INVITED REVIEW

Independent and Combined Effects of Nicotine or Chronic Tobacco Smoking and HIV on the Brain: A Review of Preclinical and Clinical Studies

Linda Chang [1,2,3,4] · Huajun Liang [1] · Suresh R. Kandel [5] · Johnny J. He [5]

Abstract

Tobacco smoking is highly prevalent among HIV-infected individuals. Chronic smokers with HIV showed greater cognitive deficits and impulsivity, and had more psychopathological symptoms and greater neuroinflammation than HIV non-smokers or smokers without HIV infection. However, preclinical studies that evaluated the combined effects of HIV-infection and tobacco smoking are scare. The preclinical models typically used cell cultures or animal models that involved specific HIV viral proteins or the administration of nicotine to rodents. These preclinical models consistently demonstrated that nicotine had neuroprotective and anti-inflammatory effects, leading to cognitive enhancement. Although the major addictive ingredient in tobacco smoking is nicotine, chronic smoking does not lead to improved cognitive function in humans. Therefore, preclinical studies designed to unravel the interactive effects of chronic tobacco smoking and HIV infection are needed. In this review, we summarized the preclinical studies that demonstrated the neuroprotective effects of nicotine, the neurotoxic effects of the HIV viral proteins, and the scant literature on nicotine or tobacco smoke in HIV transgenic rat models. We also reviewed the clinical studies that evaluated the neurotoxic effects of tobacco smoking, HIV infection and their combined effects on the brain, including studies that evaluated the cognitive and behavioral assessments, as well as neuroimaging measures. Lastly, we compared the different approaches between preclinical and clinical studies, identified some gaps and proposed some future directions.

Keywords Nicotine · Tobacco · HIV · Neuroprotective · Neurotoxicity · Neuroinflammation

✉ Linda Chang
  Linda.Chang@som.umaryland.edu

✉ Johnny J. He
  johnny.he@rosalindfranklin.edu

1 Department of Diagnostic Radiology and Nuclear Medicine, University of Maryland School of Medicine, 670 W. Baltimore Street, HSF III, Baltimore, MD 21201, USA

2 Department of Neurology, University of Maryland School of Medicine, Baltimore, MD, USA

3 Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

4 Department of Medicine, John A. Burns School of Medicine, University of Hawaii at Manoa, Honolulu, HI, USA

5 Department of Microbiology and Immunology, Chicago Medical School, Center for Cancer Cell Biology, Immunology and Infection, Rosalind Franklin University, 3333 Green Bay Road, Basic Science Building 2.300, North Chicago, IL 60064, USA

Introduction

Tobacco smoking among HIV seropositive (HIV+) adults is twice as prevalent as that in the general population (36% vs. 16–21%) (Mdodo et al. 2015; Centers for Disease Control and Prevention 2018; Jamal et al. 2018). HIV+ individuals are also more vulnerable to the adverse consequences of tobacco smoking, since population-attributable risk of smoking-related death is almost twice as high in people with HIV (PWH) than in the general population (62% vs. 34%) (Helleberg et al. 2013). Tobacco smoking also leads to poorer viral suppression (Ande et al. 2015; Gamarel et al. 2018) and more years of life lost than HIV-infection alone in PWH (Helleberg et al. 2015). Moreover, treating tobacco smoking in HIV+ individuals is challenging (Ledgerwood and Yskes 2016). For instance, since HIV-infection may accelerate nicotine metabolism in HIV+ smokers (Ashare et al. 2019), they would have stronger tobacco craving, greater withdrawal symptoms (Sofuoglu et al. 2012), and poorer response to

tobacco cessation treatment (Ledgerwood and Yskes 2016) than individuals without HIV infection.

Furthermore, tobacco smoking may have additional negative impact on brain injury and brain function. Despite effective cART, HIV-associated neurocognitive disorders (HAND) continue to be prevalent in up to half of HIV-infected individuals (McArthur et al. 2010; Saylor et al. 2016; Clifford 2017). In those with HAND, likely etiologies include sustained and widespread neuroinflammation and oxidative stress, which in turn leads to neuronal loss and axonal damage in the brain (McArthur et al. 2010; Saylor et al. 2016). Independent of HIV infection, tobacco smoking may cause increased oxidative stress (Durazzo et al. 2014b), blood-brain barrier impairment (Mazzone et al. 2010), and astroglial and microglial activation (Moreno-Gonzalez et al. 2013), which all may contribute to neuroinflammation (Bradford et al. 2011; Khanna et al. 2013) and subsequent neuronal damage. Studies that compared cognitive function in PWH who smoked and those who did not smoke found that tobacco smoking was associated with worse working memory (Bryant et al. 2013; Harrison et al. 2017), learning (Bryant et al. 2013; Monnig et al. 2016), processing speed (Bryant et al. 2013; Monnig et al. 2016; Harrison et al. 2017), and executive function (Bryant et al. 2013), as well as greater intra-individual variability on response time during the Continuous Performance Task (Harrison et al. 2017). However, two smaller studies found that tobacco smoking was not associated with cognitive decline (Akhtar-Khaleel et al. 2017; Tsima et al. 2018). The effects of smoking on cognition in PWH may be sex-specific. For example, one small study found that HIV+ women who were smokers had better executive function than non-smoking women with HIV (Wojna et al. 2007). Another study also reported tobacco smoking associated with poorer learning in HIV+ men but not in women (Bryant et al. 2013). In contrast, recent studies that evaluated the sex-specific effects on cognitive performance in PWH found that HIV+ women had the poorest performance on various cognitive domains compared to HIV+ men and seronegative men or women; 70% of the HIV+women were tobacco smokers (Liang et al. 2020). Nevertheless, the underlying cellular and molecular mechanisms for these divergent findings are not fully understood.

Although preclinical studies rarely studied the effects of tobacco smoking in HIV models, extensive *in vitro* and *in vivo* studies were conducted to evaluate the effects of nicotine with HIV viral proteins or in HIV transgenic rodents. In this review, we will first highlight the major findings from preclinical studies that evaluated nicotine's effects on the brain, neuroprotective (including its cognitive enhancing effects) versus nicotine or tobacco smoke's neurotoxic or neuroinflammatory effects, both from *in vitro and in vivo* animal models. To evaluate whether some of the animal models have clinical relevance, we will also review the current knowledge from cognitive, behavioral and neuroimaging studies in human tobacco smokers. Since many tobacco smokers also co-use alcohol and marijuana, data regarding the neurocognitive or neuroimaging outcomes in these co-users will also be reviewed. Next, we will review the current literature on HIV-associated brain injury, including *in vitro* studies that utilized HIV viral proteins or animal models, such as transgenic rodents that over-expressed HIV viral proteins, as well as behavioral and neuroimaging studies from PWH.

Lastly, we will discuss the limited data on the combined effects of nicotine/tobacco and HIV infection on the brain. Very few studies have evaluated the combined effects of nicotine / tobacco smoke and HIV (e.g., with HIV viral proteins) on neurotoxicity/neuroinflammation in preclinical models. Similarly, only a few neurobehavioral and neuroimaging studies specifically evaluated the independent and combined effects of HIV brain infection and chronic tobacco smoking. We will summarize these studies, discuss the discrepancies and the gaps in our knowledge, and hence the research opportunities, that are needed to bridge the preclinical and clinical findings. We will also identify opportunities for future research that are needed to elucidate the mechanisms involved in the highly prevalent co-morbid condition of tobacco smoking in PWH, which will be useful in guiding future preventions or treatments.

Effect(s) of Nicotine/tobacco on Neuroprotection or Neurotoxicity/neuroinflammation

Nicotine and its Receptors

Nicotine is the major component of tobacco and is an addictive chemical. It is metabolized by many enzymes including cytochrome p450 enzymes, uridine 5'-diphospho–glucuronosyl-transferase, flavin-containing monooxygenase, and amine-N-methyltransferase (Albuquerque et al. 2009; Benowitz 2015). Genetics, diets, age, sex, kidney and liver diseases, pregnancy, and oral contraceptives all can affect its metabolism (Dani 2015). Nicotine selectively binds to the nicotinic acetylcholine receptors (nAChR) and triggers psychoactive rewarding effects (Benowitz 2015). Structurally, neuronal nAChR are homopentamers or heteropentamers. Eight α-like subunits, namely α2, α3, α4, α5, α6, α7, α9, and α10 (α8, not found in mammals), and three β subunits (β2- β4) have been cloned from neuronal tissues. The most abundant nAChR subtype receptors are α4β2, α3β4, and α7. Because of multiple subunit combinations, these receptors have distinct functional properties. The structure and function of mammalian nAChR are reviewed in more detail elsewhere (Hukkanen et al. 2005; Albuquerque et al. 2009). Neuronal nAChR are distributed throughout the nervous system but most abundantly in the hippocampus, thalamus, and cerebral

cortex (Albuquerque et al. 2009), which correspond to brain regions critical for memory, and brain regions associated with motor and sensory pathways or higher level cognitive function. Therefore, it is conceivable that any alterations in these receptors may lead to neurological and psychiatric disorders.

Neuroprotective Effects of Nicotine

Beneficial effects of nicotine have been noted in patients with mild cognitive impairments (Newhouse et al. 2012) and in patients with Parkinson's disease (Barreto et al. 2015; Nicholatos et al. 2018). Nevertheless, the underlying cellular and molecular mechanisms are not fully understood. In order to delineate nicotine-mediated neuroprotective mechanisms, extensive *in vitro* and *in vivo* studies have been conducted. Major findings from these studies are highlighted below and in Table 1.

Nicotine has been shown to be neuroprotective *in vitro*. For example, nicotine protected primary rat neurons against glutamate-induced cytotoxicity through α7-nAChR (Kaneko et al. 1997). In primary hippocampal cultures, α7-nAChR promoted neuronal survival following exposure to excitotoxic stimuli via NMDA receptor activation and a calcium-dependent mechanism (Dajas-Bailador et al. 2000). In mouse cortical neurons, both β2 and α7 nAChR were involved in nicotine-mediated neuroprotective effects (Stevens et al. 2003). Moreover, nicotine activated the growth-promoting enzymes Janus Kinase 2 (JAK2) in PC12 cells, as JAK2-specific inhibitor AG-490 blocked the nicotine-induced neuroprotection against amyloid peptide β1–42 (Shaw et al. 2003). In a rat genome Affymetrix GeneChi array analysis, nicotine treatment induced up-regulation or down-regulation of several genes such as orphan nuclear receptor (Nr4a1), early growth response (Egr-1), and early growth response 2 (Egr-2) (Belluardo et al. 2005).

The other mechanism of nicotine-mediated neuroprotection is through its upregulation of glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) (Belluardo et al. 2005; Ghitza et al. 2010; Takarada et al. 2012; Wei et al. 2015). In rat cortical astrocytes, nicotine increased GDNF expression in a dose-dependent manner (Belluardo et al. 2005; Takarada et al. 2012). In the rat hippocampus, nicotine also significantly increased BDNF mRNA expression (Wei et al. 2015). Given the major neuroprotective roles of GDNF and BDNF in the central nervous system (CNS) (Ghitza et al. 2010), nicotine likely has therapeutic potential for CNS diseases.

Additionally, *in vivo* studies demonstrated the neuroprotective effects of nicotine. For instance, in rat models of focal cerebral ischemia, nicotine administration up-regulated cannabinoid (CB1) receptor expression, ameliorated neurological deficits and reduced the infarct volumes (Chen et al. 2013). CB1 receptor is the first cannabinoid receptor cloned in 1990

(Matsuda et al. 1990) and is rich in neuronal tissue (Straiker et al. 1999), but how it interplays with nicotine is not yet understood. Furthermore, in the intracerebral hemorrhage (ICH) mouse model, intraperitoneal administration of nicotine inhibited apoptosis, increased anti-apoptotic B cell lymphoma-2, and decreased proapoptotic protein Bax in the brain (Hijioka et al. 2011). Nicotine also decreased microglia activation, neutrophil infiltration, and the increased oxidative stress associated with ICH, without changes in hematoma expansion and brain edema (Hijioka et al. 2011). Moreover, in a compressive spinal cord injury (SCI) model, nicotine markedly attenuated inducible nitric oxide synthase (iNOS) expression (Lee et al. 2009). The overexpression of iNOS in SCI increased nitrosylation formation, up-regulated inflammatory genes, and decreased the number of neuronal nuclei (NeuN)-immunoreactive cells. Furthermore, knocking out α4 nAChR in 6-OHAD-induced Parkinson rat models (Ryan et al. 2001) and α7 nAChR in oxygen and glucose deprivation mouse model (Egea et al. 2007) abrogated the neuroprotective effects of nicotine. In non-human primates, chronic nicotine treatment also increased nAChR expression such as α4β2 and α3/α6β2 and protected dopaminergic neurotoxin-induced lesions in the brain (Bordia et al. 2006; McCallum et al. 2006; Quik et al. 2006; Quik et al. 2010). Taken together, these studies provide overwhelming and compelling evidence for the neuroprotective effects of nicotine in the brain, which are likely mediated by α4 and α7nAChR.

Nicotine as a Cognitive Enhancer

Despite the strong and consistent findings of the neuroprotective effects of nicotine, very few preclinical studies assessed the possible beneficial cognitive effects of nicotine. Given the high prevalence of tobacco smoking in schizophrenic patients, one study evaluated a maternal immune activation (MIA) schizophrenia rat model, and found that nicotine self-administration significantly ameliorated the cognitive deficits induced by MIA, lending support to the self-medication hypothesis of schizophrenia (Waterhouse et al. 2018). However, in another repeated phencyclidine (PCP) schizophrenia rat model, rats that received chronic nicotine-treatment without the PCP showed improved performance on an attention task, with shortened response latencies, more correct responses and fewer omissions, but nicotine did not attenuate the PCP-induced cognitive deficits (Amitai and Markou 2009). Therefore, although chronic nicotine administration had some pro-cognitive effects, it could not overcome the PCP-induced deficits. In a chronic unpredictable mild stress mouse model, nicotine increased sucrose consumption, but partially rescued the spatial learning and reversal learning deficits on the Morris water maze test in the stressed animals (Shang et al. 2017). In the same study, nicotine also ameliorated the depression-like symptoms and improved the

Table 1 Pre-clinical studies that evaluated nicotine-mediated neuroprotection

Author(s) / Journal(s)	Cell or Animal Model (s)/ Nicotine Dose	Primary Findings
In Vitro Models		
Kaneko et al., *Brain Res* 1997	Primary culture rat neurons; Nicotine: 10 μM for 8–24 h	Nicotine protected primary culture rat neurons against glutamate-induced cytotoxicity through α7 neuronal receptor
Dajas-Bailador et al., *Neuropharmacology* 2000	Primary hippocampal culture; Nicotine: 10 μM for 1 h	Nicotine counteracted the NMDA-induced cell death through a Ca^{2+} dependent mechanism.
Stevens et al., *J Neurosci* 2003	Mouse primary cortical neurons; Nicotine: 10 μM	Both β2 and α7 nAChR mediated nicotine's neuroprotective effects
Shaw et al., *J Neurosci* 2003	PC 12 cells; Nicotine: 10 μM	Nicotine-activated Janus Kinase 2, protected against amyloid (1–42)-induced neurotoxicity
Takarada et al., *J Neurosci Res* 2012	Wistar rat cortical astrocytes; Nicotine: 10–100 μM	Nicotine increased GDNF mRNA and protein in astrocytes after nicotine (10–100 μm) exposure
Revathi- Kumar et al., *J Neuroinflammation* 2016	Cryopreserved human fetal astrocytes; Nicotine: 1, 10, 100 μM	Nicotine diminished the production of inflammatory cytokines (IL-6, IL-1β, and TNF-α) in IL-1β-activated astrocytes
Rodent Models		
Nizri et al., *J Immunol* 2009	Experimental autoimmune encephalomyelitis C57BL/6 mice; Nicotine: 2 mg/kg/day x 28 days	Nicotine attenuated neuroinflammation via suppressing Th1 and Th17 response
Hijioka et al., *J Pharmacol Exp Ther* 2011	C57BL/6J collagenase-induced Intracerebral Hemorrhage mice; Nicotine: 1 or 2 mg/-kg/day, i.p.	Intraperitoneal administration of nicotine inhibited apoptosis, increased the relative expression level of antiapoptotic B cell lymphoma-2
Lee et al., *J Neurosci Res* 2009	Spinal cord injury mouse; Nicotine: 0.35, 3.5, 7 mg/kg 2 h after trauma	Nicotine attenuated SCI- associated iNOS production
Ryan et al., *Br J Pharmacol.* 2001	6-hydroxydopamine rats; Nicotine: 1 mg/kg, s.c.	Knocking out of α4 nAChR induced neurodegeneration (indirect evidence for the neuroprotective effect of the receptor)
Egea et al., *Neuroscience* 2007	OGD or α7 nAChR mutant mice; Nicotine: 10–100 μM	Nicotine did not mediate neuroprotection in α7 nAChR knockout mice.
Bellurado et al., *Neuroscience* 2005	Male Wistar rats (3 months old); Nicotine: 1 mg/kg, i.p.	Acute intermittent nicotine treatment up-regulated several novel candidate genes such as Nr4a1 (Nurr77), Egr-1 and Egr-2
Huang et al., *Neuroscience* 2007	Rat Pups from Timed-pregnant Sprague-Dawley rat dams; Nicotine: 6 mg/kg/day x 7 days (P1- P7)	Nicotine led to differential expression of nAChR subtypes in hippocampus and cerebellum.
Wei et al., *Neurosci Lett* 2015	Male Wistar rats; Nicotine: 0.5 mg/kg, i.p.	Nicotine increased GDNF expression in astrocytes and increases BDNF mRNA expression in hippocampus. Acute nicotine administration attenuated LPS-induced cognitive dysfunction.
Simian Models		
Bordia et al., *J Pharmacol Exp Ther* 2006; McCallum et al., *J Neurochem* 2006	Squirrel Monkeys (*Saimiri sciureus*) Nicotine: 25 μg/ml to gradual increased to 650 μg/ml over a 3-month period, Oral	Chronic nicotine treatment increased α4β2 nAChR by > 50% in striatum of both lesioned and un-lesioned monkeys, but the increase was significantly higher in lesioned than in un-lesioned monkeys.
Quick et al., *J Mol Neurosci* 2010	Female Squirrel Monkeys (*Saimiri sciureus*) Nicotine: 25 μg/ml to gradual increase to 650 μg/ml over a 3 to 4-month period, Oral	Chronic treatment led to increased expression of α3/α6β2* and α4β2* nAChR in lesioned monkeys compared to controls. Nicotine also elevated microglia and reduced extracellular deposition of neuromelanin in the substantia nigra of MPTP-lesioned monkeys.
Quick et al., *J Neurochem* 2006	Squirrel Monkeys (*Saimiri sciureus*) Nicotine: Started with 25 μg/mL-1 for one week then 50 μg/mL, up to 650 μg/mL, Oral	Chronic nicotine treatment elevated the levels of striatal tyrosine hydroxylase, dopamine transporter, vesicular monoamine transporter, dopamine and nicotinic receptors in MPTP-lesioned animals

S.C.: subcutaneous injection; i.p. intraperitoneally injection

hippocampal synaptic plasticity, as assessed by the long-term potentiation (LTP) and depotentiation recorded in the hippocampal dentate gyrus (Shang et al. 2017). However, at a dose of 0.4 mg/kg subcutaneous injection, nicotine impaired the contextual learning memory in the F344 rats (Nesil et al. 2015). Furthermore, nicotine administration also increased

behavioral impulsivity in rodent studies (Hosking et al. 2014). Overall, the findings from preclinical models are somewhat controversial regarding the beneficial cognitive enhancing effects of nicotine.

Human laboratory studies also showed that acute nicotine (e.g. nicotine patch) improved performance on fine motor and attentional tasks in both non-smokers and non-deprived smokers (Heishman et al. 2010). However, this improvement has a speed-accuracy tradeoff; nicotine-treated subjects had faster motor response but were less selective and more distracted (Vangkilde et al. 2011). In chronic smokers, they became slower, with longer reaction time, during a spatial attention task at the higher difficulty level after the administration of nicotine (Vossel et al. 2011). The potential benefits of nicotine for improving cognition and mood regulation (Newhouse et al. 2012; Gandelman et al. 2018; Lewis et al. 2018) have led to the current standard treatment of using cholinesterase inhibitors to enhance nAChR activation for the memory deficits in Alzheimer's disease (Jasinska et al. 2014). Furthermore, nicotine patch improved response speed and recall memory in patients with mild cognitive impairment (Newhouse et al. 2012), and reduced depressive symptoms in patient with late life depression (Gandelman et al. 2018). Neural correlates of these therapeutic effects of nicotine were studied using pharmacological functional imaging techniques (Bentley et al. 2011; Sutherland and Stein 2018). After nicotine administration, functional connectivity increased within the executive control network but reduced within the default mode network, with improved to normal levels cognitive performance in nicotine-deprived smokers (Lesage et al. 2017; Lesage et al. 2020). The effects of nicotine on healthy non-smokers were less consistent; while some reported improved cognitive performance (e.g. increased n-back accuracy) and brain network efficiency (Kumari et al. 2003; Lesage et al. 2020), others reported no effects on either their performance or functional connectivity (Lesage et al. 2017). Currently, none of the nAChR ligands has succeeded the phase III clinical trials for treatments of cognitive deficits or mood disorders (Bertrand and Terry 2018). Moreover, changes in the brain after long term NRT have not yet been examined.

