstrated to effectively decrease the behavioral expression of the hypothesized facilitatory effects of opioids on cytokine activity. Ibudilast has been shown to shift the dose--response curve for morphine-induced analgesia leftward, thereby decreasing the minimum effective analgesic dose [34]; it has also been shown to delay the development of morphine tolerance and hyperalgesia [35]. The mechanism by which ibudilast exerts these effects is suggested to be through direct inhibition of toll-like receptor 4 (TLR4) immune signaling [34,42]. Administration of minocycline attenuated the development of hyperalgesia in rodent models of neuropathy [36–38] and local injection of propentofylline decreased pain behavior and mechanical allodynia [39,40]. Propentofylline and ibudilast have many neuronal and non-neuronal effects acting both as phosphodiesterase inhibitors and glial modulators [35,41]. Minocycline is a microglial preferring activation inhibitor with no known phosphodiesterase inhibitory activity [34]. Studies with the glial metabolic inhibitors flurocitrate and pentoxifylline have similarly yielded promising findings for the potential therapeutic effects of glial modulators [43]. When delivered in combination with chronic morphine, fluorocitrate partially attenuated the development of tolerance to morphine analgesia in rodent behavioral pain models (paw withdrawal). Fluorocitrate also blocked the morphine-associated production of the astroglial activity marker, GFAP, as measured by immunostaining [18]. Additionally, local injections of the cytokine inhibitor pentoxifylline reduced inflammatory pain behavior in rodents by decreasing proinflammatory cytokine mRNA [39].

Inhibiting downstream effects of glial activation using proinflammatory cytokine receptor antagonists or by genetically impairing cytokine signaling restores analgesia and reduces opioid-induced hyperalgesia, allodynia and behavioral signs of withdrawal [31,43,44]. In some studies, attenuation of these behavioral effects related to opioid dependence was also associated with decreases in markers of astrocyte and microglial activation [31,45].

The above series of studies demonstrate that inhibiting opioid-induced glial activation increases the analgesic effects of these agonists, making them a more viable option for long-term treatment of pain. Many of these same inhibitors are hypothesized to also decrease the rewarding effects of opioids. Ibudilast co-administered with morphine significantly reduced the magnitude of opioid-induced dopamine release in the NAcc [46], a neurobiological substrate thought to mediate the rewarding effects of drugs [21]. Also, both ibudilast and minocycline reduced opioid-induced conditioned place preference, without altering conditioned place preference when given alone [34,47]. Propentofylline also blocked the development of the effects of morphine in the conditioned place preference procedure [22]. These findings demonstrate the therapeutic potential of glial cell modulators not only for maximizing the analgesic effectiveness of opioids, but also for suppressing effects that are thought to be correlated with the abuse liability of these drugs.

### 3. Stimulants

Stimulant use and abuse continues to be a public health concern. With the rise in non-medical use of prescription stimulants and methamphetamine, 17% of people seeking treatment for substance-use disorders in 2009 reported stimulants, including amphetamines and cocaine, as their primary substance of abuse [10]. A distinctive feature of animal models of stimulant ‘addiction’ is an effect called sensitization, whereby the behavioral response to a specified dose of drug increases with repeated administration. Reports have demonstrated that administration of amphetamine and methamphetamine induce region-specific astrogliosis and activation of microglia, and that these neuroadaptations are associated with behavioral sensitization [48].

### 3.1 Exposure to stimulants alters glial cell activity and behavior

In rats, repeated exposure to amphetamine produced neuroadaptations marked by increased GFAP immunoreactivity in the dorsal and ventral caudate putamen [49]. This increase in GFAP expression was observed up to 10 days after the final amphetamine injection when sensitization to the locomotor-stimulating effects of the drug were also observed, thus demonstrating the relationship between the long-lasting neuroadaptive and behavioral consequences of amphetamine exposure [49]. Another study demonstrated that astrocyte proliferation and/or migration, as indicated by increased expression of endogenous basic fibroblast growth factor (bFGF) [50] expressed in the VTA and substantia nigra compacta (SNc), was related to the magnitude of amphetamine-induced sensitization in rats [51,52]. Administration of a neutralizing antibody to bFGF infused directly into the VTA prior to amphetamine administration blocked sensitization to its locomotor-stimulating effects, providing evidence for the role of bFGF in the development of amphetamine-induced sensitization [52]. These studies supported the hypothesis that increased astrocytic expression of bFGF acts as a direct contributing factor to amphetamine's long-lasting behavioral changes.

