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Glial Cells as Therapeutic Targets for Smoking Cessation

Mohit Kumar^{1,#}, Adewale Adeluyi^{1,2,#}, Erin L. Anderson^{3,#}, Jill R. Turner^{1,*}

¹Department of Pharmaceutical Sciences, University of Kentucky College of Pharmacy, Lexington, KY, USA

²Ann Romney Center for Neurologic Disease, Department of Neurology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

³Department of Drug Discovery and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, Columbia, USA

Abstract

Smoking remains the leading cause of morbidity and mortality in the United States, with less than 5% of smokers attempting to quit succeeding. This low smoking cessation success rate is thought to be due to the long-term adaptations and alterations in synaptic plasticity that occur following chronic nicotine exposure and withdrawal. Glial cells have recently emerged as active players in the development of dependence phenotypes due to their roles in modulating neuronal functions and synaptic plasticity. Fundamental studies have demonstrated that microglia and astrocytes are crucial for synapse formation and elimination in the developing brain, likely contributing to why glial dysfunction is implicated in numerous neurological and psychiatric disorders. Recently, there is increasing evidence for the involvement of glial cells in drug dependence and its associated behavioral manifestations. This review summarizes the newly evaluated role of microglia and astrocytes as molecular drivers of nicotine dependence and withdrawal-associated phenotypes.

Keywords

Astrocytes; Dependence; Microglia; Nicotine; Withdrawal; Smoking

1. INTRODUCTION

Tobacco smoking is the principal cause of preventable disease and premature death in the world. Globally, over 5 million premature deaths are attributable to tobacco smoking every

*Corresponding author: Dr. Jill R Turner, PhD, Assistant Professor, University of Kentucky, College of Pharmacy, 789 S. Limestone Street, 473 Lee T. Todd Jr. Building, Lexington, KY 40536-0596, P: 859-218-1025, Jill.Turner@uky.edu.

#indicates equal contribution

Authorship contribution statement

Writing-original draft (MK, AA, JRT)

Conceptualization, Investigation, Resources, Writing-review & editing, Visualization (MK, AA, ELA, JRT)

Supervision, Funding acquisition (JRT)

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single year, and nearly one-tenth – about 480,000 of these deaths occur in the United States (Changeux, 2010; Reitsma et al., 2017). Despite decreased smoking prevalence in the United States over the last decade, the disease burden attributable to tobacco smoking continues to rise. This is due in part to population growth, but also the high failure rate in quit attempts made by current smokers (Ng et al., 2014). Currently, over 42 million Americans still smoke, and nearly 80% of these smokers have made serious attempts to quit without success (Benowitz, 2010). While quitting smoking significantly reduces the risk of smoking-related disease and death, successful smoking cessation remains a major challenge in the United States with only a 3% success rate in quit attempts each year (Benowitz, 2010; Changeux, 2010). Today, it has become clearer that nicotine and its withdrawal (during smoking cessation) have complex effects in the brain, complicating successful smoking cessation. This review summarizes the molecular effects of nicotine in reward pathway circuitry and explores the potential role of glial cells in nicotine dependence.