Nicotine or Tobacco Smoke-mediated Neurotoxicity and Neuroinflammation in Preclinical Models

Although prior preclinical studies primarily demonstrated nicotine's neuroprotective effects *in vitro* and *in vivo*, and to a lesser degree in some clinical studies, nicotine can also be neurotoxic in certain conditions. In the developing brain, a brief period of continuous or intermittent nicotine exposure led to cellular and neurite damage and altered nAChR expression (Huang et al. 2007; Dwyer et al. 2009). Nicotine exhibited neurotrophic effects on undifferentiated cells but became neurotoxic to differentiated cells (Abreu-Villaca et al. 2005). During development from fetal-neonatal brain to the mature adult brain, exposure of low-dose nicotine led to irreversible changes in adult brain function (Eriksson 1997). The molecular mechanisms of why and how nicotine is toxic to the developing brain are not well understood. The opposing effects of nicotine on the developing brain compared to the adult brain might, in part, be due to differences in the expression and distribution of nAChR in these different stages of brain development.

Another mechanism for nicotine or tobacco smoke-mediated neurotoxicity is the induction of neuroinflammation. Increased expressions of pro-inflammatory cytokines are major hallmarks of neuroinflammation (Ransohoff 2016; Gilhus and Deuschl 2019). Nicotine suppressed the production of pro-inflammatory cytokines IL-6, IL-1β, and TNF-α in IL-1β-activated astrocytes (Revathikumar et al. 2016), and lessened neuroinflammation by suppressing Th1 and Th17 responses (Nizri et al. 2009). However, one study found that nicotine subcutaneous injections led to increased TNF-α expression in rats (Royal et al. 2018). Acute administration of nicotine attenuated lipopolysaccharide-induced cognitive dysfunction, via the suppression of the strong LPS-induced release of IL-1β, IL-6, and TNF-α (Wei et al. 2015). Additionally, nicotine activation of nAChR, particularly α7-nAChR, is attributed to its anti-inflammatory activity (Ryan et al. 2001; Wei et al. 2015). Thus, α7-nAChR agonists such as nicotine are expected to ameliorate neuroinflammation, and benefit those afflicted by neurodegenerative conditions. Overall, the myriad of *in vitro* and preclinical studies demonstrated that nicotine is generally neuroprotective and anti-neuroinflammatory except for its effects in the developing brain (Fig. 1; Table 1).

However, nicotine is typically consumed via tobacco smoking in humans. Besides nicotine, tobacco smoke contains more than 5,000 toxic and carcinogenic chemicals (Talhout et al. 2011). Surprisingly, preclinical studies on tobacco smoke and its effects on the brain are scare and inconsistent. One study showed a neuroprotective effect, since pre-exposure of cigarette smoke significantly reduced kainic acid-induced mortality and neuron loss in CA1 and CA3 regions of hippocampus in rats (Kim et al. 1999). In contrast, in other rodent studies, tobacco smoke exposure led to increased brain pro-inflammatory cytokines (Khanna et al. 2013) and gene expression (Royal et al. 2018), astroglial and microglial activation (Moreno-Gonzalez et al. 2013) and higher levels of inflammatory cytokines (Bradford et al. 2011). Furthermore, studies of the developing brain in rodents again found that tobacco smoke or its extract exhibited deleterious effects to their brain development, affecting both the cholinergic and the serotonergic systems (Slotkin et al. 2015, 2019).
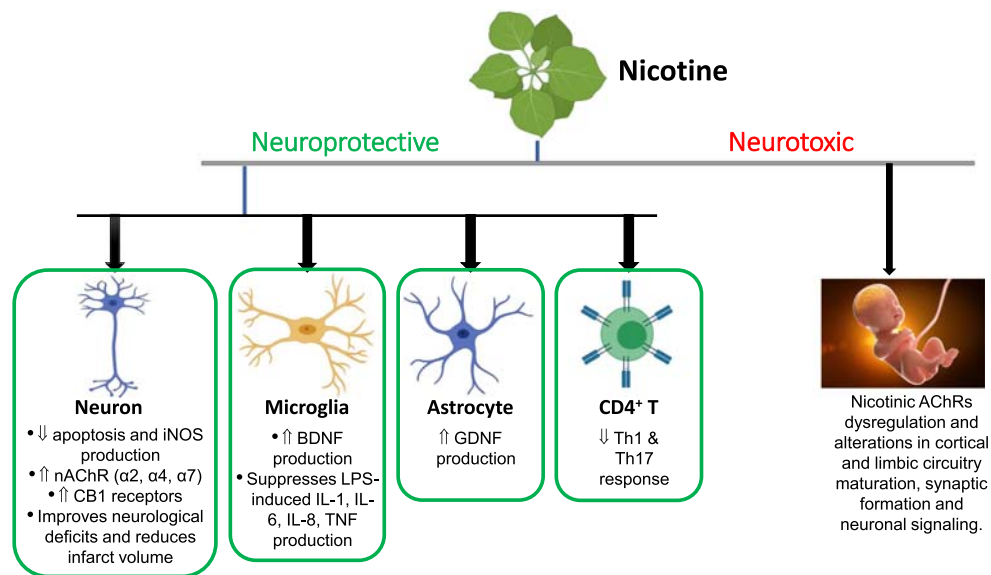
Fig. 1 Nicotine-mediated neuroprotective and neurotoxic effects. Nicotine is neuroprotective through up-regulation of the expression of CB1 receptors, nAChR, and BDNF, and decrease apoptosis and iNOS production in neurons, LPS-induced pro-inflammatory cytokines in microglia, increase GDNF production in astrocytes, and suppression of Th1 and Th17 response in CD4^{+} T cells. In contrast, nicotine may be neurotoxic through alteration of nAChR expression and damage to neurites in the developing brain. CB1, cannabinoid beta 1 receptor; iNOS, inducible nitric oxide synthase; nAChR, nicotine acetyl choline receptors; LPS, lipopolysaccharide; BDNF, brain-derived neurotrophic factor; GDNF, glial cell line derived neurotrophic factor. Images in the figure were created with BioRender.Com under a paid subscription to SK

Clinical Studies of Tobacco Smoking on Neuroinflammation and Neurotoxicity (Table 2)

In contrast to the sparse preclinical data on the effects of tobacco smoke, a vast number of clinical and epidemiological studies indicate the deleterious effects of tobacco cigarette smoking on human health. Smoking is the leading preventable cause of death and is associated with increased risks for many clinical conditions including chronic obstructive pulmonary disease, cardiovascular disorders, stroke and cancer. Furthermore, cigarette smoking induces repetitive inflammatory insults and leads to chronic and progressive activation of the immune system (Madani et al. 2018). Similar to the deleterious effects of nicotine or tobacco smoke on the developing brain in preclinical studies, infants or children prenatally-exposed to nicotine, from maternal tobacco smoking during the pregnancy, also showed delayed white matter maturation and lower levels of glial metabolites, especially the girls (Chang et al. 2012; Chang et al. 2016). Furthermore, even second-hand exposure to tobacco smoke during pregnancy increased the risk of developing neurological disorders (Slotkin et al. 2017). However, to understand the mechanisms involved in the neurotoxic effects, more studies are warranted to define the effects of tobacco smoke on the brain, particularly in co-morbid brain disorders.

Tobacco smoke contains high concentrations of free radicals that cause oxidative damage on tissues (Ambrose and Barua 2004). Tobacco smokers showed higher than normal levels of oxidative stress markers in the brain (Ambrose and Barua 2004; Durazzo et al. 2014b), and a higher level of oxidative stress biomarker (F2-isoprostanes) in the cerebrospinal fluid than non-smokers (Durazzo et al. 2014c), as well as depletion of antioxidants (Ambrose and Barua 2004). Typical oxidative responses include lipid peroxidation, which in turn leads to cell membrane damage (Betteridge 2000). In the brain, lipid peroxidation of the vascular endothelial cells contributes to blood-brain barrier damage, a major pathophysiological consequence following tobacco smoking (Mazzone et al. 2010). These oxidative responses may also lead to direct neuronal damage and promote neuroinflammation. Although it is well known that tobacco smoking promotes systematic inflammation (Levitzky et al. 2008; Madani et al. 2018), few clinical studies directly examined neuroinflammation in tobacco smokers.

Proton magnetic resonance spectroscopy (^{1}H-MRS) is a non-invasive technique that could measure the concentrations of brain metabolites, including those that reflect the glial contents in the brain, such as the glial marker myoinositol, and metabolites that are found in much higher levels in glia, including soluble choline-containing compounds (Cho) and total creatine. Although elevated Cho and myoinositol levels are considered markers of glial activation indicating neuroinflammation, they also result from ongoing neural repair (Chang et al. 2013). In tobacco smokers, both higher (Gallinat et al. 2007; Durazzo et al. 2016), lower (Mennecke et al. 2014), or normal (Wheelock et al. 2014) Cho concentrations in the anterior cingulate cortex (ACC) were reported when compared

Table 2 Neuroimaging studies in chronic tobacco smokers

Authors, Journal & year	Participant Characteristics (age, sex, smoking pattern)	Methods / Assessments	Findings
Structural MRI / morphometry			
Brody et al. *Biol Psychiatry* 2004	**19 smokers**: 39 ± 10 years, 42% women; >20 cigarettes/day; average 31 pack-years; FTND = 5.1 ± 1.9; **17 non-smokers**: age = 38 ± 13; 41% women	**Structural MRI**: 1.5 T; ROI analyses: DLPFC, ventrolateral PFC, dorsal and ventral ACC, ventral striatum, and thalamus; Voxel-Based Morphometry	• Smokers had smaller PFC, ACC, and right cerebellum. • Greater smoking pack-year was correlated with lower gray matter densities in PFC.
Durazzo et al. *Drug Alcohol Depend* 2017,2018	**40 smokers**: 22–64 years; 15% women; >10 cigarettes/day; average 26 pack-years; FTND = 5 ± 2; **43 non-smokers**: 22–70 years, 17% women	**Structural MRI**: 4T; FreeSurfer; ROIs in bilateral total subcortical lobar white matter (WM) and sub-cortical nucleus volumes (normalized to ICV)	• Smokers had thinner cortices (PFC, entorhinal, insula, fusiform and middle temporal gyrus) and greater age-related volume reduction in the thalamus, cerebellar cortex, corpus callosum and subcortical lobar white matter compared with non-smokers. • Greater pack-year correlated with smaller amygdala and thinner ACC and middle frontal cortex.
Diffusion tensor imaging (DTI)			
Gons et al. *Brain* 2011	All subjects with small-vessel disease: **149 non-smokers**: 66 ± 9 yrs; 32% men; **275 former smokers**: 66 ± 9 years; 67% male; average 22.6 pack-years; abstinent 22 ± 13 years **75 current smokers**: 64 ± 9 years; 65% men; average 23.5 pack-years	**DTI**: 1.5 T; 30 directions; **White matter volume measurements**	• Adult smokers had larger white matter lesion volumes, higher mean diffusivity (MD) and lower fractional anisotropy (FA) in both normal and abnormal white matter regions compared with non-smokers. • Lower FA and higher MD correlated with poorer cognition. • Former smokers who quit > 20 years had similar white matter FA and MD compared with non-smokers.
Lin et al. *Drug Alcohol Depend* 2013	**34 smokers**: 47 ± 9 years; 79% men; 38 ± 12 cigarettes/day for 26 ± 9 years; FTNC = 8.8 ± 0.7 **34 non-smokers**: 47 ± 7; 82% men	**DTI**: 3T, 12 directions	• Smokers had lower FA and axial diffusivity, and higher radial diffusivity (RD) in the anterior corpus callosum. • Longer smoking duration was correlated with higher RD and MD in the genu and rostral body of corpus callosum.
Proton MR Spectroscopy (^{1}H MRS)			
Janes et al., *Neuropsychopharmacology* 2013	**15 smokers**: 26 ± 5 years; 16 ± 4 cigarettes/day; FTNC = 6.1 ± 1.1	**^{1}H MRS with 2D-JPRESS** 2.5 h after smoking, **Smoking Emotional Stroop task-fMRI**: ~4.5 h after smoking; withdrawal-induced craving; 3T	• Lower GABA in the dorsal ACC in smokers correlated with withdrawal-induced poorer performance on Smoking Emotional Stroop and greater negative affect.
Wheelock et al., *Frontiers Pharmacology* 2014	**22 smokers**: 36 ± 12 years, 54% women; average 17 cigarettes /day. FTND = 5.9 ± 2	**^{1}H MRS PRESS** (Glx/tCr in the dACC) **Stroop Color-Naming task-fMRI**: 3T; both at baseline and 12 weeks after varenicline treatment	• Glx/tCr decreased in the dACC after 12 weeks varenicline treatment. • At baseline, higher Glx/tCr correlated with greater nicotine dependence and craving. Stroop task-related activation decreased in the rostral ACC/mOFC and precuneus after varenicline treatment. • dACC-IPC/r-SOFC connectivity increased and dACC- rostral ACC/mSPFC/MCC/PCC/precuneus connectivity decreased after varenicline treatment.
Durazzo et al., *Biol Psychiatry* 2016	**35 smokers**: 49 ± 12 years, 11% women; averaged 18 cigarettes /day; 27 pack-years; FTND = 5 ± 2	**^{1}H MRS with STEAM**: 4T: Localized voxels: R_DLPFC and ACC for NAA, tCr, mI, Cho and	• Smokers showed lower NAA in the DLPFC and ACC, lower glutamate

Table 2 (continued)

Authors, Journal & year	Participant Characteristics (age, sex, smoking pattern)	Methods / Assessments	Findings
	30 non-smokers: 49 ± 10 years, 13% women	Glu; **Spectroscopic Imaging at 1.5T;** three 15-mm thick parallel slices for NAA, Cho, Cr and mI.	in the DLPFC, higher Cho in the ACC compared with non-smokers. • Smokers showed greater age-related glutamate reduction in the ACC and DLPFC, and greater age-related NAA reduction in the DLPFC. • Longer duration of smoking was related to lower Glu in the ACC and DLPFC. • Higher glutamate and NAA in the DLPFC related to better cognition
Schulte et al., *Drug and Alcohol Depend* 2017	**30 Smokers**: 36 ± 10 years, averaged 155 cigarettes/week, FTND = 6.1 ± 2; **61 Smoker + polydrug**: 33 ± 7 years, 115 cigarettes/week; FTND = 5.8 ± 2 **61 Never-Smokers**: 32 ± 10 years; All men for all groups	^{1}H MRS with MEGA PRESS, **edited for GABA** (J-difference spectra);	• Smokers had higher Glx, and similar GABA, Cho, NAA and Cr in the ACC compared with non-smokers. • No correlation between metabolites levels and drug use pattern and impulsivity
Positron Emission Tomography (PET)			
Domino et al. *Neuroscience* 2000	**11 Smokers**: 34 ± 11 years, all men, average 22.5 ± cigarettes/day	**PET with** ^{18}**[F]fluoro-deoxy-glucose (**^{18}**FDG).** Withheld smoking overnight and immediately after nicotine or pepper spray	• Compared to placebo (pepper), nicotine led to increased glucose metabolism in left IFC, PCC and right thalamus, but decreased glucose metabolism in left insular and right inferior occipital gyrus.
Brody et al. *Am J Psychiatry* 2004	**10 Smokers at satiety**: 37 ± 16 years, 20% women, average 1.5 ± 0.6 packyear. **10 Smokers at withdrawal**: 35 ± 9 years, 30% women, average 1.2 ± 0.4 packyear.	**PET with** ^{11}**[C]raclopride for dopamine D2 receptors.** Both groups smoked a cigarette immediately before the first scan (baseline). Satiety group smoked another cigarette prior to 2nd scan.	• Cigarette smoke decreased binding in the left ventral caudate/putamen/nucleus accumbens. • Decreased binding in above regions correlated with decreased craving on Urge to Smoke Scale.
Zubieta et al. *Am J Psychiatry* 2005	**19 Smokers**: 27 ± 10 years, 58% women, average 16 ± 5 cigarettes/day, FTND = 3.6 ± 2	**PET with** H_2^{15}**O** Withheld smoking for 12 hours and scanned every 12 minutes after provided research cigarette to smoke up to 6 scans.	• Smoking cigarette increased blood flow (rCBF) in visual cortex and cerebellum and decreased in ACC, right hippocampus and nucleus accumbens. After the first cigarette, decreased rCBF correlated decreased FTND score.
Brody et al. *Arch Gen Psychiatry* 2006	**11 Smokers**: 37 ± 12 years, 3 women, average 22 ± 3 cigarettes/day, FTND = 5.9 ± 2.2	**PET with 2-FA for** $\alpha_4\beta_2$ **nAChRs** Withheld smoking for 2 days and scanned at 5 different levels of cigarette smoking.	• Typical daily smokers had $\alpha_4\beta_2$ nAChRs fully occupied throughout the day. Carving scores reduced when $\alpha_4\beta_2$ nAChRs was almost fully occupied.
Durazzo et al., *Nicotine & Tobacco Res* 2016	**31 *APOE*-ε4 + Smokers**: 75 ± 6 years, 52% women; **79 *APOE*ε4- Smokers**: 77 ± 6 years, 41% women; **41 *APOE*ε4 + Never smokers**: 74 ± 8 years, 58% women; **113 *APOE*ε4- Never smokers**: 76 ± 7 years, 55% women; **All smokers**: averaged 26 ± 12 pack-years; 11% were current smokers, formal smokers stopped smoking for 34 ± 12 years	**PET with** ^{18}**Florbetapir and fluorodeoxyglucose (**^{18}**FDG)** ADNI neurocognitive battery (learning, memory and executive function domains)	• Tobacco smoking and *APOE*ε4 carrier status had synergetic lower glucose metabolism, poorer learning and memory function and greater cortical amyloid deposition.
Brody et al. *Psychopharmacology (Berl)* 2017	**30 Smokers**: 52 ± 8 years, 20% women, average 14 ± 4 cigarettes/day, FTND = 4 ± 2.3; **15**	**PET with TSPO tracer** ^{11C}**DAA1106** Smokers at satiety status	• Smokers had less TSPO binding globally, suggested less microglial activation than non-smokers.