Studies with methamphetamine have demonstrated that the neuroanatomical substrates for drug-induced glial activation are also localized to regions that have been shown to play a role in the behavioral effects of stimulants indicative of their abuse liability, such as the striatum, frontal cortex and hippocampus [53–55]. In mice, repeated methamphetamine administration decreased levels of dopamine, DOPAC (3,4-dihydroxyphenyl-acetic acid) and dopamine transporter (DAT) immunoreactivity in the striatum while also producing behavioral sensitization [56]. These changes were accompanied by microglial activation in the striatum, identified by increased immunoreactivity of the activated microglia marker, Mac1-CD11b [56]. Much like the findings with amphetamine, markers indicative of glial activation were accompanied by drug-induced behavioral sensitization. For instance, methamphetamine increased microglia expression in the striatum of mice, as measured by histochemical analysis [55]. This effect was dose and time dependent; greater expression was observed at higher doses relative to lower doses, peaked 48 h after the final methamphetamine injection and returned to baseline 7 days after treatment [55]. The association between methamphetamine-induced increases in microglial activation and its neurotoxic effects was determined using HPLC (high-performance liquid chromatography) with electrochemical detection to measure changes in striatal dopamine content as a function of methamphetamine exposure. Interestingly, dopamine levels were found to decrease in a dose-dependent manner, with high methamphetamine doses inducing greater dopamine depletion than lower doses [55]. Because methamphetamine-induced microglia activation and dopamine depletion were inversely correlated, a direct association between the two effects was postulated [55]. The radioligand [<sup>3</sup>H]PK11195, which binds to the peripheral benzodiazepine receptor expressed primarily by astrocytes and microglia in the CNS, is used to measure changes in glial activity under potentially neurotoxic conditions [57]. In rats, repeated methamphetamine administration using a procedure that induces sensitization, increased [<sup>3</sup>H]PK11195 binding in the striatum and cortex, indicative of increased density of the peripheral benzodiazepine receptor in these areas [54]. Additionally, the same dosing regimen produced increases in expression of the 27-kD heat shock protein (HSP27) in the cortex, striatum and hippocampus [54], a protein that is expressed in reactive microglia and astroglia after insult [58].

Recently, positron emission tomography (PET) studies in human methamphetamine users have confirmed that methamphetamine's effects on glia are preserved across species [59]. Using [<sup>11</sup>C](R)-PK11195, a radiotracer identified to be selective for activated microglia [60], differences in expression of activated microglia between human methamphetamine users relative to non-drug using, age- and gender-matched controls were characterized [59].

Relative to control participants, the binding potential of the radioligand was higher among the participants with a history of methamphetamine abuse reflecting increases in activated microglia in this population [59]. The binding potential of [<sup>11</sup>C](R)-PK11195 was also found to be inversely correlated with duration of methamphetamine abstinence suggesting that these neuroadaptations were not permanent [59]. These findings are similar to those discussed earlier in rodent models of methamphetamine neurotoxicity and demonstrate, to some degree, the preservation of these neurotoxic effects across species.

### 3.2 Glial cell modulators attenuate the behavioral effects of stimulants

Studies with laboratory animals have suggested that pharmacological modulation of glial activity can attenuate methamphetamine-induced behavioral and neuroadaptive effects [22,56,61,62]. In mice, pre- and post-treatments of minocycline attenuated both methamphetamine-induced behavioral sensitization and the neurotoxic effects of methamphetamine discussed above (decreased striatal dopamine, DOPAC and DAT, and increased activated microglia) [56]. *In vivo* microdialysis demonstrated that a pretreatment of minocycline also dampened methamphetamine-induced increases in striatal extracellular dopamine, suggesting a mechanism for minocycline's blunting of methamphetamine-induced behavioral sensitization [56]. When minocycline was administered only after methamphetamine exposure, methamphetamine's effects on striatal DAT density, and dopamine and DOPAC levels were only partially reversed [56] corresponding to only a partial blockade of methamphetamine-induced sensitization [62]. These findings demonstrated that the protective effects of minocycline seem to be contingent on timing of administration relative to methamphetamine exposure. However, though the post-treatment alone failed to reverse methamphetamine-induced sensitization and neurotoxic effects, it attenuated deficits in long-term recognition memory associated with repeated methamphetamine treatment [62].