2. NEUROBIOLOGY OF NICOTINE DEPENDENCE

2.1. Nicotine and Neuronal Nicotinic Acetylcholine Receptors (nAChRs)

Nicotine is the major psychoactive component in tobacco that drives the neurobiological effects underlying the sustainment and reinforcement of tobacco smoking (Balfour, 2008; Benowitz, 2010; Grunberg, 2007). Smoke particles containing nicotine are inhaled into the lungs, where about 90% of nicotine present in the inhaled smoke is immediately absorbed into the pulmonary venous circulation (Armitage et al., 1975; Matta et al., 2007). Following absorption, it takes seven seconds for nicotine to reach the brain (Rose et al., 2010), where it binds and activates nicotinic acetylcholine receptors (nAChRs). These receptors are ionotropic and are comprised of 5 subunits. There are 12 nAChR subunits expressed in the mammalian brain, including $\alpha 2$ - $\alpha 10$ and $\beta 2$ - $\beta 4$. Neuronal nAChRs can exist as either homomeric nAChR subtypes, in which all subunits are the same (e.g. $\alpha 7$ nAChRs), or as heteromeric nAChR subtypes, which are a mix of different subunits (e.g. $\alpha 4\beta 2$ nAChRs). This varying subunit composition influences the pharmacological features of nAChRs and ultimately their responses to nicotine stimulation (D'Souza and Markou, 2011; Govind et al., 2009). Activation of nAChRs opens the cationic channel, allowing passage of potassium, sodium, and calcium ions. Because nAChRs are preferentially located at the presynaptic terminal, their effects are primarily modulatory and result in the release of nearly every major neurotransmitter (Benowitz, 2010; Markou, 2008). For example, dopamine release is increased during nicotine administration, where it is proportional to the hedonic response to cigarette smoking in humans (Barrett et al., 2004; D'Souza and Markou, 2011), and dopamine concentrations are decreased during nicotine withdrawal (Watkins et al., 2000). Other neurotransmitters modulated by nAChRs include γ -aminobutyric acid (GABA), β -endorphin, glutamate, serotonin, acetylcholine, and norepinephrine (Singer et al., 2004), which all have behavioral outcomes. The release of GABA or β -endorphins results in attenuation of anxiety and depression (Hegadoren et al., 2009; Nuss, 2015; Turner et al., 2018). Acetylcholine release enhances cognition, while increased norepinephrine levels suppress appetite (Benowitz, 1999). Glutamate levels are associated with learning and memory (McEntee and Crook, 1993) and serotonin release is connected to mood change (Berger et al., 2009). The ability of nAChRs to promote release of myriad neurotransmitters

provides a functional basis for nicotine's modulation of a wide variety of behaviors, contributing to the difficulty of developing efficacious smoking cessation aids (Benowitz, 2008; Feduccia et al., 2012; Picciotto et al., 2015).

2.2. Nicotinic Modulation of the Dopamine Reward Pathway

The mesocorticolimbic pathway, also known as the dopamine reward pathway, is the critical neural pathway for reinforcing rewarding behaviors, including those behaviors underlying nicotine dependence (Markou, 2008; Volkow and Li, 2004; Volkow and Morales, 2015). All drugs of abuse, including nicotine, are believed to enhance the effect of dopaminergic function in the mesocorticolimbic pathway. This is particularly true at dopaminergic nerve terminals arising from the ventral tegmental area (VTA) and terminating in the nucleus accumbens (NAc) – a key neural substrate of motivation and reward (D'Souza and Markou, 2011; Dani and De Biasi, 2013; Hyman et al., 2006; Volkow and Li, 2004). For example, nicotine activates the dopaminergic VTA-NAc pathway either (1) directly via stimulation of nAChRs on VTA dopaminergic terminals in the NAc or (2) indirectly through stimulation of nAChRs on glutamatergic nerve terminals innervating dopaminergic neurons in the VTA (D'Souza and Markou, 2011). This dopaminergic neurotransmission in the NAc terminates on the GABAergic medium spiny neurons, which account for ~95% of the NAc neuronal population (Yager et al., 2015). In addition to dopaminergic input, medium spiny neurons also receive glutamatergic input from the prefrontal cortex (PFC), where these dopaminergic and glutamatergic inputs interact physically at different sites on the dendritic spines of these GABAergic cells (Bamford et al., 2018; Wang et al., 2012). The PFC glutamatergic terminals also possess nAChRs, further increasing the complex actions of nicotine in this circuit. However, broadly, studies investigating how these interactions impact nicotine dependence have shown that inhibition of glutamatergic neurotransmission or stimulation of GABAergic neurotransmission attenuates nicotine intake and nicotine-seeking behavior in rodents (D'Souza and Markou, 2013).