Table 2 (continued)

Authors, Journal & year	Participant Characteristics (age, sex, smoking pattern)	Methods / Assessments	Findings
	Non-Smokers: 48 ± 14 years, 26% women		• Smoking more cigarettes per day correlated with lower TSPO binding globally.
Functional MRI (fMRI)			
Durazzo et al. *Addict Biol.* 2015	**34 Smokers**: 47 ± 11 years, 13% women averaged 18 cigarettes/day, 25 pack-years; FTND = 5 ± 2) **27 Never-Smokers**: 47 ± 11 years; 15% women	**Perfusion MRI with arterial spin labeling (ASL)** at 4T	• Perfusion was reduced in smokers compared with non-smokers in regions within frontal, parietal & temporal cortices • Longer duration of smoking negatively correlated with regional perfusion (bilateral and medial OFC, bilateral STG, left cuneus and left frontal pole)
Weiland et al. *Hum Brain Mapp* 2015	**452 Smokers**: 31 ± 9 years, 39% women, average 10.8 ± 7 pack-years **198 Non-smokers**: 30 ± 9 years, 41% women	**Resting-state fMRI (rs-fMRI) at 3T**. Withheld smoking for 2 hours prior to the scan.	• Smoker had reduced connectivity within ECN (to DLPFC and parietal nodes); ECN connectivity strength correlated negatively with pack years of cigarette use.
Lerman et al. *JAMA Psychiatry* 2014	**37 Smokers**; 41 ± 13 years, 51% women, average 16 ± 5 cigarette/day for 24 ± 14 years; FTND = 4.9 ± 1.6	**Resting-state & n-back working memory task fMRI at 3T**. Withheld smoking for 24 hours vs. smoking satiety	• Lower interactions among SAL-ECN-DMN during abstinence than the satiety status. • The lower SAL-ECN-DMN interaction during abstinence in the right hemisphere correlated with greater craving, as well as with less working memory task-induced DMN deactivation.
Pharmacological fMRI			
Kumari et al. *Neuroimage* 2003	**12 non-smokers**: 20–40 years; right-handed; all men.	**Pharmacological task-activated fMRI**; verbal and spatial 3-back task; Single nicotine dose (12 µg/kg body weight, single) or saline injection.	• Nicotine led to increased accuracy at all condition and faster response on 3-back. • Nicotine increased activation in right ACC, SFG and SPG and decreased activation in the right SPG. • Nicotine increased baseline activity in the posterior cingulate, medial occipital lobe, parahippocampal gyrus, cerebellum, and decreased that in the medial prefrontal cortex.
Hong et al. *Arch Gen Psychiatry.* 2009	**19 smokers**: 36 ± 11 years; 5 women; 6 smoked 10–15 cigarettes/day; 13 smoked ≥ 15 cigarettes/day; FTND = 4.3 ± 2.4	**Pharmacological rs-fMRI at 3T**; verbal and spatial 3-back task; Single dose nicotine patch (21 mg for light smoker or 35 mg for heavier smoker) vs. placebo patch	• Dorsal ACC-striatum FC negatively correlated with FTND, which was not affected by nicotine administration. • Nicotine enhanced cingulate-neocortical functional connectivity (SPG, Medial FC).
Lesage et al. *JAMA Psychiatry.* 2017	**24 Smokers**: 36 ± 10 years; 12 women; 18 ± 10.6 years of daily smoking; 17 ± 7.9 cigarettes/day; FTND = 5 ± 1.9 **20 Non-smokers**: 30 ± 7 years; 10 women	**Pharmacological rs-fMRI at 3T**; probabilistic reversal learning task; single nicotine patch, flexible dose for smokers/ repeated dose of varenicline pill/placebo patch or pill	• Smokers had decreased reward sensitivity (vs. nonsmokers) in the dorsal striatum and ACC that were not changed by nicotine or varenicline. • Abstinent smokers showed decreased meso-corticolimbic activity and impaired cognitive flexibility, which were restored to the level of non-smokers on both drugs.
Lesage et al.	**24 Smokers**: 36 ± 10 years; 12 women, 18 ± 10.6 years of daily smoking;	**Pharmacological rs-fMRI at 3T**; Flanker and Go-NoGo task; single	• Smokers performed poorer when not taking nicotine patch; Non-smokers

Table 2 (continued)

Authors, Journal & year	Participant Characteristics (age, sex, smoking pattern)	Methods / Assessments	Findings
Neuropsychopharmacology 2020	17 ± 7.9 cigarettes/day; FTND = 5 ± 1.9; **20 Non-smokers**: 30 ± 7 years; 10 women	dose nicotine patch right before scan with flexible dose for smokers/ repeated dose of varenicline pill/placebo patch or pill	performed poorer on varenicline on Flanker task, performed better on Go-NoGo when given nicotine patch. • Smokers had less activation in right anterior insula and right putamen during correct NoGo inhibition (in both drug and placebo conditions) compared to non-smokers. • Nicotine lowered activation in the dorsal ACC-preSMA during NoGo inhibition among all participants, indicating increased efficiency compared to placebo.

Abbreviations in alphabet order: ACC = anterior cingulate cortex; Cho = choline-containing compounds; DLPFC = dorsal lateral prefrontal cortex; DMN = default mode network; ECN = executive control network; FC = functional connectivity; FTNC = Fagerström Test for Nicotine Dependence; GABA = Gamma aminobutyric acid; Glx/Cr = the ratio of glutamate + glutamine over total creatine; IFC = inferior frontal cortex; IPC = inferior parietal cortex; MCC = middle cingulum; Mofc = medial orbitofrontal cortex; mSPFC = medial superiorfrontal; NAA = N-acetyl aspartate; OFC = occipital frontal cortex; PCC = posterior cingulum; PFC = prefrontal cortex; preSMA = supplementary motor area; SAL = salience network; SPG = superior parietal gyrus; STG = superior temporal gyrus; SOFC = superior occipital cortex.

with non-smokers. However, none of these studies found elevated myoinositol in tobacco smokers. The lower or normal Cho and myoinositol levels in smokers suggest the lack of greater than normal microglial content, which is consistent with findings from two PET studies on tobacco smokers (Brody et al. 2017; Brody et al. 2018). Both of these PET studies used [11C]DAA1106 to measure the translocator protein (TSPO) expression, and both studies found lower TSPO tracer binding globally in chronic tobacco smokers compared to the non-smokers, which indicated less activated microglia in the smokers (Brody et al. 2017). Furthermore, higher levels of smoking correlated with lower TSPO tracer binding levels, which is similar to the lower TSPO binding levels in alcoholdependent individuals with greater alcohol consumption (Hillmer et al. 2017; Kalk et al. 2017; Kim et al. 2018). In addition, lower TSPO tracer binding levels also correlated with blunted peripheral immune response (Hillmer et al. 2017) and poorer cognitive function (Kalk et al. 2017). Therefore, the current interpretation of these findings is that since TSPO is located on microglia, the lower TSPO binding levels might indicate microglial cells loss (Hammoud et al. 2019) or microglial dysfunction (Brody et al. 2017; Brody et al. 2018). These findings from MRS and PET studies suggest that tobacco smokers might have impaired microglial function in the brain, which might reflect decreased repair capacity to neurotoxicity or other forms of brain injury.

Consistent with the brain's decreased capacity for repair, neuronal damage or alterations in brain function were consistently found in tobacco smokers from neuroimaging studies (See Table 2 for selected studies). PET studies with [^{18}F]fluorodeoxyglucose demonstrated that nicotine may lead to both regional increases and regional decreases in glucose metabolism (Domino et al. 2000), and a study that additionally used florbetapir further demonstrated that tobacco smokers with *APOEε*4 carrier-status had even lower glucose metabolism, poorer learning and memory function and greater cortical amyloid deposition (Durazzo et al. 2016). Nicotine also occupies the dopaminergic D2 receptors in the basal ganglia region, leading to decreased binding with [^{11}C]raclopride (Brody et al. 2004b), as well as the $\alpha_4\beta_2$ nAChRs, as shown by decreased binding with 2-[18F]fluoro-3-(2(S)-azetidinylmethoxy) pyridine (or 2-FA) after nicotine administration (Brody et al. 2006). Therefore, smoking may lead to prolonged binding and desensitization of these receptors.

Structural MRI were most widely used to assess brain injury in tobacco smokers. Compared with non-smokers, chronic tobacco smokers showed smaller volumes (Brody et al. 2004a; Almeida et al. 2008; Yu et al. 2011; Kuhn et al. 2012; Liao et al. 2012; Fritz et al. 2014; Stoeckel et al. 2016) or thinner cortices (Karama et al. 2015) in widespread cortical areas, including prefrontal cortex (Brody et al. 2004a; Almeida et al. 2008; Fritz et al. 2014; Li et al. 2015; Hanlon et al. 2016; Durazzo et al. 2018), ACC (Brody et al. 2004a; Liao et al. 2012; Fritz et al. 2014; Li et al. 2015), superior parietal cortex (Almeida et al. 2008), temporal lobe (Li et al. 2015; Durazzo et al. 2018) and insula (Fritz et al. 2014; Li et al. 2015; Hanlon et al. 2016; Stoeckel et al. 2016; Durazzo et al. 2018), as well as subcortical regions, particularly the thalamus (Almeida et al. 2008; Liao et al. 2012; Durazzo et al. 2017), parahippocampus (Li et al. 2015; Hanlon et al.

2016), hippocampus (Durazzo et al. 2013) and cerebellum (Brody et al. 2004a; Yu et al. 2011; Kuhn et al. 2012). The smaller than normal cortical gray matter regions were observed in both younger (Liao et al. 2012; Li et al. 2015) or older (Almeida et al. 2008; Karama et al. 2015), light or heavy smokers, and current or ex-smokers (Karama et al. 2015). Furthermore, tobacco smoking is a known risk factor for arteriosclerosis, which may lead to reduced brain perfusion and white matter hyperintensities or lesions on T2-weighted MRI scans (Zubieta et al. 2005; Power et al. 2015).

In addition to the macrostructural changes reported with structural MRI, microstructural abnormalities can be assessed with diffusion tensor imaging (DTI), which consistently demonstrated brain abnormalities, even in normal-appearing white matter in tobacco smokers (Gons et al. 2011; Lin et al. 2013; Savjani et al. 2014; Viswanath et al. 2015; Baeza-Loya et al. 2016). DTI measures the degree and direction of water diffusion in tissue, which is affected by axonal density, axonal diameter, and the level of myelination in the white matter (Feldman et al. 2010). Adult tobacco smokers consistently showed lower fractional anisotropy (FA) (Gons et al. 2011; Lin et al. 2013; Savjani et al. 2014; Viswanath et al. 2015; Baeza-Loya et al. 2016) and higher water diffusivity (mean diffusion or radial diffusion) (Gons et al. 2011; Lin et al. 2013; Savjani et al. 2014) in the white matter brain regions compared with those measured in non-smokers. Lower FA indicates lesser axonal fiber coherence or fiber loss, while the elevated diffusivities more often reflect neuroinflammation. These white matter abnormalities are widespread in the brain, but most frequently reported in the corpus callosum (Paul et al. 2008b; Lin et al. 2013; Savjani et al. 2014; Viswanath et al. 2015) and frontal brain regions (Liao et al. 2011; Zhang et al. 2011; Huang et al. 2013; Savjani et al. 2014; Baeza-Loya et al. 2016). These DTI findings are consistent with animal studies that showed cigarette smoke exposure led to lipid oxidative injury and myelination impairment in the frontal white matter regions (Nunez et al. 2016; Yu et al. 2016). Furthermore, nicotine-derived nitrosamine ketone (NNK), one of the tobacco-specific nitrosamines caused fiber degeneration and decreased myelinated fiber density in white matter brain regions in rat models (Yalcin et al. 2015; Papp-Peka et al. 2017).

The brain regions that showed structural abnormalities are also critical hubs in functional networks that were abnormal in smokers. Blood-oxygenation-level-dependent (BOLD) functional MRI (fMRI) studies in smokers consistently found reduced connectivity between the ACC and the striatum, which was recognized as a trait for nicotine addiction; the reduced connectivity also correlated with craving and tobacco dependence severity (Hong et al. 2009; Lerman et al. 2014; Lesage et al. 2017). During withdrawal from tobacco, functional connectivity was elevated in both the insula-default mode and insula-amygdala networks, but reduced in the default mode-

executive control network, as well as the insula-executive control network (Jasinska et al. 2014; Weiland et al. 2015; Sutherland and Stein 2018). These functional connectivity changes were associated with withdrawal symptoms such as negative emotion and impaired inhibitory control and cognitive flexibility, but were restored after tobacco/nicotine re-use (Hong et al. 2009; Lesage et al. 2017; Sutherland and Stein 2018).

^{1}H-MRS studies in tobacco smokers also showed evidence of neuronal injury since they had lower levels of the neuronal marker N-acetylaspartate (NAA) in multiple brain regions, including the dorsal lateral prefrontal cortex (DLPFC) (Durazzo et al. 2016), ACC (Durazzo et al. 2016), midbrain (Durazzo et al. 2004), hippocampus (Gallinat et al. 2007), and frontal white matter (Wang et al. 2009). Several studies also found elevated levels of Glx [glutamate (Glu) + glutamine (Gln)] in the ACC (Mason et al. 2006; Wheelock et al. 2014), which appeared to normalize during withdrawal (Mennecke et al. 2014; Wheelock et al. 2014; Schulte et al. 2017). Furthermore, higher Glx levels correlated with more severe nicotine dependency and heavier smoking (Wheelock et al. 2014). Most previous studies only reported Glx levels and were not able to differentiate Glu from Gln, due to their overlapping proton resonances on MRS. However, more recent studies tried to desegregate these resonances and found higher Gln (Gutzeit et al. 2013) but lower Glu (O'Neill et al. 2014; Durazzo et al. 2016) in the cortical gray matter of tobacco smokers compared to non-smokers. In contrast to the elevated Glx and its association with smoking severity, lower Glu levels also correlated with more number of cigarettes smoked/day over the last 30 days and pack-years of smoking (O'Neill et al. 2014), as well as poorer cognitive function (Durazzo et al. 2016). Abnormally elevated Glx or low Glu levels indicate an interrupted Gln-Glu metabolite cycle between astrocytes (e.g., reduced reuptake of Glu) and neurons (e.g., decreased Glu synthesis or release). Future studies using edited ^{1}H-MRS that can separately measure Gln and Glu levels are needed to further clarify the pathophysiology involved in the alterations of these metabolites in tobacco smokers. Cortical γ-aminobutyric acid (GABA) is an important inhibitory neurotransmitter in the brain that can be measured with edited MRS, and was found to be reduced (Mason et al. 2006; Janes et al. 2013) or relatively normal (Schulte et al. 2017) in the cortical regions of smokers. The study that found smokers with reduced GABA levels also reported that those with lower levels also had poorer Smoke-Stroop performance and more smoking cue-induced negative emotional affects (Janes et al. 2013). The mechanisms of low GABA levels in tobacco smokers is unclear but might be due to reduced neuronal GABA synthesis, which declines with age and may lead to aging-related cognitive decline (McQuail et al. 2015). In addition, reduced tobacco use was associated with increased glutathione (antioxidant) levels in the ACC,

suggesting that tobacco cessation may lead to recovery from oxidative stress (Chitty et al. 2015).

A likely neurotoxic outcomes of tobacco smoking is poorer cognitive function. Tobacco smoking is a risk factor for dementia (Ott et al. 1998; Wingbermuhle et al. 2017). Chronic smokers typically showed poorer memory, attention, processing speed, executive functions and greater impulsivity compared to non-smokers (Durazzo et al. 2010; Campos et al. 2016; Chang et al. 2017; Conti et al. 2019), although such cognitive impairments or behavioral deficits may recover with cessation (Gons et al. 2011). Evidence of brain or functional recovery in the abstainers were shown consistently across various neuroimaging studies. For example, current smokers showed smaller brain volumes compared with ex-smokers (Karama et al. 2015; Elbejjani et al. 2019), suggesting recovery from abstinence. The abnormal DTI metrics found in smokers also appeared to improve after prolonged abstinence from smoking (Gons et al. 2011). In addition, the elevated Glx (Mason et al. 2006; Mennecke et al. 2014; Wheelock et al. 2014; Schulte et al. 2017) or glutathione (Chitty et al. 2015) normalized following smoking cessation, while the abnormal frontostriatal functional connectivity also normalized in tobacco smokers after they stopped or reduced smoking (Garrison et al. 2017).

Clinical Studies of Tobacco Use Disorder With Alcohol or Marijuana Co-use

Tobacco smoking is a strong risk factor for drinking behavior and vice versa (Grucza and Bierut 2006; Weinberger et al. 2013). In one study, 63.1% of current alcohol users were also smokers while 61.9% current smokers were also using alcohol (de Silva et al. 2011). Postmortem studies that evaluated alcoholics with and without tobacco smoking, and tobacco smokers without alcohol use, found generalized white matter atrophy and neuronal loss in the prefrontal cortex only in the alcoholic brains, with no independent effect or additional brain volume loss associated with tobacco smoking (McCorkindale et al. 2016; McCorkindale et al. 2019). However, more recent structural MRI studies, using quantitative morphometry, found smaller brain volumes in multiple brain regions in tobacco smokers than in non-smokers, and greater age-related volume losses in alcoholic smokers (Durazzo et al. 2014b). Further, concurrent excessive alcohol use and smoking showed synergistic effect on deficits in time based prospective memory and worse scores on executive function tasks than users of either substance alone (Luhar et al. 2013; Marshall et al. 2016). In addition, after abstinence from alcohol, cortical gray matter perfusion (Mon et al. 2009) and cognition (Durazzo et al. 2014a; Durazzo et al. 2015) improved in non-smokers but not in smokers. Furthermore, greater number of cigarettes smoked per day correlated with lower cortical perfusion even 5 weeks after abstinence from alcohol (Mon et al. 2009). The mechanisms for these

combined effects might be explained partly from rodent model studies. Alcohol and nicotine-derived nitrosamine ketone (NNK) synergistically induced myelinated white matter fiber tract degeneration and interrupted myelin synthesis and regulation (Tong et al. 2015; Yalcin et al. 2015; Papp-Peka et al. 2017). Specifically, NNK seemed to disrupt myelin sheath specifically, whereas ethanol more likely affected axonal structure (Papp-Peka et al. 2017), and co-administration of NNK and ethanol could lead to severe fiber loss (Tong et al. 2015; Yalcin et al. 2015; Papp-Peka et al. 2017).

Another highly co-used substance in tobacco users is marijuana (or cannabis). Between 2002 and 2014, daily cannabis use increased rapidly from 5% to 9%, most notably amongst current smokers (Goodwin et al. 2018); given the legalization of cannabis, greater co-use of tobacco and cannabis will continue to rise. Approximately 70% of adult marijuana users concurrently smoke tobacco (Schauer et al. 2017; Smith et al. 2020). Δ^9-tetrahydrocannabinol (THC) is the main psychoactive component of marijuana (Brumback et al. 2016). THC is a major exogenous cannabinoid CB1 receptor agonist, and CB1 receptors are widely distributed in the brain, with higher densities in the cortex, hippocampus, basal ganglia and cerebellum (Brumback et al. 2016). Chronic marijuana use leads to CB1 receptor downregulation mainly in the cortical regions (Hirvonen et al. 2012). Although tobacco smokers also showed wide spread CB1 receptor downregulation in the brain on a PET study (Hirvonen et al. 2018), no study evaluated the combined effects of tobacco and marijuana use on CB1 receptors to determine whether these individuals have even lower levels of CB1 receptors. In addition, heavy marijuana use appears to up-regulate α4β2* nAChR in tobacco smokers (Brody et al. 2016), and co-users also tended to have lower dopamine transporter availability than tobacco only smokers (Leroy et al. 2012).

Chronic marijuana use was consistently linked to smaller hippocampi (Chye et al. 2017; Lorenzetti et al. 2019), with smaller volumes in those with greater usage (Chye et al. 2017). Co-users of tobacco and marijuana tended to have even smaller right hippocampal volumes compared with those that used only tobacco, only cannabis or non-using healthy controls (Filbey et al. 2015), but no such additive effect on the brain volume was found in other brain regions (Filbey et al. 2015; Wetherill et al. 2015a; Wetherill et al. 2015b). Acute marijuana use may impair learning and attention/working memory (Broyd et al. 2016); however, this effect may be counteracted with concurrent tobacco smoking as shown in an ecological momentary assessment study of working memory (Schuster et al. 2016). Chronic marijuana use is also associated with cognitive deficits, but the deficits are evident only in those with earlier age of onset, during adolescence, and in regular users (Brumback et al. 2016; Meier et al. 2018). Very few studies examined the cognitive outcomes in co-users. Combined adverse effects on cognition were not found in adult co-users of

cannabis and tobacco (Filbey et al. 2015; Schuster et al. 2015). However, adolescent co-users performed poorer in challenging situations, such as tasks with higher cognitive demand or during nicotine withdrawal (Jacobsen et al. 2007).

Independent Effect(s) of HIV on Neurotoxicity and Neuroinflammation

HIV Proteins and Their Neurotoxicity

HIV infects the CD4 cells, macrophages and monocytes in the periphery. The infected monocytes and macrophages traffic to the brain and infect microglia and astrocytes, which in turn release neurotoxic viral proteins, various cytokines and chemokines, along with other neurochemicals that are related to oxidative stress or excitotoxicity, that ultimately cause HAND. Several HIV viral proteins, particularly those expressed in the era of combination antiretroviral therapy, are important contributors for HIV-associated brain injury that may lead to HAND; HIV proteins Tat, gp120, and Nef, and their neurotoxic effects will be discussed (Table 3; Fig. 2).