Minocycline's protective effect against methamphetamine neurotoxicity were further established in non-human primates using PET imaging, showing that minocycline blunted methamphetamine-induced decreases of the DAT in the striatum [61]. Behavioral evidence supporting the potential role of glial modulators for methamphetamine-use disorders comes from a recent study demonstrating that ibudilast suppresses stress- and methamphetamine-induced reinstatement of extinguished responding that was previously paired with methamphetamine reinforcement in rats, a model for relapse [63]. Also, propentofylline decreases preference for methamphetamine-associated cues in mice [22]. Given that this behavioral end point has strong face-validity for the positive subjective effects of the drug in humans, it is behaviorally relevant when addressing whether inhibition of methamphetamine-induced glial activity predicts decreased abuse liability. In line with these findings, minocycline was found to attenuate the positive subjective effect ratings of oral D-amphetamine in healthy volunteers [64].

The preclinical and clinical findings supporting the relationship between the glial activating and behavioral effects of amphetamine and methamphetamine provided the foundation for recent studies investigating whether this association exists with cocaine. In rodents, both acute and repeated cocaine administration increase GFAP expression in various regions including the dentate gyrus [65], noradrenaline (NA) and prefrontal cortex [66]. However, few reports have established an association between such neuroadaptations and cocaine-induced behavioral effects. One study designed to assess the effects of minocycline on cocaine-induced sensitization found that it prevented the development of cocaine-induced sensitization in mice [67]. However, similar to the inhibitor's effects on methamphetamine sensitization [62], minocycline failed to reverse cocaine-induced sensitization when it was administered as a post-treatment [67].

A single study conducted in humans probing the use of glial cell modulators for substance-use disorders investigated pentoxifylline, a drug that is structurally similar to propentofylline, as a potential pharmacotherapy for cocaine dependence [68]. For this pilot study, cocaine-dependent participants seeking treatment for their substance-use disorder were recruited to participate in a modified-blinded, placebo-controlled study investigating the effects of various pharmacotherapies on cocaine use and addiction severity, measured by urine toxicology tests and the addiction severity index (ASI), respectively. A decreasing trend in cocaine use and addiction severity was observed over the study period in participants randomized to receive pentoxifylline (n = 16) relative to those receiving placebo (n = 16), however, medication compliance was not verified [68]. Although the effect did not reach statistical significance, these preliminary findings provide evidence that the role of glial inhibitors for cocaine dependence warrants further exploration in controlled laboratory settings.

## 4. Alcohol

In 2009, about 50% of Americans aged 12 years and older reported drinking alcohol. Of this population, 45% reported binge drinking during the previous month, defined as five or more standard alcoholic drinks during one occasion. Rates of heavy drinking, defined as consuming five or more drinks on at least five days of the previous 30-day period, were also high with 13% of the drinking population reporting heavy alcohol use [10]. The deleterious behavioral and physiological effects of alcohol are widespread, as reflected in a 3.81% prevalence rate of alcohol dependence among a representative sample of US civilians 18 years of age and older as assessed by the 2001 – 2002 National Epidemiologic Survey on Alcohol and Related Conditions conducted by the National Institute of Alcohol Abuse and Alcoholism [69].