2.3. Neurobiology of Nicotine Tolerance and Withdrawal

Repeated exposure of nicotine leads to distinct neuronal adaptations including up-regulation and desensitization of nAChRs (Quick and Lester, 2002), which are the major factors contributing to the development of nicotine tolerance and withdrawal effects following smoking cessation (Gould et al., 2014; Grieder et al., 2012; Hildebrand et al., 1998; Picciotto et al., 2008). Exposure to chronic nicotine produces tolerance to most of its pharmacological effects such as hypothermia, antinociception and convulsive effects, except its effects on memory and attention (Benowitz, 2008). Furthermore, nicotine withdrawal symptoms manifest a few hours after discontinuation of nicotine use, and the collection of these negative symptoms during abstinence from nicotine is the major determinant preventing smokers from successful quitting (McLaughlin et al 2015). In humans, symptoms of nicotine withdrawal encompass affective, cognitive, and somatic domains. Affective symptoms include anxiety, depression, irritability, anhedonia, hyperalgesia, dysphoria, severe craving for nicotine, insomnia, increased appetite and weight gain (Benowitz, 2008), while memory impairment and difficulty concentrating are cognitive manifestations (McLaughlin et al 2015). Together, the biological basis of nicotine addiction can be regarded as a mix of positive reinforcement, such as mood enhancement, and avoidance of negative

effects associated with nicotine withdrawal (Benowitz 2010, Cosci 2011, Markou 2008). Similar behavioral phenotypes are also observed in rodents that have undergone chronic nicotine exposure and withdrawal (Adeluyi et al., 2019; Hussmann et al., 2014; Turner et al., 2013b; Yohn et al., 2014). Work in rodent models has highlighted the importance of multiple brain regions and their contribution to mechanisms underlying nicotine withdrawal-induced behavioral deficits, including the medial habenula (Salas et al., 2009), interpeduncular nucleus (Zhao-Shea et al., 2013), central amygdala (Panagis et al., 2000; Watkins et al., 2000), PFC (Addicott et al., 2012; Cole et al., 2020) and NAc (Gipson et al., 2013; Hildebrand et al., 1999; Morud et al., 2018). Although the neuronal contributions to withdrawal-related behaviors have been well characterized, less is understood about glial cell involvement. Extra neuronal cells may provide an untapped therapeutic target for new front-line pharmacotherapeutic treatments.

3. NOVEL TARGETS FOR SMOKING CESSATION: GLIAL CELLS IN NICOTINE DEPENDENCE

Much of our mechanistic understanding of nicotine dependence pertains exclusively to neurons, particularly in the mesocorticolimbic neurocircuitry. However, while much less is understood regarding glial cells and their contribution to these processes, recent work has highlighted a prominent role of astrocytes and microglia in drug dependence. For example, multiple drugs of abuse produce alterations in astrocytic metabolism and physiology, which are in turn associated with modulation of reward circuitry function and development of drug dependence phenotypes (Corkrum et al., 2019; Goins and Bajic, 2018; Kim et al., 2018; Scofield and Kalivas, 2014; Scofield et al., 2016). Similarly, more recent studies have shown that microglial activation and microglia mediated inflammatory responses following chronic nicotine treatment and withdrawal are major contributing factors for nicotine withdrawal-induced behavior deficits (Adeluyi et al., 2019; Saravia et al., 2019). These findings suggest that impaired interplay amongst these brain cell-types as a consequence of nicotine dependence may be a major determinant underlying high relapse rates among smokers.

3.1. Astrocytes: Morphology and Physiology

Astrocytes have been documented as part of the larger population of extra-neuronal cells in central nervous system (CNS) called “Glial” (Kettenmann and Verkhratsky, 2008). These extra-neuronal cells include microglia, oligodendrocytes and other specialized cells in addition to astrocytes (Allen and Lyons, 2018). Astrocytes are the most abundant and morphologically complex glial cells in the mammalian brain with numerous processes. However, the proportion of astrocytes varies between brain regions exhibiting regional heterogeneity with respect to their ratio to that of neurons (Ero et al., 2018). The current estimates suggest that astrocytes represent ~19–40% of brain cells (Von Bartheld et al., 2016). They exist in high density in some brain regions, such as the cerebral cortex which possesses a high astrocyte-to-neuron ratio, and low density in other regions like the cerebellum, which has a low astrocyte-to-neuron ratio (Khakh and Sofroniew, 2015b). The functional relevance of this regional diversity is still unclear. However, it has been suggested that a single cortical astrocyte can physically interact with hundreds of neuronal dendrites and wraps over 100,000 synapses (Sofroniew and Vinters, 2010), while similar studies in the