Tat is one of the three early-encoded HIV-1 proteins, and is critical for HIV-1 gene transcription and replication (Schwartz et al. 1990). Depending on the viral strain, Tat contains 86 to 101 amino acids that are encoded from two separate exons (Jeang 1996). Tat is detected in the HIV-infected brain (Hudson et al. 2000) and is secreted from HIV-infected microglia and astrocytes (He et al. 1997). Multiple studies have documented Tat-mediated neurotoxicity, but our knowledge regarding the cellular and molecular mechanisms for the neurotoxicity are still evolving. Most recent studies used astrocyte-specific doxycycline-inducible HIV-1 Tat transgenic mice (iTat) (Kim et al. 2003). Tat induced lysosomal exocytosis in astrocytes and directly contributed to astrocyte-mediated neurotoxicity (Fan and He 2016a). Tat also increased glial fibrillary acidic protein (GFAP) and its aggregation, activated unfolded protein response (UPR) and endoplasmic reticulum stress (ER) stress, which ultimately contributed to neurotoxicity (Fan and He 2016b). In addition, Tat impaired neurogenesis through notch signaling pathway (Fan et al. 2016). Tat was also found to be secreted by astrocytes in the form of exosomes (Rahimian and He 2016a), which caused shortened neurites and decreased neuron survival through microRNA-132 (Rahimian and He 2016b).

Tat-mediated neurotoxicity also occurs through a myriad of other mechanisms. For instance, Tat disrupted endolysosomal membrane integrity and affected the function of endolysosomes, which also led to neurotoxicity (Hui et al. 2012). Tat contributed to neuroinflammation through direct induction of both IL-6 and IL-8 production (Nookala and Kumar 2014). In addition, Tat increased the expression of purinergic P2Y4 receptor in astrocytes and receptor signaling

to mediate neuroinflammation via PI3K/AKT- and ERK1/2-dependent pathways (Zhou et al. 2019). Furthermore, Tat activated NMDA receptors (Li et al. 2008), through its interaction with the low-density lipoprotein receptor-related protein (Liu et al. 2000). Activation of NMDA receptors caused neuronal cell death, while NMDA antagonist blocked HIV infection-mediated neuronal toxicity (Shin et al. 2012). Chronic Tat expression induced both oxidative stress and neuroinflammation (Guha et al. 2012).

HIV gp120 is also neurotoxic through multiple mechanisms. Even a brief exposure to gp120 caused neuronal apoptosis (Capo-Velez et al. 2018). Dynamin-dependent endocytosis was critical for gp120 neurotoxicity (Wenzel et al. 2017). gp120 impaired neuronal mitochondrial function and distribution (Avdoshina et al. 2016). In addition, gp120 decreased mature BDNF expression and promoted intracellular and extracellular accumulation of pro-BDNF by affecting furin levels, which altered the balance between anti-apoptotic and pro-apoptotic neurotrophins (Bachis et al. 2012). Furthermore, gp120 modulated the expression of pro-inflammatory chemokine IL-8 in astrocytes via NF-κβ pathway (Shah and Kumar 2010). Binding of gp120 to CXCR4 evoked the release of IL-1β in microglia and increased the number of inhibitory synapses (Zhang et al. 2019). Collectively, these studies suggest that gp120 is directly involved in neurotoxicity and neuroinflammation. In gp120 transgenic mice, α7-nAChR were up-regulated on striatal neurons, which led to increases in both intracellular calcium and apoptotic cells (Capo-Velez et al. 2018). The blockade of these events by α7-nAChR antagonists further confirmed that α7-nAChR were involved in gp120-induced neurotoxicity (Capo-Velez et al. 2018).

Nef is a 27–32 kDa early viral protein that down-regulates important immune molecules such as MHC-I (Blagoveshchenskaya et al. 2002) and CD4 receptor (Chen et al. 1996; Poe and Smithgall 2009), and promotes HIV infectivity (Rosa et al. 2015; Usami et al. 2015). In the CNS, Nef is abundantly expressed in HIV-infected brains (Saito et al. 1994; Ranki et al. 1995); therefore, Nef likely contributes to HAND. Ectopic expression of Nef increased CCL5 expression in astrocytes involving activation of PI3K/Akt and p38 MAPK pathways, and transcription factors such as NF-kB, CEBP, and AP-1 (Liu et al. 2014). The increased CCL5 expression then activated microglia to express and release pro-inflammatory cytokines (Skuljec et al. 2011), which further promoted neuroinflammation. Moreover, expression of HIV Nef caused axonal and neurite degeneration, decreased the levels of phosphorylated-Tau but increased the total level of Tau in primary neurons (Sami Saribas et al. 2017). Injection of Nef-transfected astrocytes into the rat hippocampus increased the permeability of blood-brain barrier and up-regulated IL-1β (Rivera et al. 2019). The increased IL-1β also led to inflammatory effects in distal organs including lungs and ileum.

Table 3 Pre-clinical studies showing HIV protein-mediated neurotoxicity and/or neuroinflammation

Author(s)	HIV protein	Model (s) used	Primary Findings
In-Vitro Models			
Rahimian et al., *J Neuroinflammation* 2016; Rahimian et al., *J Neurovirol* 2016	**Tat**	human astrocytoma cells U373.MG and U138.MG	Secreted Tat from astrocytes in exosomes caused shortened neurites and decreased neuronal survival through microRNA-132
Hui et al., *ASN neuro* 2012		Primary cultures of hippocampal neurons embryonic (day 18 Sprague–Dawley rats)	Tat disrupted endo-lysosome membrane integrity and affected functions
Nookala et al., *J Neuroinflammation* 2014		SVG astrocytes	Tat induced both IL-6 and IL-8 production
Zhou et al., *J Neuroinflammation* 2019		primary mouse astrocytes from 0- to 1-day-old C57BL/6 mice	Tat increased the expression of purinergic P2Y4 receptor in astrocytes and increased receptors signaling to mediate neuroinflammation via PI3K/AKT and ERK 1/2 dependent pathway.
Li et al., *J Neurosci* 2008; Liu et al., *Nat Med*.2000; Shin et al., *Br J Pharmacol*2012		SVGA cells and Primary rat hippocampal neuronal cultures	Tat activated NMDA receptor by interacting low-density lipoprotein receptor related proteins. Activation of NMDA receptors led to neuronal cell death.
Fan et al., *J Biol Chem* 2016		Primary astrocytes from HIV-1 Tat transgenic (iTat) mice	Tat induced lysosomal exocytosis in astrocytes and directly contributed to astrocytes-mediated neurotoxicity
Bachis et al., *J Neurosci* 2012	**gp120**	Rat cortical neurons	gp120 decreased mature BDNF expression and promoted accumulation of intracellular and extracellular pro-BDNF.
Shah et al., *J Neuroinflammation* 2010		SVGA astrocytes	Gp120 modulated the expression of IL-8 in astrocytes via NF-Kβ pathway
Zhang et al., J Neurochem 2019		Rat primary hippocampal neurons	Binding of gp120 to CXCR4 led to the release of IL-1β in microglia and increased the number of inhibitory synapses
Liu et al., *Sci Rep* 2014	**Nef**	SVGA astrocytic cell line	Nef induced CCL5 expression in astrocytes through p38-MAPK and PI3K/Akt pathways along with transcription factors NF-kβ, CEBP & AP-1
Sami et al., *Cell Death Dis* 2017		Primary human fetal astrocytes and neurons	Nef caused axonal and neurite degeneration and enhanced total tau protein in primary neurons.
Rivera et al., *PLoS One* 2019		Primary rat astrocytes	Injection of Nef-transfected astrocytes into the rat hippocampus increased the permeability of blood-brain barrier and upregulated IL-1β production
Rodent Models			
Fan et al., *J Biol Chem* 2016	**Tat**	iTat mice	Tat increased GFAP and its aggregation, activated unfolded protein response, and ER stress
Fan et al., *J Biol Chem* 2016		iTat mice	Tat impaired neurogenesis via notch signaling pathway
Wenzel et al., *J Neuroimmune Pharmacol*.2017	**gp120**	Gp120 transgenic mice	Upregulated expression of α7 nAChRs on striatal neurons led to increases in both intracellular calcium and percentage of apoptotic cells
Avdoshima et al., *Neurotox Res.* 2016		Gp120 transgenic mice	gp120 impaired neuronal mitochondrial function and distribution
Chompre et al., *J Neuroinflammation* 2013	**Nef**	Sprague Dawley rats implanted with astrocytes expressing Nef	Nef increased CCL2 expression and infiltration of peripheral macrophages that caused neuronal damage and cognitive impairment
Chompre et al., *Neurobiol Dis.* 2019		Sprague Dawley rats transfused with astrocytes expressing Nef	Nef utilized TGFβ signaling pathway and induced neuroinflammation

Moreover, primary rat astrocytes expressing Nef protein implanted into the hippocampus of rats led to increased chemokine ligand 2 (CCL2) expression that correlated with impaired novel location and novel object recognition, as well as infiltration of peripheral macrophages that eventually caused neuronal damage and cognitive impairment (Chompre et al. 2013), which was shown to involve the TGFβ signaling pathway (Chompre et al. 2019). Therefore, the continued expression of Nef by infected astrocytes, even in the absence of viral replication, could lead to ongoing neuroinflammation and
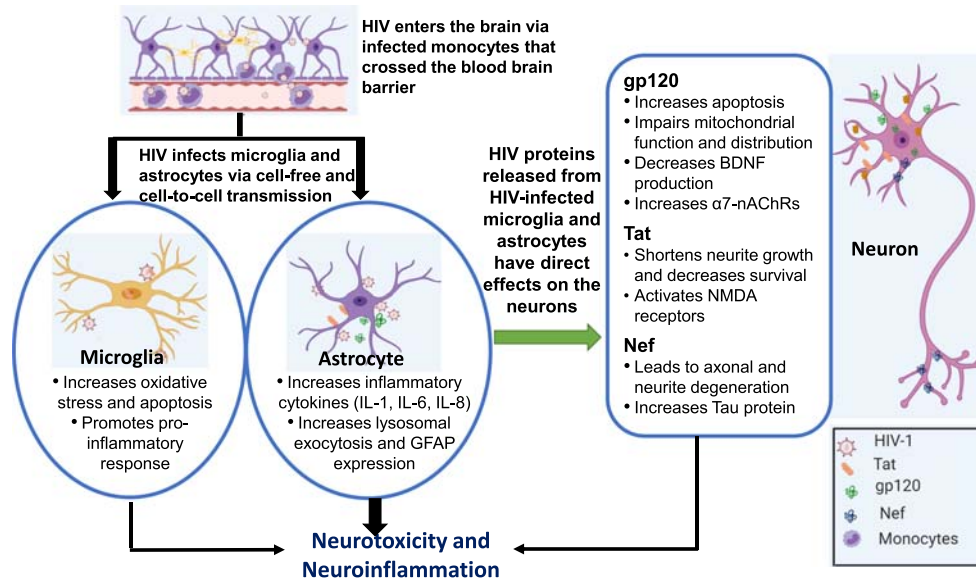
Fig. 2 HIV protein-mediated neurotoxic and neuroinflammatory effects. HIV enters the brain through blood-brain barrier and infects microglia and astrocytes via cell-free and cell-to-cell transmission. Infected microglia and astrocytes then express and secret viral proteins. Tat increases oxidative stress and apoptosis in microglia. Tat also leads to the up-regulation of GFAP, IL-6, IL-8 and causes oxidative stress and lysosomal exocytosis, induces apoptosis and P2Y4 receptors expression in astrocytes. Furthermore, secreted Tat from microglia and astrocytes activates NMDA receptors and shortens neurite growth and decreases neuronal survival. gp120 elevates IL-8 and IL-1 secretion in astrocytes. gp120 also leads to increased apoptosis, impairs mitochondrial function and distribution, decreases BDNF production, and increases α7-nAChR in neurons. Nef up-regulates the production of total tau-protein and leads to the axonal and neurite degeneration in neurons. Images were created with BioRender.com.

eventual neuronal damage and ultimately contribute to HAND.

Taken together, the expression of HIV-1 proteins Tat, gp120 and Nef in infected microglia and astrocytes would result in up-regulated gene expression, increased apoptosis, decreased BDNF production, and increased production of inflammatory cytokines (Fig. 2; Table 3). These combined effects could lead to neurotoxicity and neuroinflammation, and ultimately HAND.

HIV-associated Neuroinflammation and Neurotoxicity - Neuroimaging and Behavioral Studies (See Table 4)

Consistent with the preclinical studies that delineated the neurotoxic and neuroinflammatory effects of HIV viral proteins, HIV+ individuals often demonstrated neuroinflammation (Chang et al. 2002; Paul et al. 2008a), disrupted white matter microstructural integrity (Chang et al. 2008b; O'Connor et al. 2017; Liang et al. 2018) and neuronal damage (Paul et al. 2008a). Postmortem studies in HIV + patients consistently showed evidence of neuronal loss, neuroinflammation with activated microglia and astrocytes, and HIV-related leukoencephalopathy (Vago et al. 2002; Silva et al. 2012). Numerous cognitive and neuroimaging studies in PWH also demonstrated these brain abnormalities, both in patients with or without HAND (Chang and Shukla 2018).

For example, neuroinflammation with glial activation was observed in medication-naïve HIV patients, as well as in PWH maintained on cART, as elevated levels of glial markers on ^{1}H MRS, including Cho (Chang et al. 1999a; Chang et al. 2002; Paul et al. 2008a) or myoinositol (Chang et al. 1999a; Chang et al. 2002) in the frontal lobe or basal ganglia (Chang et al. 1999a; Chang et al. 2002; Paul et al. 2008a; Chang et al. 2014). These elevated MRS glial markers may normalize or improve in HIV patients who were initiated and maintained on cART for 9 months (Chang et al. 1999b). However, elevated MRS glial markers may also reflect ongoing repair since newly diagnosed HIV + patients who were started and maintained on cART showed persistently or even higher levels of total creatine, Cho and myoinositol 3-months later (Chang et al. 2003), and higher Cho compounds were also observed in those with well-controlled viral loads and milder forms of HAND (Alakkas et al. 2019). Furthermore, with prolonged cART, chronically HIV-infected individuals again showed elevated frontal white matter myoinositol that correlated with poorer fine motor function, and worse fluency, especially in those with the $APOE\varepsilon 4$ allele (Chang et al. 2014).

Recent PET studies also demonstrated greater binding of various tracers to TSPO, a marker of microglial activation, in the frontal, temporal, parietal and occipital lobes, and globus pallidus of HIV-infected persons (Coughlin et al. 2014; Vera et al. 2016). Higher TSPO binding in some of the brain regions also correlated with poorer cognitive performance in HIV-infected individuals (Vera et al. 2016;

Table 4 Selected Neuroimaging Studies in HIV + Individuals

Author Journal-Year	Participant Characteristics (Age, Sex, HIV Disease)	Methods / Assessments	Findings
Structural MRI / Morphometry Studies			
Tate et al. *J Neurovirol.* 2011	**216 HIV**: 48 ± 9 years, 88% men, 181 ADC < 0.5/ANI; 35 ADC ≥ 1/MND/HAD; HIV duration = 12 ± 6 years) **139 SN**: 37 ± 14 years, 48% men	**Structural MRI** at 1.5 T; manually traced; width and area of the corpus callosum (CC)	• Symptomatic HIV+ showed reduced width and area across all sub-regions of CC than SN and shorter width in isthmus and genus than asymptomatic group. • No difference between asymptomatic group and SN group • Lower nadir CD4 correlated with smaller area in the posterior middle body of CC; lower CD4 correlated with shorter width in genu, isthmus and splenium of CC. Advanced ADC stage correlated with smaller area in genu and isthmus of CC.
Chang et al. *Neuroimage* 2011	**47 HIV + *APOEε4*-**: 47 ± 1 years, 94% men, 79% on cART; **22 HIV + *APOEε4*+**: 48 ± 3 years, 86% men, 82% on cART; **54 SN *APOEε4*-**: 46 ± 2 years, 91% men; **16 SN *APOEε4*+**: 46 ± 3 years, 81% men	**Structural MRI** at 3T; FreeSurfer	• HIV+ subjects had smaller brain volumes than SN subjects regardless of HAND status, although smaller white matter, amygdala, thalamus and hippocampal volumes did correlate with cognitive deficits. • Only SN subjects with *APOEε4* showed antagonistic pleiotropy effect on cognition and brain volume. • HIV + with *APOEε4* showed premature aging with neurodegeneration.
Guha et al. *J Acquir Immune Defic Syndr* 2016	**146 HIV**: 41 ± 17 years, 55% men; HIV duration = 9 ± 7 years, 84% on cART, 63% viral suppression, HAND status not available **51 SN**: 35 ± 15 years, 45% men	**Structural MRI** at 3T; FreeSurfer (regional volumes were composited into cortical, subcortical and non-specific components); ICV normalized volume, **NPTs** (execution, memory and processing speed)	• HIV had smaller cortical volume and greater age-related volume reduction in the subcortical region than SN individuals. • Age and nadir CD4 predicted cortical volume while age and recent viral load predicted subcortical volumes. • Cortical volume was positively correlated with global cognition.
Sanford et al. *J Acquir Immune Defic Syndr* 2017	**125 HIV**: 47 ± 12 years, 64% men, HIV duration = 11 ± 8 years, 90% on cART, 75% viral suppression, HAND status not available; **62 SN**: 45 ± 12 years, 55% men	**Structural MRI** at 3T; voxel-based morphometry and deformation-based morphometry; **NPTs** (execution, memory and processing speed)	• HIV had thinner cortex, lower subcortical white matter densities and worse cognition compared with SN participants. Worse cognition was correlated with thinner cortex but not subcortical volume. • Lower Nadir CD4 was correlated with smaller subcortical volume and larger 3rd ventricle. • Current CD4, current plasma viral load and cART treatment were not correlated with regional brain volume.
Diffusion Tensor Imaging (DTI) studies			
Chang et al. *J Neuroimmune Pharmacol* 2008	**39 HIV**: 47 ± 2 years, HIV duration = 13 ± 1 years, 56% viral suppression **32 SN**: 47 ± 1 years, participants in both groups were predominantly men.	**DTI** (12 directions) **at baseline and 1-year follow-up; 3T;.NPTs** (all 7 relevant cognitive domains)	• At baseline, HIV showed higher MD in the frontal WM, parietal WM than SN participants. • At 1-year follow-up: HIV showed increased MD in genu CC, but not SN. • Typical age-related increases in MD in genu of CC and age-related increases in FA in putamen were seen only in SN.

Table 4 (continued)

Author Journal-Year	Participant Characteristics (Age, Sex, HIV Disease)	Methods / Assessments	Findings
			• Changes in FA in the frontal WM and putamen, as well as changes in MD in the genu of CC correlated with changes in global cognitive deficit scores.
Pfefferbaum et al. *AIDS* 2009	**42 HIV**: 42 ± 10; 69% men, HIV duration = 8 ± 6 years, 19% viral suppression, cognitive intact **88 SN**: 45 ± 10; 48% men	**DTI** (6 directions) at 1.5 T; FA AD, and RD on major white matter fiber tracts	• Cognitively intact HIV + had higher AD than SN in the posterior CC, internal and external capsules, superior cingulate bundles and was even higher in those with AIDS. • Higher log viral load and self-reported peripheral neuropathy were associated with higher AD.
Ragin et al. *Ann Clin Transl Neurol* 2015	**15 HIV**: 35 ± 11 years, 93% men, HIV duration < 100 days, 8 on cART, **20 SN**: 32 ± 9 years; 80% men	**Structural MRI** at 3T; FreeSurfer ROIs based **DTI** (64 directions), ROIs included cerebral cortex, cerebral white matter, CC, caudate, putamen, thalamus, and hippocampus	• HIV+ had smaller total parenchyma, larger third ventricle and brainstem than SN. • HIV+ had lower FA in the CC and higher MD in the caudate than SN. • Higher CD4 count correlated with higher FA in the CC and higher MD in the caudate. • Higher level of plasma immune markers correlated with smaller parenchyma volume and higher caudate-MD but did not correlated with CC-FA
Su et al. AIDS 2016	**100 HIV**: *49–61 years*, aviraemic ≥ 12 months, 93% men who have sex with men (MSM), HIV duration 13.4 years. 16% cognitively impaired; **70 SN**: *49–59 years*, 87% MSM	**DTI (64 directions)** at 3T; analyzed with tract-based spatial statistics (TBSS)	• HIV+ showed lower average FA, higher average MD in the whole brain and lower FA in widespread white matter regions.
Proton MR Spectroscopy Studies (^{1}H-MRS)			
Ernst et al. *J Magn Reson Imaging* 2010	**27 HIV**: 47 ± 2 years, 96% men, HIV duration = 13 ± 1 years; **18 HIV-cognitive deficits**: 45 ± 3 years, 89% men, HIV duration = 10 ± 1 years; **46 SN**: 43 ± 2 years, 80% men	^{1}H **MRS** TE-averaged PRESS at 3T; Glutamate levels in the frontal GM, frontal WM and basal ganglia; **NPTs**: processing speed, learning, memory, verbal fluency, executive function, attention, and fine motor	• Glutamate levels in the parietal gray matter:.HIV + with cognitive deficit < HIV normal cognition < SN • Lower glutamate levels in the parietal WM, frontal cortex and basal ganglia correlated with poorer processing speed. Lower glutamate in the parietal WM also correlated with poorer memory while lower glutamate the frontal cortex correlated with poorer working memory.
Chang et al. *Neurology* 2014	**80 HIV**: 47.3 6 1.1 years, 91.3% men, 23 APOE e4+ **97 SN**: 44.7 6 1.3 years, 87.6%; men; 28 APOE- e4+	^{1}H **MRS, PRESS** at 3T in four brain regions (ACC, frontal and parietal WM, basal ganglia) **NPTs** assessed all relevant cognitive domains	• HIV+ subjects showed persistent elevation and lack of normal age-dependent increase in myo--inositol, which suggest that persistent glial activation attenuated the typical antagonistic pleiotropic effects of APOEε4 on neuroinflammation. • APOEε4 negatively affects energy metabolism (tCr) in brain regions rich in dopaminergic synapses. • The combined effects of HIV infection and APOEε4 may lead to greater cognitive deficits, especially in those with greater neuroinflammation.