### 4.1 Effects of alcohol on glial cell activity and behavior

Research has demonstrated that the deleterious neurobiological and behavioral effects of alcohol exposure are dose and time dependent, and also contingent on duration of abstinence [70]. In order to investigate the neurobiological and behavioral effects of alcohol in laboratory animals, an ethanol-dosing schedule was developed to model human alcohol dependence. Intra-gastric ethanol administration several times a day for multiple days induces profound tolerance to the behavioral effects of the drug despite high blood ethanol levels, thus mimicking the behavioral tolerance to alcohol observed in alcoholics. Rats exposed to ethanol administered according to this procedure demonstrated specific cognitive deficits related to performance on the Morris Water Maze [71]. Although ethanol exposure did not affect the acquisition of spatial reference and working memory tasks, deficits in reversal learning were observed in the ethanol-treated group relative to the ethanol-naïve group [71]. Neurodegeneration identified with amino cupric silver staining was apparent immediately after ethanol exposure in the corticolimbic areas, a system that was also marked by increased microglial proliferation measured by [<sup>3</sup>H]PK11195 binding 3 weeks after ethanol exposure [71]. In a second study investigating the neurobiological effects of ‘binge drinking’, rats were exposed to high alcohol concentrations (2 – 3 g/kg, three times/day for 2 days) followed by a 5-day period of abstinence [72]. Ethanol’s effects were determined using immunohistological staining with an antibody against major histocompatibility complex class II antigens, molecules known to be expressed on activated microglia under certain neuropathological states. After three binge cycles, a robust increase in immunopositive cells was observed in the dentate gyrus of the hippocampus, supporting the area as a neuroanatomical substrate for memory deficits observed after repeated alcohol exposure [72].

## 4.2 Glial cell modulators attenuate the behavioral effects of alcohol

The effects of alcohol exposure on microglia have also been explored in humans using postmortem brain tissue from alcoholics and moderate drinkers. Region specific expression of two microglia-specific proteins, glucose transporter type 5 (GluT<sub>5</sub>) and ionized calcium binding adaptor protein-1 (Iba-1), revealed increased markers of microglia in the cingulate cortex, VTA and midbrain in tissue from alcoholics relative to the moderate drinkers [73]. The extent to which these alcohol-associated changes in glial activity correspond to abuse and dependence has not yet been verified in humans. However, evidence for glial modulation on alcohol's effects has recently been reported in laboratory animals. Minocycline, discussed previously for its ability to prevent opioid-induced conditioned place preference and methamphetamine- and cocaine-induced sensitization, was found to decrease ethanol drinking in mice, an effect hypothesized to be mediated by the drug's actions on microglia [74]. Also, administration of the thiazolidinediones (TZDs) pioglitazone and rosiglitazone to rats decreased ethanol dependence and ethanol self-administration without affecting food- and saccharin-maintained responding [75]. These drugs activate peroxisome proliferator-activated receptor-gamma (PPAR $\gamma$ ) receptors, which are expressed in both neurons and glia in brain regions associated with drug reward and reinforcement. Though the direct mechanism mediating the effects of PPAR $\gamma$  receptor activators on ethanol self-administration is unknown, one hypothesis is that PPAR $\gamma$  receptor activation decreases ethanol reinforcement and dependence by attenuating glia-mediated inflammatory response [75]. Although this area is relatively uncharted, these recent findings in conjunction with data discussed earlier derived from opioid and stimulant studies provide a foundation and rationale for further investigations into establishing glial modulation of ethanol's behavioral effects and the therapeutic potential of glial inhibitors for alcoholism.

## 5. Potential clinical use of glial modulators

Preventing and treating drug-use disorders continues to be sociologically and clinically relevant. Current pharmacotherapy modalities that have shown promise in treating substance dependence include agonist treatment, antagonist treatment and other therapeutics that directly or indirectly modify the actions of the abused drug. Regardless of modality, treatment for drug dependence and abuse is directly aimed at achieving and maintaining abstinence. These medications have favorable attributes including decreasing withdrawal symptoms and drug craving, and blocking the reinforcing effects of the abused substance. However, present treatment challenges include inconvenience and social stigma (methadone), poor patient compliance (naltrexone, disulfiram), the need for a detoxification or induction phase (buprenorphine, naltrexone) and the potential risk of abuse (agonist and replacement therapies).

By affecting the neuroadaptive and behavioral changes that are associated with repeated exposure to commonly abused drugs, glial cell modulators potentially provide a novel pharmacotherapeutic approach to not only treat existing drug dependence, but also to curb the abuse liability of opioids and stimulants while maximizing their therapeutic potential.