hippocampus demonstrated that ~57% of synapses are in physical contact with astrocytes (Harada et al., 2015). Because close physical contact between astrocytes and neurons has been shown to influence neuronal synaptic plasticity (Allen and Eroglu, 2017), these unequal astrocytic densities may represent important differential contributions to plasticity. Astrocytes also have functional interactions with the blood brain barrier (BBB) and other glial cells, impacting processes such as metabolic support (Abbott et al., 2006), neurotransmitter clearance (Murphy-Royal et al., 2017), and ion homeostasis (Khakh and Sofroniew, 2015a; Simard and Nedergaard, 2004) (Figure 1). As such, astrocytes act as metabolic bridge between vascular glucose supply and neurons by metabolizing glucose via glycolysis to generate adenosine triphosphate (ATP) and lactate as byproduct, which is used by neurons as energy source to generate ATP and fulfills the energy demand of synaptic transmission (Turner and Adamson, 2011). Astrocytes also help to regulate glutamatergic synaptic transmission by clearing glutamate from the synaptic cleft through ion mediated glutamate transporters (GLT-1) (Albrecht et al., 2010; Verkhratsky et al., 2017). The glutamate taken up by astrocytes is converted to glutamine by glutaminase and then subsequently released into the extracellular space to make it available to neurons for synthesis of glutamate. The glutamate synthesized by neurons is then loaded into the vesicles by the vesicular glutamate transporter for the next cycle of glutamatergic synaptic transmission (Niciu et al., 2012).

3.1.1. Astrogliosis and Classification of Reactive Astrocytes—Astrocytes at rest are involved in synaptic plasticity by providing metabolic and trophic support to neurons (Allen and Eroglu, 2017). However, environmental perturbations such as exposure to drugs of abuse can trigger astrocytes to become reactive, resulting in extended process length, hypertrophy, and increased expression of GFAP, β -catenin, Nestin, N-cadherin, matrix metalloprotease (MMP) 2, Plaur, MMP13, Axin2, Nes, and Ctnnb1 (Hagino et al., 2003; Hara et al., 2017). Once reactive, these astrocytes can proliferate, migrate, and transform into scar-forming astrocytes, which possess a molecular signature of Cdh2, Sox9, and chondroitin sulphate proteoglycan-related genes such as Csgalnact1, Chst11, Pcanwas, Acan, and Slit2 (Nathan and Li, 2017; Silver and Miller, 2004; Sun et al., 2017). Activation of astrocytes and their altered functioning have been implicated in numerous aspects of drug dependency, including drug-seeking behavior (Fouyssac and Belin, 2019) and reward memory (Gomez et al., 2019). Recently, reactive astrocytes have been categorized into two classes (A1 & A2) according to their function, mode of activation, and genetic signature (Figure 2). A1 astrocytes are suggested to induce neurotoxicity and promote death of oligodendrocytes, whereas A2 astrocytes have neuroprotective effects and promote neuronal survival (Liddelow et al., 2017). A number of genes are differentially expressed in A1 and A2 subtypes of reactive astrocytes, with Complement 3, Complement factor B (CFB) and MX1S as specific markers of A1 astrocytes, and the S100 protein family member S100A10 as a specific marker of A2 astrocytes (Fujita et al., 2018; Liddelow et al., 2017). Moreover, mode of activation is another criterion to differentiate A1 and A2 reactive astrocytes. A1 astrocytes are activated via stimulation by IL-1 α , TNF α , and C1q cytokines released from activated microglia (Liddelow et al., 2017), while A2 astrocytic reactivity is triggered by Stat3-signaling, resulting in astrocytic proliferation and increased support for neuronal regeneration (Anderson et al., 2016). While A1 reactive astrocytes are reported to induce

neuronal death, A2 reactive astrocytes exert neuroprotective effects by secreting several trophic factors and anti-inflammatory cytokines, including S100A10 (an essential factor for cell proliferation, membrane repair, and inhibition of cell apoptosis) and transforming growth factor beta (TGF- β ; participates in synaptogenesis) (Liddel et al., 2017; Xu et al., 2018). However, it should be noted that the phenotypic A1/A2 designation is fluid, as recent work has shown that a switch between A1 & A2 types of astrocytes can occur via milk fat globule epidermal growth factor 8 (MFG-E8) stimulation, resulting in upregulation of the PI3K-Akt and downregulation of the NF- κ B pathways (Xu et al., 2018).