Table 4 (continued)

Author Journal-Year	Participant Characteristics (Age, Sex, HIV Disease)	Methods / Assessments	Findings
Mohamed et al. *AJNR Am J Neuroradiol* 2018	**24 Asymptomatic HIV** (age = 60 ± 6 years, 71% men, HIV duration = 19 ± 9 years); **21 Symptomatic HIV** (age = 58 ± 5 years, 76% men, HIV duration = 19 ± 9 years)	^{1}H MRS with STEAM at 7T: NAA, Glutamate, Cho and mI in the frontal WM, PCC, precuneus, left hippocampus and left basal ganglia, **NPTs**: all relevant cognitive domains	• HIV+ symptomatic group had lower NAA/tCr in the frontal WM, PCC and precuneus as well as lower Glutamate/tCr in the precuneus compared with HIV asymptomatic group. • Lower NAA/Cr in the frontal WM and PCC, and lower Glu/Cr in the frontal WM and the precuneus all correlated with poorer cognition.
Alakkas et al. *J Neurovirol* 2019	253 HIV: 44 ± 8 years, 81% men, 50% viral suppressed, 152 no HAND, 54 ANI, 37 MND, 10 HAD) from CHARTER study.	^{1}H MRS with PRESS at 1.5T (NAA, Cho, MI and Cr in the middle FGM, right FWM and right basal ganglia), sMRI (cortical GM, subcortical GM, abnormal WM and total WM)	• HIV+ with any kind of HAND had smaller cortical and subcortical gray matter volume and more white matter lesion compared with cognitive unimpaired HIV + individuals • ANI and MND had higher Cho in the FWM and HAD and ANI HAD lower NAA in the basal ganglia.

Functional MRI (fMRI) Studies

Author Journal-Year	Participant Characteristics (Age, Sex, HIV Disease)	Methods / Assessments	Findings
Chang et al. *Ann Neurol*. 2004	**18 HIV**: 38 ± 2 years, 4 women (15 on HIV meds; average plasma Log viral load 3.2 ± 0.3; ADC stage 0–1) **18 SN**: 38 ± 2 years, 4 women	**Task-activated fMRI** at 4T; visual attention task with parametric design (tracking 2, 3 or 4 balls)	• HIV showed similar task performance vs. SN • HIV showed decreased activation in brain regions within the visual attention network, but increased activation in adjacent or contralateral brain regions. • Cognitive performance, CD4, and viral load all correlated with activated BOLD signals in brain regions that activated more in HIV subjects.
Ernst et al. *Ann Neurol* 2009	**31 HIV**: 50 ± 2 years; 1 woman; all on HIV meds, **32 SN**: 47 ± 2 years; 4 women; None of the subjects had HAND	**Task-activated fMRI** at 3T; visual attention task (tracking 2,3,4 balls) at baseline and 1-year follow-up. **NPTs** (7 cognitive domains)	• HIV showed increased brain activation while SN showed decreased brain activation on a visual attention task after one-year. These findings indicate decline neural efficiency in the HIV-infected brain since they had to compensate with greater activation to maintain normal cognitive function.
Thomas et al. *Neurology* 2013	**58 HIV**: 41 ± 14 years; 90% men; 44% on cART; 22% with substance use positive (mostly marijuana); **53 SN**: 44 ± 14 years; 51% men	**Resting fMRI** at 3T; two 6-minute; eyes open on fixed cross.	• HIV had decreased FC in DMN, CON, and SAL. • HIV had lower FC in the DMN-DAN, DMN-SAL and CON-SMN, and CON-SAL. • Clinical markers (plasma HIV viral load or CD4(+) cell count) and cognitive impairment did not correlate with FC measures. • Aging correlated with less FC in the DMN, SAL and DMN-SAL. No HIV-by-aging interaction.
Egbert et al. Prog *Neuropsycho-pharmacol Biol Psychiatry* 2019	**54 HIV+**: 25–75 years (all taking cART) **54 SN**: 25–69 years All men, None with HAND	**Resting fMRI** at 3T; 5-mins; eyes open on no target.	• HIV+: both greater or lesser FCs within DMN, FPN, SMN or DMN-SMN.

Table 4 (continued)

Author Journal-Year	Participant Characteristics (Age, Sex, HIV Disease)	Methods / Assessments	Findings
	• Correlations between NPTs and various connectivity networks: positive in HIV+ but negative in SN • HIV had age-related decreases, while SN had age-related increases. in several functional connectivity networks		

Positron Emission Tomography (PET)

Author Journal-Year	Participant Characteristics (Age, Sex, HIV Disease)	Methods / Assessments	Findings
Coughlin et al. *J Neuroviral* 2014	**23 HIV**: 47 ± 7 years, all on cART and with viral suppression. **12 SN**: 40 ± 11 years. Sex information not available.	PET with TSPO tracer [11][C]DPA-713	• HIV+ had higher TSPO binding than SN in temporal, frontal, parietal and cingulate cortex and white matter. • HIV+ with HAD had even higher TSPO binding in frontal and parietal cortex than HIV+ no-HAND • Asymptomatic HIV+ had higher TSPO binding in the temporal, occipital and cingulate cortex and white matter than SN.
Rubin et al. *AIDS* 2018	**21 HIV**: 48 ± 8 years, HIV duration average 14 years, all on cART and with viral suppression.	PET with TSPO tracer [11][C]DPA-713 NPTs (with 7 cognitive domains)	• In general, higher binging correlate with poorer cognition (e.g. higher binging in the middle frontal gyrus correlated with poorer working memory, executive function, verbal memory and motor skill). • However, higher binding in the thalamus correlated with better verbal memory.
Boerwinkle et al. *J Acquir Immune Defic Syndr* 2020	**24 HIV**: 57 ± 6 years, 75% men, HIV duration 18 ± 8 years, all on Cart and with viral suppression. **13 SN**: 58 ± 7 years, 69% men	PET with TSPO tracer [11][C]PBR28 NPTs (processing speed, executive function, learning and memory)	• TSPO binging was similar between HIV and SN in the 30 ROIs. • Among HIV+, higher binding in the frontal, parietal and occipital cortex and thalamus correlated with poorer executive function. Higher binding in the parietal cortex also correlated with poorer global function.
Ances et al. *Neurology* 2010	**10 HIV**: 52 ± 6 years, 45% men, average viral load 2.47 copies/mm³, intact cognition **20 SN**: 47 ± 6 years, 80% men	PET with amyloid tracer [11][C]-Pittsburgh compound B (PiB) CSF Aβ42	• HIV with normal or low CSF Aβ42 had similar ^{11}C-PiB binding compared to SN with normal CSF Aβ42. • SN with low CSF Aβ42 had higher binding level than other groups. • CSF Aβ42 level did not correlate with ^{11}C-PiB binding.
Mohamed et al. *J Neurovirol* 2020	**48 HIV**: 59 ± 5 years, 71% mem, HIV duration 19 ± 9 years, all on Cart, 92% viral suppression, 12 with ANI, 12 with MND and 11 with HAD **25 SN**: 64 ± 8 years, 88% men,	PET with amyloid tracer [18][F] AV-45 (florbetapir) CSF Aβ42 and tau NPTs: (processing speed, executive function, learning and memory, visual construction and memory, and motor skill)	• Global and regional binding level did not different between HIV serostatus and HAND status, except for HIV + had higher binding in the Centrum semiovale than SN. • Among those > 50 years old, HIV+ had higher binding in multiple regions than SN (frontal, parietal, PCC, precuneus, centrum semiovale and putamen).

Table 4 (continued)

Author Journal-Year	Participant Characteristics (Age, Sex, HIV Disease)	Methods / Assessments	Findings
			• Cognitive performance did not correlate with global or regional binding levels. • No difference on CSF Aβ42 and tau levels by HIV serostatus.

Multimodal Imaging

Author Journal-Year	Participant Characteristics (Age, Sex, HIV Disease)	Methods / Assessments	Findings
Cloak et al., *J Neuroimmunol* 2004	**11 HIV**: 35 ± 3 years, 10 men, 6/11 on cART, 1/11 viral suppressed, 9 had HAND, with 2 missing) **14 SN**: 31 ± 3 years 11 men)	**DWI**: mean diffusion; ^{1}**H MRS with PRESS** at 1.5T (NAA, Cr, Cho, mI in the parietal WM); **NPTs** (NPZ-8 scores)	• Compared ot SN, HIV+ individuals had higher mean diffusion and higher levels of Cho and myoinositol in the frontal WM • HIV subjects also had poorer cognitive performance than SN • HIV subjects with higher mean diffusion also had higher myoinositol and poorer cognitive performance
Paul et al. *J Int Neuropsychol Soc* 2008	**22 HIV**: 38 ± 4 years, 19 men (17 with HAND) **20 SN**: 35 ± 10 years, 9 men	^{1}**H MRS with PRESS** (MI/tCr, NAA/tCr, Cho/tCr, NAA/Cho, and NAA/MI in the putamen), **sMRI** (caudate and putamen), **NPTs** (with all relevant cognitive domains)	• HIV+ individuals had lower NAA, higher Cho and smaller volume in the basal ganglia. • Lower NAA was correlated with smaller volume in the putamen. • Both lower NAA and smaller caudate and putamen volume were correlated with poorer cognitive performance.
Vera et al. *Neurology* 2016	**12 HIV** (cognitively healthy): 42 ± 6 years, all men, HIV duration = 15 years, all on cART, all virally suppressed **10 SN**: 41 ± 9 years; all men	**PET with [^{11}C] PBR28** for TSPO; **DTI (68 directions)** at 3T; TBSS **CSF biomarkers** (HIV-RNA, eotaxin, MIP-1β, IL-8 and IL-10); TSPO genotype, **NPTs**: Speed, visual and verbal learning and executive function	• HIV+ had higher TSPO binding in the parietal, occipital lobes and globus pallidus compared with SN group • Among HIV+ individuals, greater binding in the hippocampus, amygdala and thalamus were associated with poorer global cognition. • Among HIV+ individuals, greater binding was correlated with higher mean diffusion in most white matter tracts. • TSPO binding was not correlated with CSF chemokine

Abbreviations: in alphabet order: ADC = AIDS dementia complex; ANI = Asymptomatic neurocognitive disorder; Cho = choline-containing compounds; CON = control network; tCr = total creatine; CSF = cerebrospinal fluid; DAN = dorsal attention; DMN = default mode network; FA = fractional anisotropy; GM = gray matter; HAD, HIV-associated dementia; HAND, HIV associated neuropsychological disorder; ICV, total intracranial volume; MD = mean diffusivity; mI, myo-inositol; MND, mild neurocognitive disorder;; NAA, N-acetyl aspartate; NPTs, neuropsychological tests; ROI = region of interest; SAL, salience network ; SMN, sensorimotor network; SN, HIV seronegative controls; TBSS = tract-based spatial statistics; TSPO = translocator protein; WM, white matter

Rubin et al. 2018; Boerwinkle et al. 2020). In contrast, lower TSPO binding were found in simian immunodeficiency virus (SIVsm804E)-infected macaques, suggesting marked glial loss, along with focal and diffuse microglia activation on postmortem immunostaining, especially in those with high CSF viral loads (Hammoud et al. 2019). Another MRI technique, DTI, can assess neuroinflammation and microstructural abnormalities. Elevated brain diffusivity indicates neuroinflammation while lower FA reflects lesser neuronal integrity on DTI. HIV-infected individuals consistently showed such abnormalities on DTI metrics in the white matter in various brain regions, especially in the larger white matter tracts such as the corpus callosum (Chang et al. 2008b; Pfefferbaum et al. 2009; O'Connor et al. 2017; Liang et al. 2018). The elevated brain diffusivity in HIV-infected persons were observed even in those who were newly infected (Ragin et al. 2015) or aviremic (Su et al. 2016), and correlated with elevated glial marker myoinositol (Cloak et al. 2004), and with poorer cognitive function (Wang et al. 2020), which further indicates that neuroinflammation has a mediating role in HAND.

In addition to the persistent neuroinflammation, cognitive deficits or HAND may also result from neuronal damage, which can be due to the direct neurotoxic effects from the HIV viral proteins (gp120, Tat, Nef), as well as from oxidative stress, glutamate-mediated excitotoxicity and from certain antiretroviral medications. PET studies that evaluated beta-amyloid (Aβ) deposition, using [^{11}C]-Pittsburgh compound B (PiB) (Ances et al. 2010), or [^{18}F] AV-45 (florbetapir) (Mohamed et al. 2020) did not find elevated Aβ binding in HIV+ individuals with or without HAND, suggesting that beta-amyloid did not contribute to HAND in middle aged HIV patients. However, on ^{1}H MRS, lower than normal levels of the neuronal marker NAA were found in the frontal brain regions, basal ganglia (Chang et al. 2002; Paul et al. 2008a; Alakkas et al. 2019) and parietal cortex (Chang et al. 1999a; Mohamed et al. 2018) of HIV patients with HAND, but not those without HAND (Chang et al. 2002). Repeatedly, the abnormally lower NAA levels were reported in antiretroviral-naïve patients with HIV-associated dementia (Chang et al. 2002), as well as cART treated HIV patients with cognitive impairment (Mohamed et al. 2018), and those with HAND (Alakkas et al. 2019).

Another MRS marker to assess neuronal health is the brain glutamate, which was found to be reduced in the frontal and parietal brain regions in HIV+ compared with seronegative individuals, and the lower levels of glutamate also correlated with the more severe cognitive deficits (Ernst et al. 2010; Mohamed et al. 2018). Lower brain glutamate in HIV patients reflects depletion of intracellular glutamate due to several mechanisms. First, reduced re-uptake and recycling of the released glutamate back to the neurons occurred with neuroinflammation, when the excitatory amino acid transporters on the activated astroglia could not reuptake the extracellular glutamate (Zou and Crews 2005). Second, exposure of astrocytes to HIV-1 or the envelope glycoprotein gp120 also reduced their ability to transport glutamate (Wang et al. 2004), which also could lead to excitotoxicity and neuronal injury. Third, both nucleoside reverse transcriptase inhibitors (NRTIs) and oxidized proteins from oxidative stress could lead to mitochondrial toxicity that inhibit glutamate synthesis via the TCA cycle (Dagan et al. 2002). Using an edited MRS technique, brain glutamate levels indeed negatively correlated with the number of NRTIs taken by PWH (Ernst et al. 2010).

Furthermore, evidence for brain injury that might have resulted from either neuroinflammation or neuronal injury were found with brain atrophy on MR morphometry. HIV+ individuals often showed smaller cortical or subcortical volumes (Paul et al. 2008a; Guha et al. 2016; Sanford et al. 2017) as well as smaller white matter volumes, especially in HIV+ patients with the APOEε4 allele (Chang et al. 2011). However, younger HIV+ individuals with normal diffusivity (Tate et al. 2011; Towgood et al. 2012) and HIV+ individuals without cognitive deficits (Chang et al. 2011) may also show similar brain atrophy. Furthermore, in a study that used both MRS and morphometry to assess brain abnormalities in non-demented HIV patients, lower NAA correlated with smaller putamen, and both were associated with poorer cognition (Paul et al. 2008a). Therefore, brain atrophy may correlate with cognitive deficits, but it can occur in PWH with or without cognitive impairment.

A sensitive measure of brain function that reflects the neuronal health of PWH can be derived from BOLD-fMRI studies. BOLD-fMRI consistently showed abnormal brain connectivity at resting-state (rsfMRI) or abnormal brain activation during specific tasks (task-fMRI) in those with HAND, as well as in those without HAND. Specifically, HIV+ individuals showed reduced functional connectivity in cortical networks such as the default mode network (DMN) and the salience network (SAL) (Thomas et al. 2013; Plessis et al. 2014), as well as cortical-subcortical networks (Ortega et al. 2015), even at the resting state. Compared to SN individuals, HIV+ also had lesser between-network connectivity (Thomas et al. 2013; Plessis et al. 2014), and those with lower connectivity had poorer cognitive performance (Egbert et al. 2019). Furthermore, compared to cognitively normal PWH, functional connectivity in those with HAND were even lower (Ann et al. 2016) and decreased faster with aging (Groff et al. 2020). However, clinically stable HIV+ individuals who were able to maintain normal cognitive function over a 30-month period showed no structural brain changes but increased resting functional connectivity in the visual network, frontal-parietal network and the cerebellar network, reflecting a successful neuronal adaptive response to the ongoing HIV-mediated neurotoxicity among those with good clinical outcomes (Correa et al. 2017). Neuronal adaptation to HIV infection is more readily observed in task-fMRI studies, since the tasks require greater neuronal modulation and may be applied in a parametric design to assess the cognitive load effects (i.e., analogous to a stress test). Similar to the adaptive response seen with rsfMRI, HIV+ individuals typically showed greater neuronal activation than SN controls, indicating greater usage of brain reserve to compensate for the brain injury. Therefore, even PWH without HAND show similarly greater BOLD activation on task-activated fMRI (Ernst et al. 2002). On tasks that used a parametric design with increasing attentional load, PWH showed lesser brain activation in the normal network, but greater activation in brain regions that were adjacent or contralateral to brain regions that were activated in seronegative controls (Chang et al. 2001; Chang et al. 2004), and greater activation in those reserve brain regions correlated to better performance (Chang et al. 2004). Moreover, in a longitudinal fMRI study, cognitively normal and stable HIV patients again showed greater activation than SN controls at baseline, as well as increased brain activation when performing the same cognitive task one year later, indicating declined neural efficiency, while SN controls showed decreased activation due to greater efficiency from practice effects (Ernst et al. 2009). These findings demonstrate that BOLD-fMRI is highly sensitive for detecting neuronal injury in PWH, and can demonstrate decline in neuronal function even in those without HAND.