## 6. Conclusion

This review highlights the effects of three commonly abused psychoactive drugs on glial cell activity and summarizes findings that suggest the potential therapeutic benefits of glial cell modulators for substance-use disorders. In addition to their well-known opioid agonist effects, opioid drugs enhance glial activity. The subsequent increase in the release of cytokines and chemokines is thought to contribute to the development of opioid tolerance and hyperalgesia. Antagonism of these effects increases the analgesic effectiveness of opioids and decreases their rewarding effects. As a result, compounds that inhibit glia show



clinical potential to improve the therapeutic use of opioids, while decreasing their abuse liability.

Similar findings have been reported with stimulant drugs (methamphetamine, amphetamine and less extensively, cocaine). Stimulants have been shown to increase activation of microglia and astrocytes in various brain regions. Consequently, this enhancement has been associated with stimulant neurotoxicity and behavioral sensitization. Research has shown that under specific parametric conditions, glial modulators can attenuate these effects and antagonize the increase in dopamine release thought to underlie the addictive nature of these drugs. These findings are suggestive of a potential role for glial modulators in the clinical use of prescription stimulants and also for their use as pharmacotherapies for stimulant abuse.

Finally, markers of glial expression and activity have been shown to be related to alcohol exposure and abstinence. Although research has yet to further investigate the potential benefits of glial modulators, research findings with opioids and stimulants suggest that there are potential benefits for this class of drugs as well.

## 7. Expert opinion

Research into the benefits of glial inhibitors has shown a clear progression from basic science investigations performed *in vitro*, to preclinical studies with rodent models, and clinical investigations in human volunteers. Though we are only on the cusp of characterizing the precise role that glial activity plays in behaviors predictive of a drug's abuse liability, the evidence for their association is increasing. Recent reports indicate that opioid agonist effects extend beyond classic agonist action at neuronal opioid receptors to include direct non-neuronal activity [76]; for instance, opioid agonists activate TLR4 proinflammatory signaling, affecting the agonist's pharmacodynamic actions [76]. These investigations have also helped to elucidate the precise mechanism by which glial modulators, many of which have a variety of neuronal and non-neuronal effects, disrupt behaviors associated with drug-induced alteration in glial activity [76]. For example, ibudilast, a non-selective anti-inflammatory agent, potentiates the antinociceptive effects of opioid agonists by directly inhibiting opioid-induced TLR4 signaling. When the opioid agonist is given repeatedly, it is postulated that tolerance is directly mediated by opioidergic action at TLR4 receptors, and ibudilast's effectiveness in blocking tolerance is due to its direct effects at that site [76]. Ethanol-induced neuroinflammation is also hypothesized to be associated with the drug's direct effects on TLR4 signaling, but little is known regarding cocaine and methamphetamine's direct interaction with the receptor [77]. Further exploration into the precise mechanism by which drugs alter glial activity to induce behavioral changes associated with abuse liability will help in the development of more selective, site-specific modulators targeting specific behavioral end points related to addiction.

The pivotal findings related to the potential effectiveness of glial modulators for substance-use disorders will come from additional studies investigating their effectiveness in decreasing drug self-administration in preclinical and clinical models. To date, there have been few controlled studies systematically assessing this behavioral end point. This critical gap in understanding and defining the role of glia in the reinforcing effects of abused drugs is a fundamental area for future exploration in order to determine the potential utility of glial modulators in treating drug dependence in the clinic. As discussed earlier, withdrawal symptoms are thought to contribute to relapse to drug use. Preclinical findings strongly support the potential of glial modulators, specifically ibudilast and minocycline, to decrease opioid withdrawal symptoms [34]. These reports have contributed to the development of a

Phase IIb randomized, placebo-controlled trial investigating the effectiveness of ibudilast in decreasing opioid withdrawal symptoms in morphine-dependent volunteers; findings that will verify the effectiveness of glial modulators to alter opioid dependence in a clinically meaningful fashion. Thus, as research continues to provide encouraging findings regarding the relationship between drug-induced glial activity and behaviors indicative of drug abuse and dependence, glial modulators are expected to move closer to becoming viable pharmacotherapies for substance-use disorders.

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