3.1.2. Effects of Nicotine on Astrocytes—The role of astrocytes in the brain were once thought to be limited to metabolic and trophic support for neurons, ionic and pH homeostasis, and the formation and maintenance of the BBB; however, it is now becoming more evident that astrocytes are active participants in the modulation of neuronal activity in the brain (Chung et al., 2013). Drugs of abuse such as nicotine can disrupt the interplay between astrocyte and neuron in the brain by direct or indirect modulation. For direct modulation, nicotine can directly bind to nAChRs on astrocytes. Early studies showed that astrocytes express $\alpha 3$, $\alpha 4$, $\alpha 7$, $\beta 3$, $\beta 4$ nAChR subunits, with the $\alpha 7$ subunit being the most predominantly expressed (Gotti and Clementi, 2004; Graham et al., 2003). While nicotine treatment has been reported as protective in both Alzheimer's disease and Parkinson's disease (Newhouse et al., 2012; Villafane et al., 2007), several studies have linked astrocytic nAChRs, particularly the $\alpha 7$ -subtype, to neuroinflammatory processes in these disorders (Liu et al., 2015; Quik et al., 2009; Teaktong et al., 2003). Nicotinic stimulation of astrocytes results in increased intracellular Ca^{2+} concentrations (Sharma and Vijayaraghavan, 2001), which trigger the coordinated release of vesicles containing gliotransmitters. Gliotransmitters have important functions in the brain and can directly influence the synaptic activity and function of local neurons, driving behavioral changes associated with drugs of abuse (Turner et al., 2013a). For example, a study examining nicotine's modulation of neuron-glia interaction in the hippocampus observed that the release of the astrocytic gliotransmitter D-serine following nicotine stimulation is necessary for nicotine's enhancement of both synaptic transmission and long-term memory (Lopez-Hidalgo et al., 2012).

Nicotine may also indirectly impact astrocytic function, such as by elevating local dopamine concentrations. For example, direct dopaminergic signaling onto astrocytes can trigger CTCF-dependent morphological and genomic changes in these cells, resulting in many pro-activation hallmarks, such as stellation of astrocytic processes, increased astrocytic Ca^{2+} levels, and activation of poly-ADP-ribose polymerase (Galloway et al., 2018). However, it is unknown whether these activated astrocytes are pro- or anti-inflammatory in nature. For example, nicotine has well-described anti-inflammatory effects in the brain and has been shown to attenuate IL-1 β -induced activation of human astrocytes through cyclooxygenase mediated mechanism (Revathikumar et al., 2016). It is possible that nicotine's combined direct and indirect effects result in the generation of both A1 (neurotoxic) and A2 (neuroprotective) reactive astrocytes, accounting for both the pro- and anti-inflammatory effects observed in astrocytes following nicotine exposure. However, more comprehensive

studies exploring genetic signatures of reactive astrocytes following chronic nicotine exposure are necessary to delineate this.