Combined Effect(s) of HIV and Nicotine/tobacco on Brain injury

Although tobacco smokers with PWH typically start smoking cigarettes at an early age, and were addicted to nicotine with an altered or adapted brain system, prior to their HIV infection, pre-clinical studies that evaluated the combined effects of HIV and nicotine/tobacco smoking in the brain typically used the HIV transgenic (Tg) rat models (Table 5). Such preclinical experimental designs and model thus could only evaluate how nicotine might have protected or exacerbated the brain injury associated with the ongoing HIV gene product-mediated neurotoxicity, which began since birth in these animals. The HIV-1 Tg rat was developed using a noninfectious transgene, with a portion of the viral gag-pol region deleted from the infectious HIV-1 plasmid pNL4-3, leading to expression of seven of the nine HIV genes, and similar neuropathological findings in patients with HIV encephalitis (Reid et al. 2001). In HIV-1 Tg rats, nicotine exposure showed neuroprotective effects by blunting the changes in gene expression resulting from signaling pathways (Cao et al. 2013; Li et al. 2013). Major signaling pathways involved were Wnt/β-catenin signaling and ephrin B signaling in the prefrontal cortex, cAMP-responsive element-binding protein (CREB) signaling and glutathione metabolism pathway in dorsal hippocampus, tricarboxylic acid cycle and calcium signaling in the dorsal striatum (Nesil et al. 2015). In addition, chronic nicotine treatment ameliorated the disrupted synaptic plasticity formation and calcium signaling that were mediated by the HIV gene expression in HIV-1 Tg rats (Cao et al. 2016). Furthermore, chronic nicotine treatment decreased α3, β3, β4 nAChR subunits but increased expression of α4 and α6 in the ventral tegmental area of HIV-1 Tg rats

Table 5 Combined effects of nicotine and/or tobacco smoke and HIV infection in the rat brains

Author(s)	Animal Model/ Nicotine Dose administered	Primary Findings
Rodent Model		
Li et al., *PLoS One* 2013; Cao et al., *PLoS One* 2013	HIV-1 Tg Rats Nicotine: 0.25 mg/kg, s.c. twice a day x 17 days	HIV gene expression in HIV Tg rats led to changes in many genes and signaling pathways. Nicotine restored impaired gene expression in this model.
Nesil et al., *Mol Brain* 2015	HIV-1 Tg Rats Nicotine: 0.4 mg/kg, s.c., daily x 15 days	HIV-1 proteins disrupted synaptic plasticity and chronic treatment improves synaptic plasticity.
Cao et al., *J Neurovirol* 2016	HIV-1 Tg Rats Nicotine: 0.4 mg/kg s.c., daily x 27days	Chronic nicotine treatment decreased α3, β3, β4 nAChR subunits but increased the expression of α4 and α6 in the ventral tegmental area of HIV-1 Tg rats.
Yang et al., *Nicotine Tob Res* 2017	HIV-Tg Rats Nicotine: 0.4 mg/kg s.c., daily x 27days	Nicotine significantly modulated the expression of genes in HIV-1 Tg rats that are implicated in drug addiction and novelty-seeking behavior. *Drd3* and *Grm2* in the prefrontal cortex and *Drd5* and *Gabar6* in the ventral tegmental area were significantly upregulated whereas *Drd5* was downregulated in the nucleus accumbens.
Yang et al. *J Neuroimmune Pharmacol* 0.2016	HIV-1 Tg Rats Nicotine: 0.4 mg/kg daily, s.c. x 27days	HIV-1 gene expression affected multiple immune related genes in different regions of HIV-1 Tg rats. Nicotine or HIV-1 Tg both suppressed the expression of some of these genes. However nicotine added to HIV-1 Tg rats led to interactive effect, with greater expression of IL-1 (proinflammatory), but may attenuate the excess expression of Irf7 (apoptotic) in the nucleus accumbens.
Royal W III et al., *J Neurovirol* 2018	HIV-1 Tg Rats Nicotine: 0.5 mg/kg/day, s.c., 5 days a week x 6 weeks. Cigarette Smoke: 2 cigarette (0.7 mg nicotine/cig)/day, 5 days a week x 6 weeks.	HIV-1 Tg rats showed higher expression of pro-inflammatory cytokines IL-1, IL-6, and TNF-α. Exposure of smoke shows worsen effects than nicotine alone in the HIV-1 Tg rats.
Rodent Behavioral Model		
Gonzalez-Lira et al., *Neurosci Lett* 2006	HIVgp120 intra-cerebro- ventricularly treated rats; Nicotine: 1.5 mg/kg, i.p., daily x 5 days	Nicotine prevented HIVgp120-induced equilibrium control deficit on a rotate rod task.
Vigorito et al., *J Neurovirol* 2013	HIV-1 Tg Rats; Nicotine: 0.25 mg/kg s.c., daily x 17 days	Nicotine did not ameliorate the spatial leaning deficit in HIV-1Tg rats.
Nesil et al. *Mol Brain* 2015	HIV-1 Tg Rats; Nicotine: 0.4 mg/kg s.c., daily x 15 days	Nicotine enhanced working memory performance in Y-maze and contextual fear memory in the passive avoidance test in HIV-1Tg rats.

S.C.= subcutaneous injection; i.p.= intraperitoneally injection

(Adinoff 2004; Midde et al. 2011). However, nicotine also significantly enhanced the expression of host genes in HIV-1 Tg rats that were implicated in drug addiction and novelty-seeking behavior (e.g., dopamine D4 receptor gene, DRD5) (Wingo et al. 2016; Yang et al. 2017). Collectively, these pre-clinical studies suggest that nicotine is neuroprotective in the context of HIV gene expression but may increase the addictive behaviors.

Although nicotine showed anti-inflammatory effects in cell cultures and other mouse models (Nizri et al. 2009; Revathikumar et al. 2016), there is no evidence that nicotine suppresses neuroinflammation in HIV animal models. In fact, nicotine modulated the over-expression of multiple immune-related genes that were altered by HIV gene expression in the HIV-1 Tg rats (Yang et al. 2016). Furthermore, nicotine exposure increased inflammatory cytokine gene expression in HIV-1 Tg rats (Royal et al. 2018). Activation of nicotine α7-nAChR activation is critical in the cholinergic anti-inflammatory pathway (Ryan et al. 2001; Wei et al. 2015); both chronic nicotine exposure (Marks et al. 1993; Jasinska et al. 2014) and HIV- gp120 led to upregulated α7- nAChR in peripheral immune cells from humans (Delgado-Velez et al. 2015). However, the upregulated α7-nAChR in immune cells from HIV+ women failed to induce an anti-inflammatory effect in cultured lipopolysaccharide-treated monocyte-derived macrophages (MDM), suggesting that HIV may disrupt the cholinergic-mediated anti-inflammatory response (Delgado-Velez et al. 2015). On the contrary, α7-nAChR antagonist bupropion reduced inflammatory chemokines in those MDM with up-regulated α7-nAChR (Delgado-Velez et al. 2015). Bupropion also improved locomotor impairment in gp120-transgenic mice (Capo-Velez et al. 2018). Furthermore, up-regulation of α7-nAChR led to neuronal cell death by promoting an increase in Ca2 + entry (Ballester et al. 2012; Capo-Velez et al. 2018). In another mouse study, HIV gp120-induced brain amyloid-beta accumulation was found in a α7-nAChR-dependent manner, but was blocked by an α7-nAChR antagonist (Liu et al. 2017). In addition, although nicotine was found to improve performance on a series of learning and behavior tasks, this effect was not observed in HIV-1 Tg rats (Royal et al. 2018). Several studies also showed moderate and inconsistent effects of nicotine on cognitive performance in HIV animal models. For instance, nicotine treatment did not ameliorate the spatial leaning deficit in HIV-1 Tg rats and even slightly worsened their performance (Vigorito et al. 2013). However, nicotine, but not saline, enhanced the contextual memory in HIV-1 Tg rats (Nesil et al. 2015) and prevented HIV gp120-induced motor disturbance when treated simultaneously in rats (Gonzalez-Lira et al. 2006). Furthermore, in these experiments (Vigorito et al. 2013; Nesil et al. 2015), nicotine did not enhance the performance in wild-type rats. Overall, the effects of nicotine administration on neuroinflammation and cognitive function in the HIV animal models remain unclear.

Consistent with the absence of anti-inflammatory effects of nicotine in HIV animal models, tobacco smoke promoted inflammation in the HIV-1 Tg rat model and in PWH (Table 6). In HIV-1 Tg rats, exposure to regular cigarette smoke or nicotine-free cigarette smoke induced higher pro-inflammatory cytokines TNF-α, IL-1, and IL-6 than nicotine alone in the brain lysates (Royal et al. 2018). Similarly, among individuals with HIV infection, tobacco smokers showed higher serum pro-inflammatory chemokines, microglobulin, cyclophilin A, and RANTES, which indicated more systemic immune activation than HIV + non-smokers (Steel et al. 2018). Since tobacco smoke may aggravate neuroinflammation through interrupted BBB integrity (Mazzone et al. 2010) and increased oxidative stress (Ghosh et al. 2009; Ande et al. 2013; Louboutin and Strayer 2014), these mechanisms may be even more evident in HIV+ smokers. However, no data are available regarding the neuroinflammatory cytokine or chemokine levels in relation to BBB integrity or oxidative stress in HIV + tobacco smokers.

Several clinical studies evaluated cognitive function in HIV+ individuals with or without tobacco smoking and found conflicting results. For example, several studies of PWH reported tobacco smoking was associated with worse working memory (Bryant et al. 2013; Harrison et al. 2017), learning (Bryant et al. 2013; Monnig et al. 2016), processing speed (Bryant et al. 2013; Monnig et al. 2016; Harrison et al. 2017), and executive function (Bryant et al. 2013), as well as larger intra-individual variability on response time during the Continuous Performance Task (Harrison et al. 2017), while others reported no effect of tobacco smoking on cognitive decline. (Akhtar-Khaleel et al. 2017; Tsima et al. 2018). A small study even found that tobacco smoking might have beneficial effects on cognition in HIV+ women compared with that in HIV+ men (Wojna et al. 2007; Bryant et al. 2013). However, these previous studies were not designed to evaluate the possible interactive or additive effects between HIV infection and tobacco smoking on cognition (Wojna et al. 2007; Bryant et al. 2013; Monnig et al. 2016; Tsima et al. 2018), since they mixed the current smokers with former smokers (Wojna et al. 2007; Tsima et al. 2018), and lacked seronegative smoker controls or detailed smoking patterns (Akhtar-Khaleel et al. 2017). A structural MRI study found smaller cortical gray matter volumes and poorer cognitive function in the smoking, compared to non-smoking, HIV-seropositive heavy drinkers (Durazzo et al. 2007), suggesting deleterious additive effects of HIV infection and smoking in heavy drinkers on the brain. Another study that also used a 2 × 2 designed to evaluate the independent and combined or interactive effects of tobacco smoking and HIV-serostatus found additive adverse effects on impulsivity, neurocognitive functions and psychopathological symptoms, with HIV+ smokers having the worst cognitive performance and most prevalent psychopathological symptoms among all participant groups (Chang et al. 2017). Furthermore, these HIV+ smokers showed highest brain diffusivity on DTI that indicated additive or synergistic effects on neuroinflammation and disrupted white matter integrity

Table 6 Neuroimaging or behavioral studies on the combined effects of tobacco smoking and HIV infection

Author Journal-Year	Participant Characteristics (age sex, HIV disesae	Smoking pattern	Methods / Assessments	Findings
Durazzo et al. *Alcohol*.2007	**27 HIV + S + Alcohol**: 44 ± 5 years, 89% men, 41% on HAART, 35% viral suppressed; **17 HIV + Alcohol**: 44 ± 5 years, 88%men, 82% on HAART, 35% virally suppressed); **27 SN**: 43 ± 9 years; 82% men	**Smokers**: smoking nearly daily for 6 months prior to the study.	**Structural MRI at** 1.5 T; Customized ROIs (frontal, temporal, parietal, and occipital), lateral ventricles, thalamus, caudate, lenticular nuclei, brainstem, and cerebellum; **NPTs**: Executive skills, fine motor skills, Learning and memory, working memory, visuospatial, postural stability	• HIV-seropositive smokers with heavy drinking had poorer learning, memory and execution, as well as smaller cortical volumes compared with non-smoking seropositive heavy drinkers.
Changet al. *J Neuroimmune Pharmacol* 2017	**22 HIV + S**: 46 ± 11 years, 91% men, HIV duration = 19 ± 9 year, 91% viral suppressed, 91% on cART; 14 with HAND; **25 HIV**: 47 ± 12 years, 96% men, HIV duration = 15 ± 21 year, 76% viral suppressed, 88% on cART; 8 with HAND); **26 SN + S**: 44 ± 9 years, 92% men; 9 with HAND-Equivalent **27 SN**: 44 ± 11 years, 92% men, 6 with HAND-Equivalent	**SN + S**: 18 ± 9 cigarettes/day, 22.3 pack-years; FTND = 4.8 ± 2.4 **HIV + S**: 18 ± 12 cigarettes/day, 22.6 pack--years; FTND = 3.4 ± 2.2	Psychopathological symptoms with **Symptom Check List (SCL-90)**; **NPTs** in 7 domains (processing speed, learning, memory, verbal fluency, executive function, attention, and fine motor function), **Iowa Gambling Test, Wisconsin Card Sorting Test, Barrett Impulsivity Scale**	• HIV and tobacco smoking showed additive adverse effects in all cognitive domains. impulsivity, and all psychopathological symptoms, especially depressive symptoms
Akhtar-Khaleel et al., *J Neurovirol*. 2017	**53 HIV + S; 100 HIV + FS; 65 HIV; 72 SN + S; 172 SN + FS; 120 SN**; all at 50 years old, all men. MACS Cohort Study	Smoking information not available	**NPTs**: Trail Making A, Trail Making B, and Symbol Digit Modalities	• Baseline smoking status did not correlate with cognition decline 5 years later in any participant group.
Liang et al. *J Neuroimmune Pharmacol* 2018	**24 HIV + S**: 45 ± 2 years; 92% men, 11 ± 2 years HIV duration, 96% on cART **25 HIV**: 47 ± 2 years; 100% men, 16 ± 4 years HIV duration; 92% on cART; **26 SN + S**: 44 ± 2 years, 92% men); **25 SN**: 44 ± 2 years, 88% men	**SN + S**: 18 (5–40) cigarettes/ day; 20.1 pack--years **HIV + S**: 16 (3–40) cigarettes/ day 22.8 pack--years	**DTI (12 directions)** at 3T; **NPTs** in 7 domains (Processing speed, learning, memory, verbal fluency, executive function, attention, and fine motor function)	• Additive adverse effects of HIV infection and tobacco smoking on both cognition and on the white matter integrity.

cART = combination antiretroviral therapy; FTND = Fagerstrom Test for Nicotine Dependence; HIV + S = seropositive smokers; HIV + FS = seropositive former smokers; HIV = seropositive non-smokers; SN + S = seronegative smokers; SN + FS = seronegative former smokers; SN = seronegative non-smokers; DTI = diffusion tensor imaging; NPTs = neuropsychological tests

compared to either HIV-seronegative smokers or HIV+ non-smokers, and these white matter microstructure abnormalities predicted poorer verbal fluency, attention and psychomotor speed only in HIV+ smokers (Liang et al. 2018).

Comparing Pre-clinical and Clinical Research Regarding the Interactive or Combined Effects of Nicotine/smoking and HIV on the Brain (Fig. 3; Table 7)

1 *HIV viral proteins in preclinical studies of HIV versus HIV infection in clinical studies.* As can be seen in Table 7, preclinical studies typically evaluated a single HIV viral protein to delineate the neurotoxic effects, which are necessary to elucidate the mechanism(s) or neurotoxicity involved with each of these viral proteins. However, since humans infected with HIV are exposed to the virus with all the viral neurotoxic proteins, as well as the hosts' neuroinflammatory responses to the infection for many years, the chronicity of the infection with the combined neurotoxic exposure is particularly difficult to model from preclinical studies.

2 *Timing of HIV viral protein exposure differs in preclinical versus clinical studies*: HIV-1 transgenic (HIV-1 Tg) rodent model was widely used in recent years to study how HIV-1-infection might impact the brain or other organs, as in the context of nicotine/tobacco exposure. Some investigators posited that since they have ongoing low levels of HIV expression of the viral proteins gp120 and Tat, that the resultant pathology mimics PWH who are taking cART with only plasma viral suppression but have

Table 7 Preclinical versus clinical studies of HIV and nicotine or tobacco use

Points to Consider	Preclinical Studies	Clinical Studies
HIV viral proteins	HIV viral proteins (Tat, gp120, Nef) were always evaluated individually *in vitro* or *in vivo*	Persons with HIV infection (PWH) were exposed to all the HIV neurotoxic viral proteins throughout their infection.
Timing of HIV protein exposure	7 of 9 viral genes are expressed in HIV-1 Tg rats throughout their lifespan	HIV infection is typically sexually transmitted and hence occurs after puberty; few patients with vertical transmission have the infection since birth
Nicotine vs. tobacco smoke	Typically used nicotine administration (s.c., i.p. or oral); only one study used tobacco smoke	Tobacco smoke is inhaled and absorbed through the lungs; tobacco cigarettes contains nicotine plus many other substances that may also be neurotoxic
Other drugs used concurrently	Rarely if ever would preclinical studies assess more than one drug in the model	Polysubstance use is common amongst PWH, especially with cannabis, alcohol and other stimulants
Antiretroviral medications	None of the models reviewed used antiretroviral medications	Almost all clinical studies evaluated PWH who were maintained on cART
Nicotine's cognitive enhancing or anti-depressive effects	Very few studies evaluated cognitive enhancing effects of nicotine in preclinical models	Clinical studies of nicotine's cognitive enhancing or anti-depressive effects have not passed Phase III, and none of the studies were done in PWH

ongoing residual viruses (Han et al. 2018). As described above, these HIV-1 Tg rats expresses HIV neurotoxic proteins throughout their lifespan including the critical periods of brain development. Therefore, it is perhaps a good model for studying the effects of vertical transmission of HIV during birth from their mothers, or to study the effects of HIV on adolescent brain development (Moran et al. 2012). However, this model can be used to show how an

addictive substance such as nicotine or tobacco smoke might further impact the HIV-infected brain. In our previous study of HIV + smokers, the participants had been infected with HIV on average for about 10 years, but had smoked tobacco cigarettes daily for approximately 26 years (Chang et al. 2017). Therefore, animal models to emulate the human condition should use inducible models, such as the gp120 mice that would express
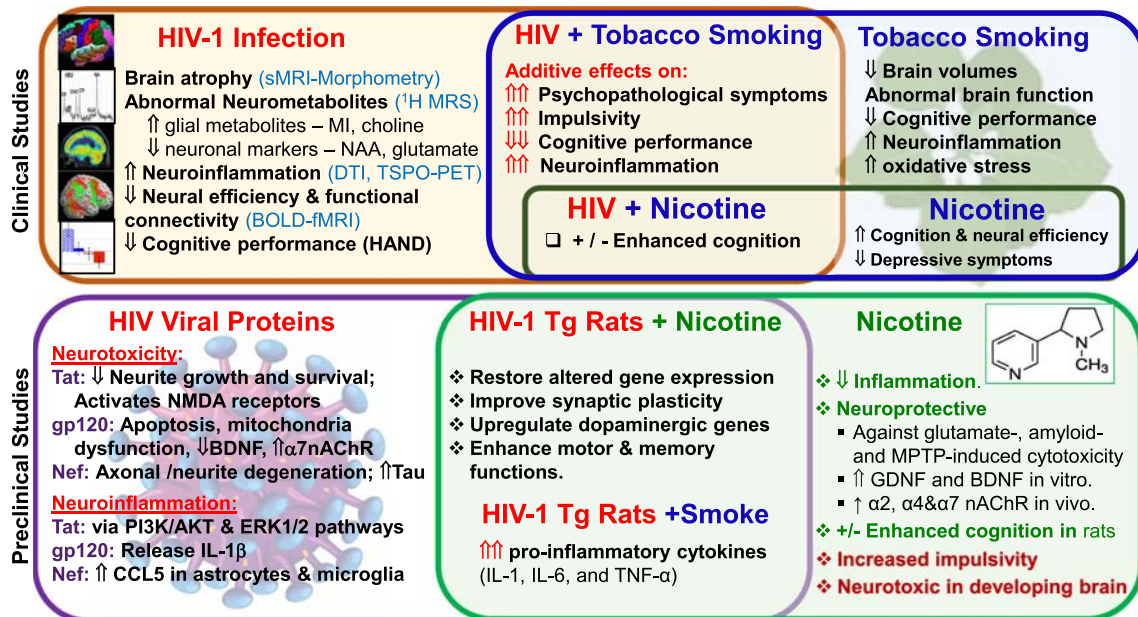

Fig. 3 Independent and combined effects of HIV and tobacco/nicotine. **Left top and bottom panels**: Both clinical studies of HIV infected persons and preclinical studies using viral proteins *in vitro* or *in vivo* in animal models showed HIV led to neurotoxicity and neuroinflammation. **Right top and bottom panels**: While clinical studies of tobacco smoking consistently showed deleterious effects of smoking, clinical and preclinical studies that used nicotine showed mild cognitive enhancement, neuroprotective and possibly anti-inflammatory effects. In the developing brain, however, nicotine is neurotoxic. **Middle overlapping panels**: Clinical studies of persons with HIV who were smokers typically showed additive deleterious effects of HIV and tobacco smoking. However, in the preclinical studies, when nicotine was administered to the HIV-1 Tg rats, the neurotoxic effects of HIV were attenuated, but tobacco smoke worsened the inflammatory cascade

gp120 (Toggas et al. 1994), or HIV-1 Tat transgenic mice (iTat mice) that would express brain-specific Tat protein, only when the animals are treated with doxycycline (Kim et al. 2003; Langford et al. 2018). The iTat mice were used to evaluate the combined or deleterious effects of opiates and cocaine use behavior in PWH, with doxycycline fed simultaneously (Hauser et al. 2009; Fitting et al. 2010) or prior to (Fitting et al. 2012; Paris et al. 2014; Mediouni et al. 2015) the study drug. However, in the human studies, the typical PWH tobacco smokers had been smoking for more than two decades. Therefore, to more accurately model the human condition, future studies should start with a drug self-administration model, prior to exposure of the HIV proteins, after the brain is in a nicotine-dependent or an "addicted" state. Many PWH also started smoking during their adolescent years when the brain is still developing and may be more vulnerable to the toxic or inflammatory effects of tobacco smoking. The vulnerability of the developing brain to neurotoxic substances was also demonstrated in those who smoked cannabis at an earlier age (Hurd et al. 2019). Hence, more studies are needed to evaluate the effects of adolescent tobacco/nicotine use before the additional impact of HIV in the adult brain.

3 **Nicotine administration versus tobacco smoking**: Almost all preclinical *in vitro* or animal model studies used nicotine administration as a model to evaluate the combined effects of HIV and nicotine dependence (i.e., tobacco smoking). In addition, not all nicotine administration evaluated the "chronic" model, such as those that administered the nicotine daily for almost a month. The dosages of nicotine used across the different animal studies also varied widely and should be tested in a systematic manner. As discussed above, the typical preclinical studies found nicotine to have neuroprotective effects, but tobacco smoke led to activation of proinflammatory cascades. Therefore, using tobacco smoke to develop the drug self-administration and addictive model, and subsequently induce the expression of HIV viral protein in the setting of an addicted brain, would be the best approach to evaluate the additional effects of HIV in the brains of chronic tobacco smokers.

4 **Nicotine or tobacco in preclinical studies versus polysubstance use in clinical studies**: Poly-substance use is common amongst PWH, and as reviewed above, marijuana and alcohol are commonly co-used in tobacco smokers and in PWH. These additional substances co-used are additional challenges to both preclinical and clinical research when the goal is to unravel or delineate the reciprocal effects of HIV neurotoxicity or neuroinflammation in the nicotine/tobacco-addicted brain. However, no preclinical study evaluated the combined effects of tobacco with these other commonly co-used substances, with or without HIV.

5 *Additional interactive effects of antiretroviral medications*: Since almost all clinical studies of PWH involved those who are taking antiretroviral medications, which can lead to additional interactive effects with HIV and nicotine/tobacco on brain pathology. These complicated interactions are reviewed in this same Special Issue (Ghura et al. 2019). For instance, nicotine might increase BBB penetration, and therefore increase the neurotoxicity of HIV medications such as NRTIs (Ernst et al. 2010) or HIV protease inhibitors (Manda et al. 2010). Furthermore, HIV infection can enhance nicotine metabolism (Earla et al. 2014; Ashare et al. 2019), interrupt dopaminergic function (Gaskill et al. 2017) and induce reward deficit (Kesby et al. 2016); all of these processes may worsen the tobacco smoking behaviors (Manda et al. 2010). In particular, striatal dysfunction might further increase the potential and severity of substances abuse or addiction, both for nicotine and other substances (Volkow et al. 2017). Whether chronic nicotine/tobacco exposure augment striatal dopaminergic dysfunction in PWH is unclear. A PET study that measured D2 receptors, using [C11]-raclopride, in HIV + patients with or without cocaine use, did find downregulation of the D2 receptors in those who also smoked tobacco cigarettes (Chang et al. 2008a).

6 *Nicotine's cognitive enhancing effects*: To study the nicotine-HIV interaction and clarify the role of nicotine in the context of HIV infection is important since nicotine replacement treatment (NRT) is the main pharmacological treatment for smoking cessation and considered the first line of treatment by providers (Shahrir et al. 2020). Other than NRT, bupropion and varenicline are the second and third most common medications for smoking cessation (Shahrir et al. 2020). However, the long-term effects of these three medications on neuroHIV are unknown. While some suggested nicotine might be useful to treat HAND by lessening the neuroinflammation in people with HIV (Han et al. 2018), others are less optimistic about this treatment approach (Capo-Velez et al. 2018). The few preclinical studies that used the schizophrenia or stress rodent models found nicotine had some cognitive enhancing effects but also led to increased impulsivity. The clinical studies that evaluated the cognitive effects of nicotine were also unclear since there were speed-accuracy trade off in the performance. No clinical study was conducted to evaluate the effects of nicotine in PWH. Therefore, more studies are needed to understand the complex interactive effects between HIV and nicotine/tobacco smoking both in the preclinical conditions, as well as in PWH. Furthermore, dopamine increased HIV replication in human macrophages via activation of dopamine receptors (Gaskill et al. 2014), and it is not known whether nicotine/tobacco smoking also contributed to the high viral

replication observed in HIV + smokers (Ande et al. 2015; Gamarel et al. 2018). Therefore, more preclinical studies are needed to elucidate the impact of chronic nicotine/tobacco on dopamine-mediated neuroinflammation and the cognitive outcomes in HIV transgenic models.

Some Gaps in Knowledge and Future Directions

Preclinical studies consistently demonstrated neuroprotective and anti-inflammatory effects of nicotine, and neurotoxic and neuroinflammatory effects of HIV viral proteins in cell cultures or animal models. These studies are useful for elucidating the individual contributions or mechanisms of nicotine or each of the viral proteins (gp 120, Tat or Nef), and how each component impacted the brain at the neuronal, receptor, specific glial cell responses or gene-expression level. However, the cell culture and the majority of the animal models did not evaluate the effects of tobacco smoking that occur in human chronic tobacco smokers. The *in vitro* studies also did not evaluate the concurrent direct and indirect effects of HIV infection on the brain, which would be exposed to all neurotoxic components of the virus and impacted by the hosts' neuroinflammatory responses from the CNS astroglia and microglia. With the *in vivo* models, HIV-1 Tg rats or the SIV models provided better simulations of the human conditions of either HIV infection or tobacco smoking; however, except for one study (Royal et al. 2018), these studies used nicotine administration rather than tobacco smoke. Therefore, these studies typically found that nicotine restored the altered gene expression, improved synaptic plasticity, restored motor deficits and enhanced memory function in HIV animal models (Fig. 3). In contrast, clinical studies showed clear evidence that chronic tobacco smoking and HIV-infection led to additive effects on cognitive deficits, more prevalent psychopathological symptoms, greater impulsivity, as well as smaller brain volumes and greater white matter microstructural abnormalities that reflected greater neuroinflammation, compared with HIV+ non-smokers. However, whether the greater brain functional and structural abnormalities are due to greater nicotine-mediated dopamine release (Brody et al. 2004b), down-regulated dopaminergic function, or neuroinflammation will need to be evaluated further. Although inflammatory markers in the CSF may reflect neuroinflammation, ^{1}H-MRS and PET with TPSO tracers that assess microglia activation can evaluate regional or global neuroinflammation in the brains of PWH or those with combined HIV and tobacco smoking. Other mechanisms that might lead to cognitive deficits in HIV+ smokers are the potential impacts of chronic tobacco/nicotine exposure and HIV infection on the cholinergic system, which has been studied in animal models but not in humans. Moreover, longitudinal studies are also necessary to monitor and assess the causation between chronic tobacco smoking/nicotine exposure and greater brain injury and higher risk of HAND that are observed in HIV infected toabcco smokers versus non-smokers. Such studies may help guide future preventive approaches or novel therapeutic treatments for HAND.

Compliance with Ethical Standards

Conflict of Interest The authors have no conflicts of interest to disclose.

References

Abreu-Villaca Y, Seidler FJ, Qiao D, Slotkin TA (2005) Modeling the developmental neurotoxicity of nicotine in vitro: cell acquisition, growth and viability in PC12 cells. Brain Res Dev Brain Res 154: 239–246

Adinoff B (2004) Neurobiologic processes in drug reward and addiction. Harv Rev Psychiatry 12:305–320

Akhtar-Khaleel WZ, Cook RL, Shoptaw S, Miller EN, Sacktor N, Surkan PJ, Becker J, Teplin LA, Beyth RJ, Price C, Plankey M (2017) Association of midlife smoking status with change in processing speed and mental flexibility among HIV-seropositive and HIV-seronegative older men: the Multicenter AIDS Cohort Study. J Neurovirol 23:239–249

Alakkas A, Ellis RJ, Watson CW, Umlauf A, Heaton RK, Letendre S, Collier A, Marra C, Clifford DB, Gelman B, Sacktor N, Morgello S, Simpson D, McCutchan JA, Kallianpur A, Gianella S, Marcotte T, Grant I, Fennema-Notestine C, Group C (2019) White matter damage, neuroinflammation, and neuronal integrity in HAND. J Neurovirol 25:32–41

Albuquerque EX, Pereira EFR, Alkondon M, Rogers SW (2009) Mammalian nicotinic acetylcholine receptors: from structure to function. Physiol Rev 89:73–120

Almeida OP, Garrido GJ, Lautenschlager NT, Hulse GK, Jamrozik K, Flicker L (2008) Smoking is associated with reduced cortical regional gray matter density in brain regions associated with incipient Alzheimer disease. Am J Geriatr Psychiatry 16:92–98

Ambrose JA, Barua RS (2004) The pathophysiology of cigarette smoking and cardiovascular disease: an update. J Am Coll Cardiol 43:1731–1737

Amitai N, Markou A (2009) Chronic nicotine improves cognitive performance in a test of attention but does not attenuate cognitive disruption induced by repeated phencyclidine administration. Psychopharmacology 202:275

Ances B, Christensen J, Teshome M, Taylor J, Xiong C, Aldea P, Fagan A, Holtzman D, Morris J, Mintun M (2010) Cognitively unimpaired HIV-positive subjects do not have increased 11C-PiB: a case-control study. Neurology 75:111–115

Ande A, McArthur C, Kumar A, Kumar S (2013) Tobacco smoking effect on HIV-1 pathogenesis: role of cytochrome P450 isozymes. Expert Opin Drug Metab Toxicol 9:1453–1464

Ande A, McArthur C, Ayuk L, Awasom C, Achu PN, Njinda A, Sinha N, Rao PS, Agudelo M, Nookala AR, Simon S, Kumar A, Kumar S (2015) Effect of mild-to-moderate smoking on viral load, cytokines,

oxidative stress, and cytochrome P450 enzymes in HIV-infected individuals. PLoS One 10:e0122402

Ann HW, Jun S, Shin NY, Han S, Ahn JY, Ahn MY, Jeon YD, Jung IY, Kim MH, Jeong WY, Ku NS, Kim JM, Smith DM, Choi JY (2016) Characteristics of resting-state functional connectivity in HIV-associated neurocognitive disorder. PLoS One 11:e0153493

Ashare RL, Thompson M, Leone F, Metzger D, Gross R, Mounzer K, Tyndale RF, Lerman C, Mahoney MC, Cinciripini P, George TP, Collman RG, Schnoll R (2019) Differences in the rate of nicotine metabolism among smokers with and without HIV. AIDS 33:1083–1088

Avdoshina V, Fields JA, Castellano P, Dedoni S, Palchik G, Trejo M, Adame A, Rockenstein E, Eugenin E, Masliah E, Mocchetti I (2016) The HIV protein gp120 alters mitochondrial dynamics in neurons. Neurotox Res 29:583–593

Bachis A, Avdoshina V, Zecca L, Parsadanian M, Mocchetti I (2012) Human immunodeficiency virus type 1 alters brain-derived neurotrophic factor processing in neurons. J Neurosci 32:9477–9484

Baeza-Loya S, Velasquez KM, Molfese DL, Viswanath H, Curtis KN, Thompson-Lake DG, Baldwin PR, Ellmore TM, De La Garza R II, Salas R (2016) Anterior cingulum white matter is altered in tobacco smokers. Am J Addict 25:210–214

Ballester LY, Capo-Velez CM, Garcia-Beltran WF, Ramos FM, Vazquez-Rosa E, Rios R, Mercado JR, Melendez RI, Lasalde-Dominicci JA (2012) Up-regulation of the neuronal nicotinic receptor alpha7 by HIV glycoprotein 120: potential implications for HIV-associated neurocognitive disorder. J Biol Chem 287:3079–3086

Barreto GE, Iarkov A, Moran VE (2015) Beneficial effects of nicotine, cotinine and its metabolites as potential agents for Parkinson's disease. Front Aging Neurosci 6:340

Belluardo N, Olsson PA, Mudo G, Sommer WH, Amato G, Fuxe K (2005) Transcription factor gene expression profiling after acute intermittent nicotine treatment in the rat cerebral cortex. Neuroscience 133:787–796

Benowitz NL (2015) Nicotine Addiction. N Engl J Med 362:2295–2303

Bentley P, Driver J, Dolan RJ (2011) Cholinergic modulation of cognition: insights from human pharmacological functional neuroimaging. Prog Neurobiol 94:360–388

Bertrand D, Terry AV Jr (2018) The wonderland of neuronal nicotinic acetylcholine receptors. Biochem Pharmacol 151:214–225

Betteridge DJ (2000) What is oxidative stress? Metabolism 49:3–8

Blagoveshchenskaya AD, Thomas L, Feliciangeli SF, Hung CH, Thomas G (2002) HIV-1 Nef downregulates MHC-I by a PACS-1- and PI3K-regulated ARF6 endocytic pathway. Cell 111:853–866

Boerwinkle AH, Strain JF, Burdo T, Doyle J, Christensen J, Su Y, Wisch JK, Cooley SA, Vaida F, Smith MD, Jafri H, Paul RH, Benzinger TLS, Ances BM (2020) Comparison of [11C]-PBR28 binding between persons living with HIV and HIV-uninfected individuals. J Acquir Immune Defic Syndr 85:244–251

Bordia T, Parameswaran N, Fan H, Langston JW, McIntosh JM, Quik M (2006) Partial recovery of striatal nicotinic receptors in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned monkeys with chronic oral nicotine. J Pharmacol Exp Ther 319:285–292

Bradford ST, Stamatovic SM, Dondeti RS, Keep RF, Andjelkovic AV (2011) Nicotine aggravates the brain postischemic inflammatory response. Am J Physiol Heart Circ Physiol 300:H1518–H1529

Brody AL, Hubert R, Mamoun MS, Enoki R, Garcia LY, Abraham P, Young P, Mandelkern MA (2016) Nicotinic acetylcholine receptor availability in cigarette smokers: effect of heavy caffeine or marijuana use. Psychopharmacology 233:3249–3257

Brody AL, Mandelkern MA, Jarvik ME, Lee GS, Smith EC, Huang JC, Bota RG, Bartzokis G, London ED (2004a) Differences between smokers and nonsmokers in regional gray matter volumes and densities. Biol Psychiatry 55:77–84

Brody AL, Olmstead RE, London ED, Farahi J, Meyer JH, Grossman P, Lee GS, Huang J, Hahn EL, Mandelkern MA (2004b) Smoking-induced ventral striatum dopamine release. Am J Psychiatry 161:1211–1218

Brody AL, Mandelkern MA, London ED, Olmstead RE, Farahi J, Scheibal D, Jou J, Allen V, Tiongson E, Chefer SI (2006) Cigarette smoking saturates brain $\alpha4\beta2$ nicotinic acetylcholine receptors. Arch Gen Psychiatry 63:907–914

Brody AL, Hubert R, Enoki R, Garcia LY, Mamoun MS, Okita K, London ED, Nurmi EL, Seaman LC, Mandelkern MA (2017) Effect of cigarette smoking on a marker for neuroinflammation: A [(11)C]DAA1106 positron emission tomography study. Neuropsychopharmacology 42:1630–1639

Brody AL, Gehlbach D, Garcia LY, Enoki R, Hoh C, Vera D, Kotta KK, London ED, Okita K, Nurmi EL, Seaman LC, Mandelkern MA (2018) Effect of overnight smoking abstinence on a marker for microglial activation: a [(11)C]DAA1106 positron emission tomography study. Psychopharmacology 235:3525–3534

Broyd SJ, van Hell HH, Beale C, Yucel M, Solowij N (2016) Acute and chronic effects of cannabinoids on human cognition-A systematic review. Biol Psychiatry 79:557–567

Brumback T, Castro N, Jacobus J, Tapert S (2016) Effects of marijuana use on brain structure and function: Neuroimaging findings from a neurodevelopmental perspective. Int Rev Neurobiol 129:33–65

Bryant VE, Kahler CW, Devlin KN, Monti PM, Cohen RA (2013) The effects of cigarette smoking on learning and memory performance among people living with HIV/AIDS. AIDS Care 25:1308–1316

Campos MW, Serebrisky D, Castaldelli-Maia JM (2016) Smoking and cognition. Curr Drug Abuse Rev 9:76–79

Cao J, Nesil T, Wang S, Chang SL, Li MD (2016) Expression profile of nicotinic acetylcholine receptor subunits in the brain of HIV-1 transgenic rats given chronic nicotine treatment. J Neurovirol 22:626–633

Cao J, Wang S, Wang J, Cui W, Nesil T, Vigorito M, Chang SL, Li MD (2013) RNA deep sequencing analysis reveals that nicotine restores impaired gene expression by viral proteins in the brains of HIV-1 transgenic rats. PLoS One 8:e68517

Capo-Velez CM, Morales-Vargas B, Garcia-Gonzalez A, Grajales-Reyes JG, Delgado-Velez M, Madera B, Baez-Pagan CA, Quesada O, Lasalde-Dominicci JA (2018) The alpha7-nicotinic receptor contributes to gp120-induced neurotoxicity: implications in HIV-associated neurocognitive disorders. Sci Rep 8:1829

Centers for Disease Control and Prevention (2018) Behavioral and Clinical Characteristics of Persons with Diagnosed HIV Infection—Medical Monitoring Project, United States, 2010 Cycle to 2015 Cycle. HIV Surveillance Special Report 9, 12, 16, 17 and 20. In, May 2018 Edition

Chang L, Shukla DK (2018) Imaging studies of the HIV-infected brain. In: Handbook of Clinical Neurology, volume 152 (3rd series) The Neurology of HIV infection, 3rd edn (Brew BJ, ed). Elsevier, Amsterdam, pp 229–264

Chang L, Munsaka SM, Kraft-Terry S, Ernst T (2013) Magnetic resonance spectroscopy to assess neuroinflammation and neuropathic pain. J Neuroimmune Pharmacol 8:576–593

Chang L, Lim A, Lau E, Alicata D (2017) Chronic tobacco-smoking on psychopathological symptoms, impulsivity and cognitive deficits in HIV-infected individuals. J Neuroimmune Pharmacol 12:389–401

Chang L, Ernst T, Leonido-Yee M, Walot I, Singer E (1999a) Cerebral metabolite abnormalities correlate with clinical severity of HIV-1 cognitive motor complex. Neurology 52:100–108

Chang L, Ernst T, Witt MD, Ames N, Gaiefsky M, Miller E (2002) Relationships among brain metabolites, cognitive function, and viral loads in antiretroviral-naive HIV patients. Neuroimage 17:1638–1648

Chang L, Cloak CC, Jiang CS, Hoo A, Hernandez AB, Ernst TM (2012) Lower glial metabolite levels in brains of young children with prenatal nicotine exposure. J Neuroimmune Pharmacol 7:243–252

Chang L, Ernst T, Leonido-Yee M, Witt M, Speck O, Walot I, Miller EN (1999b) Highly active antiretroviral therapy reverses brain metabolite abnormalities in mild HIV dementia. Neurology 53:782–789

Chang L, Tomasi D, Yakupov R, Lozar C, Arnold S, Caparelli E, Ernst T (2004) Adaptation of the attention network in human immunodeficiency virus brain injury. Ann Neurol 56:259–272

Chang L, Wang G-J, Volkow ND, Ernst T, Telang F, Logan J, Fowler JS (2008a) Decreased brain dopamine transporters are related to cognitive deficits in HIV patients with or without cocaine abuse. Neuroimage 42:869–878

Chang L, Andres M, Sadino J, Jiang CS, Nakama H, Miller E, Ernst T (2011) Impact of apolipoprotein E epsilon4 and HIV on cognition and brain atrophy: antagonistic pleiotropy and premature brain aging. Neuroimage 58:1017–1027

Chang L, Jiang C, Cunningham E, Buchthal S, Douet V, Andres M, Ernst T (2014) Effects of APOE epsilon4, age, and HIV on glial metabolites and cognitive deficits. Neurology 82:2213–2222

Chang L, Speck O, Miller EN, Braun J, Jovicich J, Koch C, Itti L, Ernst T (2001) Neural correlates of attention and working memory deficits in HIV patients. Neurology 57:1001–1007

Chang L, Wong V, Nakama H, Watters M, Ramones D, Miller EN, Cloak C, Ernst T (2008b) Greater than age-related changes in brain diffusion of HIV patients after 1 year. J Neuroimmune Pharmacol 3:265–274

Chang L, Ernst T, Witt MD, Ames N, Walot I, Jovicich J, DeSilva M, Trivedi N, Speck O, Miller EN (2003) Persistent brain abnormalities in antiretroviral-naive HIV patients 3 months after HAART. Antivir Ther 8:17–26

Chang L, Oishi K, Skranes J, Buchthal S, Cunningham E, Yamakawa R, Hayama S, Jiang CS, Alicata D, Hernandez A (2016) Sex-specific alterations of white matter developmental trajectories in infants with prenatal exposure to methamphetamine and tobacco. JAMA Psychiatry 73:1217–1227