Another indirect effect of nicotine on astrocytic function pertains to the role of these cells in the clearance of small molecule neurotransmitters, such as glutamate. Much of the research to date examining astrocyte's role in drug dependence focuses on the role astrocytic control of glutamate receptor-mediated plasticity via glutamate clearance (for review, see (Kruyer et al., 2020)). The regulation of glutamate release and clearance has been shown to directly influence the signaling of ionotropic and metabotropic glutamate receptors, emphasizing an important role for glutamate transporters in synaptic plasticity. Of the five types of excitatory amino acid transporters (EAATs) present in the brain, EAAT2, which is primarily expressed on astrocytes, plays the major role in glutamate clearance – responsible for >90% glutamate uptake (Kim et al., 2011; Spencer and Kalivas, 2017). Nicotine's effects on this aspect of astrocytic function is likely due to its regulation of synaptic glutamatergic signaling, as demonstrated by work showing that withdrawal from nicotine self-administration in rats results in glutamatergic dysregulation in the corticostriatal circuit (Gipson et al., 2013; Knackstedt et al., 2009). Furthermore, these studies observed decreased EAAT2 protein expression in the nucleus accumbens shell following withdrawal (Knackstedt et al., 2009), indicating impaired glutamate clearance. There are existing drugs that have been shown to reduce nicotine withdrawal and nicotine-seeking behaviors via restoration of glutamate homeostasis. For example, ceftriaxone, an antibiotic drug that increases EAAT2 expression and function, reduced nicotine-induced reinstatement of conditional place preference (CPP) and blocked somatic and affective nicotine withdrawal signs in mice (Alajaji et al., 2013). Additionally, chronic administration of n-acetylcysteine (NAC), a drug that acts primarily on the glutamatergic system and restores EAAT2 (McClure et al., 2014), reduced extinction responding and reinstatement of nicotine-seeking in rats that underwent nicotine self-administration (Powell et al., 2019) and also reduced nicotine conditioned rewarding effects in a mouse CPP model (Bowers et al., 2016). Similarly, a randomized controlled clinical trial showed reduction in the number of cigarettes smoked by nicotine-dependent individuals treated with NAC (Knackstedt et al., 2009). Together, these studies indicate a role for the astrocytic glutamate transporter EAAT2 in nicotine-seeking behaviors.

In summary, astrocytes are major players in the regulation of neuronal activities that are key for nicotine dependency. Targeting astrocytes to modulate nicotine dependence-associated brain circuits may add new and critically needed therapeutics to our present toolkit for smoking cessation.

3.2. Microglia: Morphology and Physiology

Microglia are the resident innate immune cells in the brain and account for ~10% of the CNS cell population in the healthy brain (Salter and Stevens, 2017). In terms of their developmental origin and functions, microglia are a unique population among the other brain cell-types. Like peripheral myeloid cells, microglia are derived from mesoderm lineage, and are unlike other brain cell-types, which are derived from neuroectodermal progenitors. Recent lineage-tracing studies provided additional characterization of microglia origin, showing that they are derived from the yolk-sac progenitors – an embryonic origin

distinct from that of the peripheral myeloid populations (De et al., 2018; Ginhoux et al., 2010; Madore et al., 2017). Microglia are highly branched, dynamic cells with relatively rapid movement compared to other brain cell-types (Nimmerjahn et al., 2005). These features, in addition to their fine motile processes, allow them to constantly scan the brain parenchyma to maintain homeostasis. Further, as CNS surveillance elements, microglia can sense and respond rapidly to extracellular signals.

3.2.1. Microglial Activation and Polarization—Similar to astrocytes, microglia can assume diverse morphological phenotypes that have been previously classified into classical M1 microglia, the pro-inflammatory activation phenotype, or the alternative M2 microglia, the anti-inflammatory activation phenotype (Figure 3). This simplistic characterization of microglia activation phenotypes is based on the presence of particular cell surface molecules and the expression of specific sets of cytokines (Butovsky and Weiner, 2018). For example, M1 microglia are associated with factors such as tumor necrosis factor alpha (TNF α), interleukin-1-beta (IL-1 β), interleukin 6 (IL-6), nitric oxide and reactive oxygen species (ROS) (Gustin et al., 2015; Janssen et al., 2018; Shen et al., 2018; Tang and Le, 2016; Yao and Zu, 2019), while M2 microglia are typified by interleukin-10 (IL-10), insulin like growth factor 1 (IGF1) and TGF- β (Butovsky and Weiner, 2018; Tang and Le, 2016). M2 activation can be further divided into additional sub-categories according to differential expression of subtype markers. The expressions of mannose receptor (MRC1) and Arg1 are considered to be indicators of M2a activation whereas, increased expression of CD86 and the major histocompatibility complex II (MHCII) receptor are the key markers of M2b activation state. The M2c phenotype is characterized by elevated expressions of TGF- β 1 and sphingosine kinase 1 (Sphk1) (Zhang et al., 2018). Despite these established sub-classifications, the differential functions performed by M2 microglia sub-phenotypes are not well understood and future investigations are needed. However, evidence from more recent studies that re-characterize microglial diversity (De Biase et al., 2017; Stratoulis et al., 2019) have shown that the simplistic view of microglial phenotype does not sufficiently represent the intricate physiology of microglial cells (Ransohoff, 2016; Werry et al., 2019), underscoring the need for better functional understanding of these sub-categorizations.

In addition to the phenotypic complexities of microglia, they are also largely heterogeneous across diverse neural circuits and brain regions (De Biase et al., 2017). For example, a recent study that examined microglial phenotypes among a group of subcortical nuclei revealed that microglia across the basal ganglia are very diverse in terms of their density and morphology, metabolic and activation state, and transcriptome (De Biase et al., 2017). This implicates local cues as a critical mediator of microglia phenotype across neural circuits (McCarthy, 2017). Further, regional microglial heterogeneity may also indicate that diverse functional phenotypes are required for the sustained integrity of the various local CNS environments.

3.2.2. Effects of Nicotine on Microglia—Microglia were once considered only as support cells in the brain (Streit, 2002), but recent evidence demonstrates they are active players in both brain function and dysfunction (Salter and Stevens, 2017). In addition to their immune functions, microglia are critical elements in synaptic remodeling and plasticity

(Miyamoto et al., 2016; Torres et al., 2016), placing them as potential participants in the neuroplasticity underlying drug addiction. Similar to astrocytes, nicotine and many other drugs of abuse can directly modulate microglial morphology and function via interaction with nAChRs on microglia.

To date, the $\alpha 7$ -nAChR is the only nAChR subtype reportedly found on microglia and nicotine has been established to inhibit microglial function (Egea et al., 2015). Studies in immortalized microglia lines and primary cell culture have reported $\alpha 7$ -nAChR expression (De Simone et al., 2005; King et al., 2017; Mencil et al., 2013; Shytle et al., 2004; Suzuki et al., 2006), and Shytle et al., observed $\alpha 7$ expression both in primary murine microglial cortical culture and brain slices (Shytle et al., 2004). Shytle et al. further described a protective or anti-inflammatory effect of nicotine in an LPS activation model of TNF α release or microglial activation that could be attenuated via an $\alpha 7$ selective nicotinic antagonist, α -bungarotoxin. These anti-inflammatory effects were subsequently confirmed using both methyllycaconitine (MLA), another $\alpha 7$ -nAChR antagonist, and NS6740, a very weak $\alpha 7$ partial agonist (Li et al., 2016; Noda and Kobayashi, 2017; Thomsen and Mikkelsen, 2012). This neuroprotective effect on microglia activation was also observed in rat models of ischemia, where nicotine treatment prevented both microglial proliferation and thrombin-induced microglial activation (Guan et al., 2015; Ohnishi et al., 2009). Interestingly, this neuroprotective effect of nicotine can be observed reducing microglial activation in adult brains but increasing microglial activation in the adolescent brain (Linker et al., 2019).

In addition to playing a role in nicotine-mediated neuroprotection, microglia's direct involvement in nicotine withdrawal-related behaviors has been demonstrated in two recent *in vivo* studies in rodents (Adeluyi et al., 2019; Saravia et al., 2019). Adeluyi and colleagues demonstrated that withdrawal from chronic nicotine treatment induced microglial morphological changes in the NAc, promoting microglial activation via Nox2-mediated increases in ROS. Furthermore, these activated microglia released pro-inflammatory cytokines (TNF α and IL-1 β) in the NAc, which were correlated with exaggerated withdrawal behaviors; all of these effects were absent in mice with microglial depletion by PLX5622. These findings were complementary with a study from (Saravia et al., 2019), which linked nicotine withdrawal-induced cognitive deficits to microglial activation.

In addition to the *in vivo* animal studies described above, Brody et al. conducted a recent study in human subjects using [^{11}C]DAA1106, a ligand for TSPO and putative indicator of microglial activation (Brody et al., 2017). While their results showing less TSPO binding in active smokers may seem contradictory to the current rodent literature, it should be noted that Adeluyi et al. reported reduced microglial activation in animals chronically treated with nicotine and only observed the pro-inflammatory microglial activation following 48h withdrawal from nicotine. This suggests that future investigation is needed examining inflammatory responses and TSPO binding in human smokers during acute withdrawal in order to evaluate the therapeutic potential of microglial modulators as smoking cessation aids. Furthermore, these pro-inflammatory effects during nicotine withdrawal may be additive in nature as well. For example, (Liddel et al., 2017) demonstrated that activated microglia are necessary to induce A1 astrocyte activation. Therefore, region specific

microglia-mediated A1 astrocyte activation might be another possible downstream mechanism of nicotine dependence, further broadening potential therapeutic targets for smoking cessation.

While microglia may actively contribute to nicotine dependence, research investigating the role of microglia in nicotine dependence is still in the early stages. However, despite its infancy, microglia potentially represent an untapped opportunity to develop more potent and specific drugs to treat nicotine dependence and withdrawal.

4. CONCLUSION

Currently, there are three FDA-approved first-line smoking cessation medications: Nicotine Replacement Therapy (NRT), bupropion, and varenicline (Fiore et al., 2008; Prochaska and Benowitz, 2016; Singer et al., 2004). However, despite years of innovation and advancement in the development of smoking cessation medications, a large population of smokers attempting to quit still fail. This may be largely attributable to the inability of current smoking cessation therapies to address some critical aspects of nicotine dependency. For instance, all the three FDA-approved smoking cessation medications were developed to mechanistically target neuronal nAChRs, neglecting the possible contributions of targets on glial cells like astrocytes and microglia. To advance nicotine dependence research, studies exploring astrocyte and microglia function in nicotine dependence should be prioritized. Long-term, this may represent an untapped therapeutic avenue for smoking cessation.

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HIGHLIGHTS

- Tobacco use disorder accounts for 5 million premature deaths globally.
- Recent work suggests that extra-neuronal cells in the CNS may contribute significantly to withdrawal symptomology.
- This review examines the potential therapeutic potential of targeting astrocytes and microglia for treatment of nicotine withdrawal.

Figure 1:

Schematic representation of the neuron-astrocyte lactate shuttle and glutamate-glutamine cycle. Most of the glutamate released at the synapse is taken up by astrocytes via excitatory amino acid transporters (EAATs) where it gets converted to glutamine by the action of glutamine synthetase (GS) utilizing ammonia (NH₄) and ATP. Glutamine produced by astrocytes shuttled back to pre-synaptic neurons via Na⁺-coupled glutamine transporters (slc38a7) where it gets converted back to glutamate by glutaminases (GLS) enzyme and released at the synapses. Glutamate activates post-synaptic glutamatergic receptors (GluR) and increase the energy demand for neuronal transmission. To fulfill increased energy demand, neurons utilize lactate as an energy source to synthesize ATP through oxidative phosphorylation in mitochondria. This lactate is synthesized by lactate dehydrogenase 5 from pyruvate, which is synthesized from glucose utilization in astrocytes and glucose uptake from the circulation through GLUT1 glucose transporter.

Figure 2:

Pictorial illustration of drug of abuse-induced astrogliosis in brain and classification of reactive astrocytes with different molecular markers. A1 astrocytes are neurotoxic with increased expression of C3, CFB, and MX1S. A2 astroglia are neuroprotective with upregulation in S100a10, TGFβ and Stat3.

Figure 3:

Illustration showing drug of abuse-induced microgliosis in brain and classification of reactive microglia with different molecular markers. Activated microglia with M1 phenotype are neurotoxic with increased expression of pro-inflammatory markers like TNF α , NO, IL-1 β , ROS and Ccl5. Activated microglia with M2 phenotype are neuroprotective with enhanced expression of anti-inflammatory markers including IL-10, IGF1 and TGF- β . The M2 microglia can further be sub-classified into M2a, M2b and M2c phenotypes depending upon the expression of their specific markers.