Chen BK, Gandhi RT, Baltimore D (1996) CD4 down-modulation during infection of human T cells with human immunodeficiency virus type 1 involves independent activities of vpu, env, and nef. J Virol 70:6044–6053

Chen Y, Nie H, Tian L, Tong L, Yang L, Lao N, Dong H, Sang H, Xiong L (2013) Nicotine-induced neuroprotection against ischemic injury involves activation of endocannabinoid system in rats. Neurochem Res 38:364–370

Chitty KM, Lagopoulos J, Hickie IB, Hermens DF (2015) A longitudinal proton magnetic resonance spectroscopy study investigating oxidative stress as a result of alcohol and tobacco use in youth with bipolar disorder. J Affect Disord 175:481–487

Chompre G, Martinez-Orengo N, Cruz M, Porter JT, Noel RJ (2019) TGFβRI antagonist inhibits HIV-1 Nef-induced CC chemokine family ligand 2 (CCL2) in the brain and prevents spatial learning impairment. J Neuroinflammation 16:262

Chompre G, Cruz E, Maldonado L, Rivera-Amill V, Porter JT, Noel RJ (2013) Astrocytic expression of HIV-1 Nef impairs spatial and recognition memory. Neurobiol Dis 49:128–136

Chye Y, Suo C, Yucel M, den Ouden L, Solowij N, Lorenzetti V (2017) Cannabis-related hippocampal volumetric abnormalities specific to subregions in dependent users. Psychopharmacology 234:2149–2157

Clifford DB (2017) HIV-associated neurocognitive disorder. Curr Opin Infect Dis 30:117–122

Cloak CC, Chang L, Ernst T (2004) Increased frontal white matter diffusion is associated with glial metabolites and psychomotor slowing in HIV. J Neuroimmunol 157:147–152

Conti AA, McLean L, Tolomeo S, Steele JD, Baldacchino A (2019) Chronic tobacco smoking and neuropsychological impairments: A systematic review and meta-analysis. Neurosci Biobehav Rev 96:143–154

Correa DG, Zimmermann N, Ventura N, Tukamoto G, Doring T, Leite SC, Fonseca RP, Bahia PR, Lopes FC, Gasparetto EL (2017) Longitudinal evaluation of resting-state connectivity, white matter integrity and cortical thickness in stable HIV infection: Preliminary results. Neuroradiol J 30:535–545

Coughlin JM, Wang Y, Ma S, Yue C, Kim PK, Adams AV, Roosa HV, Gage KL, Stathis M, Rais R (2014) Regional brain distribution of translocator protein using [11 C] DPA-713 PET in individuals infected with HIV. J Neurovirol 20:219–232

Dagan T, Sable C, Bray J, Gerschenson M (2002) Mitochondrial dysfunction and antiretroviral nucleoside analog toxicities: what is the evidence? Mitochondrion 1:397–412

Dajas-Bailador FA, Lima PA, Wonnacott S (2000) The alpha7 nicotinic acetylcholine receptor subtype mediates nicotine protection against NMDA excitotoxicity in primary hippocampal cultures through a Ca(2+) dependent mechanism. Neuropharmacology 39:2799–2807

Dani JA (2015) Neuronal nicotinic acetylcholine receptor structure and function and response to nicotine. Int Rev Neurobiol 124:3–19

de Silva V, Samarasinghe D, Hanwella R (2011) Association between concurrent alcohol and tobacco use and poverty. Drug Alcohol Rev 30:69–73

Delgado-Velez M, Baez-Pagan CA, Gerena Y, Quesada O, Santiago-Perez LI, Capo-Velez CM, Wojna V, Melendez L, Leon-Rivera R, Silva W, Lasalde-Dominicci JA (2015) The alpha7-nicotinic receptor is upregulated in immune cells from HIV-seropositive women: consequences to the cholinergic anti-inflammatory response. Clin Transl Immunol 4:e53

Domino E, Minoshima S, Guthrie S, Ohl L, Ni L, Koeppe R, Cross D, Zubieta J-K (2000) Effects of nicotine on regional cerebral glucose metabolism in awake resting tobacco smokers. Neuroscience 101:277–282

Durazzo TC, Meyerhoff DJ, Nixon SJ (2010) Chronic cigarette smoking: implications for neurocognition and brain neurobiology. Int J Environ Res Public Health 7:3760–3791

Durazzo TC, Meyerhoff DJ, Nixon SJ (2013) Interactive effects of chronic cigarette smoking and age on hippocampal volumes. Drug Alcohol Depend 133:704–711

Durazzo TC, Meyerhoff DJ, Yoder KK (2018) Cigarette smoking is associated with cortical thinning in anterior frontal regions, insula and regions showing atrophy in early Alzheimer's Disease. Drug Alcohol Depend 192:277–284

Durazzo TC, Gazdzinski S, Banys P, Meyerhoff DJ (2004) Cigarette smoking exacerbates chronic alcohol-induced brain damage: a preliminary metabolite imaging study. Alcohol Clin Exp Res 28:1849–1860

Durazzo TC, Pennington DL, Schmidt TP, Meyerhoff DJ (2014a) Effects of cigarette smoking history on neurocognitive recovery over 8 months of abstinence in alcohol-dependent individuals. Alcohol Clin Exp Res 38:2816–2825

Durazzo TC, Meyerhoff DJ, Yoder KK, Murray DE (2017) Cigarette smoking is associated with amplified age-related volume loss in subcortical brain regions. Drug Alcohol Depend 177:228–236

Durazzo TC, Mon A, Gazdzinski S, Yeh PH, Meyerhoff DJ (2015) Serial longitudinal magnetic resonance imaging data indicate non-linear regional gray matter volume recovery in abstinent alcohol-dependent individuals. Addict Biol 20:956–967

Durazzo TC, Rothlind JC, Cardenas VA, Studholme C, Weiner MW, Meyerhoff DJ (2007) Chronic cigarette smoking and heavy drinking in human immunodeficiency virus: consequences for neurocognition and brain morphology. Alcohol 41:489–501

Durazzo TC, Mon A, Pennington D, Abé C, Gazdzinski S, Meyerhoff DJ (2014b) Interactive effects of chronic cigarette smoking and age on brain volumes in controls and alcohol-dependent individuals in early abstinence. Addict Biol 19:132–143

Durazzo TC, Meyerhoff DJ, Mon A, Abe C, Gazdzinski S, Murray DE (2016) Chronic cigarette smoking in healthy middle-aged

individuals is associated with decreased regional brain N-acetylaspartate and glutamate levels. Biol Psychiatry 79:481–488

Durazzo TC, Mattsson N, Weiner MW, Korecka M, Trojanowski JQ, Shaw LM, Alzheimer's Disease Neuroimaging I (2014c) History of cigarette smoking in cognitively-normal elders is associated with elevated cerebrospinal fluid biomarkers of oxidative stress. Drug Alcohol Depend 142:262–268

Dwyer JB, McQuown SC, Leslie FM (2009) The dynamic effects of nicotine on the developing brain. Pharmacol Ther 122:125–139

Earla R, Ande A, McArthur C, Kumar A, Kumar S (2014) Enhanced nicotine metabolism in HIV-1-positive smokers compared with HIV-negative smokers: simultaneous determination of nicotine and its four metabolites in their plasma using a simple and sensitive electrospray ionization liquid chromatography-tandem mass spectrometry technique. Drug Metab Dispos 42:282–293

Egbert AR, Biswal B, Karunakaran KD, Pluta A, Wolak T, Rao S, Bornstein R, Szymanska B, Horban A, Firlag-Burkacka E, Sobanska M, Gawron N, Bienkowski P, Sienkiewicz-Jarosz H, Scinska-Bienkowska A, Lojek E (2019) HIV infection across aging: Synergistic effects on intrinsic functional connectivity of the brain. Prog Neuropsychopharmacol Biol Psychiatry 88:19–30

Egea J, Rosa AO, Sobrado M, Gandía L, López MG, García AG (2007) Neuroprotection afforded by nicotine against oxygen and glucose deprivation in hippocampal slices is lost in alpha7 nicotinic receptor knockout mice. Neuroscience 145:866–872

Elbejjani M, Auer R, Jacobs DR Jr, Haight T, Davatzikos C, Goff DC Jr, Bryan RN, Launer LJ (2019) Cigarette smoking and gray matter brain volumes in middle age adults: the CARDIA Brain MRI substudy. Transl Psychiatry 9:78

Eriksson P (1997) Developmental neurotoxicity of environmental agents in the neonate. Neurotoxicology 18:719–726

Ernst T, Chang L, Jovicich J, Ames N, Arnold S (2002) Abnormal brain activation on functional MRI in cognitively asymptomatic HIV patients. Neurology 59:1343–1349

Ernst T, Jiang CS, Nakama H, Buchthal S, Chang L (2010) Lower brain glutamate is associated with cognitive deficits in HIV patients: A new mechanism for HIV-associated neurocognitive disorder. J Magn Reson Imaging 32:1045–1053

Ernst T, Yakupov R, Nakama H, Crocket G, Cole M, Watters M, Ricardo-Dukelow ML, Chang L (2009) Declined neural efficiency in cognitively stable human immunodeficiency virus patients. Ann Neurol 65:316–325

Fan Y, He JJ (2016) HIV-1 Tat promotes lysosomal exocytosis in astrocytes and contributes to astrocyte-mediated Tat neurotoxicity. J Biol Chem 291:22830–22840

Fan Y, He JJ (2016) HIV-1 Tat induces unfolded protein response and endoplasmic reticulum stress in astrocytes and causes neurotoxicity through Glial Fibrillary Acidic Protein (GFAP) activation and aggregation. J Biol Chem 291:22819–22829

Fan Y, Gao X, Chen J, Liu Y, He JJ (2016) HIV Tat impairs neurogenesis through functioning as a notch ligand and activation of notch signaling pathway. J Neurosci 36:11362–11373

Feldman HM, Yeatman JD, Lee ES, Barde LH, Gaman-Bean S (2010) Diffusion tensor imaging: a review for pediatric researchers and clinicians. J Dev Behav Pediatr 31:346–356

Filbey FM, McQueeny T, Kadamangudi S, Bice C, Ketcherside A (2015) Combined effects of marijuana and nicotine on memory performance and hippocampal volume. Behav Brain Res 293:46–53

Fitting S, Scoggins KL, Xu R, Dever SM, Knapp PE, Dewey WL, Hauser KF (2012) Morphine efficacy is altered in conditional HIV-1 Tat transgenic mice. Eur J Pharmacol 689:96–103

Fitting S, Xu R, Bull C, Buch SK, El-Hage N, Nath A, Knapp PE, Hauser KF (2010) Interactive comorbidity between opioid drug abuse and HIV-1 Tat: chronic exposure augments spine loss and sublethal dendritic pathology in striatal neurons. Am J Pathol 177:1397–1410

Fritz HC, Wittfeld K, Schmidt CO, Domin M, Grabe HJ, Hegenscheid K, Hosten N, Lotze M (2014) Current smoking and reduced gray matter volume-a voxel-based morphometry study. Neuropsychopharmacology 39:2594–2600

Gallinat J, Lang UE, Jacobsen LK, Bajbouj M, Kalus P, von Haebler D, Seifert F, Schubert F (2007) Abnormal hippocampal neurochemistry in smokers: evidence from proton magnetic resonance spectroscopy at 3 T. J Clin Psychopharmacol 27:80–84

Gamarel KE, Westfall AO, Lally MA, Hosek S, Wilson CM, Adolescent Medicine Trials Network for HIVAI (2018) Tobacco use and sustained viral suppression in youth living with HIV. AIDS Behav 22:2018–2025

Gandelman JA, Kang H, Antal A, Albert K, Boyd BD, Conley AC, Newhouse P, Taylor WD (2018) Transdermal nicotine for the treatment of mood and cognitive symptoms in nonsmokers with late-life depression. J Clin Psychiatry 79

Garrison KA, Yip SW, Balodis IM, Carroll KM, Potenza MN, Krishnan-Sarin S (2017) Reward-related frontostriatal activity and smoking behavior among adolescents in treatment for smoking cessation. Drug Alcohol Depend 177:268–276

Gaskill PJ, Yano HH, Kalpana GV, Javitch JA, Berman JW (2014) Dopamine receptor activation increases HIV entry into primary human macrophages. PLoS One 9:e108232

Gaskill PJ, Miller DR, Gamble-George J, Yano H, Khoshbouei H (2017) HIV, Tat and dopamine transmission. Neurobiol Dis 105:51–73

Ghitza UE, Zhai H, Wu P, Airavaara M, Shaham Y, Lu L (2010) Role of BDNF and GDNF in drug reward and relapse: a review. Neurosci Biobehav Rev 35:157–171

Ghosh D, Mishra MK, Das S, Kaushik DK, Basu A (2009) Tobacco carcinogen induces microglial activation and subsequent neuronal damage. J Neurochem 110:1070–1081

Ghura S, Gross R, Jordan-Sciutto K, Dubroff J, Schnoll R, Collman RG, Ashare RL (2019) Bidirectional associations among nicotine and tobacco smoke, neuroHIV, and antiretroviral therapy. J Neuroimmune Pharmacol

Gilhus NE, Deuschl G (2019) Neuroinflammation - a common thread in neurological disorders. Nat Rev Neurol 15:429–430

Gons RA, van Norden AG, de Laat KF, van Oudheusden LJ, van Uden IW, Zwiers MP, Norris DG, de Leeuw FE (2011) Cigarette smoking is associated with reduced microstructural integrity of cerebral white matter. Brain 134:2116–2124

Gonzalez-Lira B, Rueda-Orozco PE, Galicia O, Montes-Rodriguez CJ, Guzman K, Guevara-Martinez M, Elder JH, Prospero-Garcia O (2006) Nicotine prevents HIVgp120-caused electrophysiological and motor disturbances in rats. Neurosci Lett 394:136–139

Goodwin RD, Pacek LR, Copeland J, Moeller SJ, Dierker L, Weinberger A, Gbedemah M, Zvolensky MJ, Wall MM, Hasin DS (2018) Trends in daily cannabis use among cigarette smokers: United States, 2002–2014. Am J Public Health 108:137–142

Groff BR, Wiesman AI, Rezich MT, O'Neill J, Robertson KR, Fox HS, Swindells S, Wilson TW (2020) Age-related visual dynamics in HIV-infected adults with cognitive impairment. Neurol Neuroimmunol Neuroinflamm 7(3):e690

Grucza RA, Bierut LJ (2006) Cigarette smoking and the risk for alcohol use disorders among adolescent drinkers. Alcohol Clin Exp Res 30:2046–2054

Guha A, Brier MR, Ortega M, Westerhaus E, Nelson B, Ances BM (2016) Topographies of cortical and subcortical volume loss in HIV and aging in the cART Era. J Acquir Immune Defic Syndr 73:374–383

Guha D, Nagilla P, Redinger C, Srinivasan A, Schatten GP, Ayyavoo V (2012) Neuronal apoptosis by HIV-1 Vpr: contribution of proinflammatory molecular networks from infected target cells. J Neuroinflammation 9:138

Gutzeit A, Froehlich JM, Hergan K, Graf N, Binkert CA, Meier D, Brugger M, Reischauer C, Sutter R, Herdener M, Schubert T, Kos

S, Grosshans M, Straka M, Mutschler J (2013) Insula-specific H magnetic resonance spectroscopy reactions in heavy smokers under acute nicotine withdrawal and after oral nicotine substitution. Eur Addict Res 19:184–193

Hammoud DA, Sinharay S, Shah S, Schreiber-Stainthorp W, Maric D, Muthusamy S, Lee DE, Lee CA, Basuli F, Reid WC, Wakim P, Matsuda K, Hirsch V, Nath A, Di Mascio M (2019) Neuroinflammatory changes in relation to cerebrospinal fluid viral load in simian immunodeficiency virus encephalitis. MBio 10(3): e00970–e00919

Han H, Yang Z, Chang SL, Li MD (2018) Modulatory effects of nicotine on neuroHIV/neuroAIDS. J Neuroimmune Pharmacol 13:467–478

Hanlon CA, Owens MM, Joseph JE, Zhu X, George MS, Brady KT, Hartwell KJ (2016) Lower subcortical gray matter volume in both younger smokers and established smokers relative to non-smokers. Addict Biol 21:185–195

Harrison JD, Dochney JA, Blazekovic S, Leone F, Metzger D, Frank I, Gross R, Hole A, Mounzer K, Siegel S, Schnoll RA, Ashare RL (2017) The nature and consequences of cognitive deficits among tobacco smokers with HIV: a comparison to tobacco smokers without HIV. J Neurovirol 23:550–557

Hauser KF, Hahn YK, Adjan VV, Zou S, Buch SK, Nath A, Bruce-Keller AJ, Knapp PE (2009) HIV-1 Tat and morphine have interactive effects on oligodendrocyte survival and morphology. Glia 57:194–206

He J, Chen Y, Farzan M, Choe H, Ohagen A, Gartner S, Busciglio J, Yang X, Hofmann W, Newman W, Mackay CR, Sodroski J, Gabuzda D (1997) CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. Nature 385:645–649

Heishman SJ, Kleykamp BA, Singleton EG (2010) Meta-analysis of the acute effects of nicotine and smoking on human performance. Psychopharmacology 210:453–469

Helleberg M, Afzal S, Kronborg G, Larsen CS, Pedersen G, Pedersen C, Gerstoft J, Nordestgaard BG, Obel N (2013) Mortality attributable to smoking among HIV-1-infected individuals: a nationwide, population-based cohort study. Clin Infect Dis 56:727–734

Helleberg M, May MT, Ingle SM, Dabis F, Reiss P, Fatkenheuer G, Costagliola D, d'Arminio A, Cavassini M, Smith C, Justice AC, Gill J, Sterne JA, Obel N (2015) Smoking and life expectancy among HIV-infected individuals on antiretroviral therapy in Europe and North America. AIDS 29:221–229

Hijioka M, Matsushita H, Hisatsune A, Isohama Y, Katsuki H (2011) Therapeutic effect of nicotine in a mouse model of intracerebral hemorrhage. J Pharmacol Exp Ther 338:741–749

Hillmer AT, Sandiego CM, Hannestad J, Angarita GA, Kumar A, McGovern EM, Huang Y, O'Connor KC, Carson RE, O'Malley SS, Cosgrove KP (2017) In vivo imaging of translocator protein, a marker of activated microglia, in alcohol dependence. Mol Psychiatry 22:1759–1766

Hirvonen J, Goodwin RS, Li CT, Terry GE, Zoghbi SS, Morse C, Pike VW, Volkow ND, Huestis MA, Innis RB (2012) Reversible and regionally selective downregulation of brain cannabinoid CB1 receptors in chronic daily cannabis smokers. Mol Psychiatry 17:642–649

Hirvonen J, Zanotti-Fregonara P, Gorelick DA, Lyoo CH, Rallis-Frutos D, Morse C, Zoghbi SS, Pike VW, Volkow ND, Huestis MA (2018) Decreased cannabinoid CB1 receptors in male tobacco smokers examined with positron emission tomography. Biol Psychiatry 84: 715–721

Hong LE, Gu H, Yang Y, Ross TJ, Salmeron BJ, Buchholz B, Thaker GK, Stein EA (2009) Association of nicotine addiction and nicotine's actions with separate cingulate cortex functional circuits. Arch Gen Psychiatry 66:431–441

Hosking JG, Lam FC, Winstanley CA (2014) Nicotine increases impulsivity and decreases willingness to exert cognitive effort despite

improving attention in "slacker" rats: insights into cholinergic regulation of cost/benefit decision making. PLoS One 9:e111580

Huang LZ, Abbott LC, Winzer-Serhan UH (2007) Effects of chronic neonatal nicotine exposure on nicotinic acetylcholine receptor binding, cell death and morphology in hippocampus and cerebellum. Neuroscience 146:1854–1868

Huang W, DiFranza JR, Kennedy DN, Zhang N, Ziedonis D, Ursprung S, King JA (2013) Progressive levels of physical dependence to tobacco coincide with changes in the anterior cingulum bundle microstructure. PLoS One 8:e67837

Hudson L, Liu J, Nath A, Jones M, Raghavan R, Narayan O, Male D, Everall I (2000) Detection of the human immunodeficiency virus regulatory protein tat in CNS tissues. J Neurovirol 6:145–155

Hui L, Chen X, Haughey NJ, Geiger JD (2012) Role of endolysosomes in HIV-1 Tat-induced neurotoxicity. ASN Neuro 4:243–252

Hukkanen J, Jacob P III, Benowitz NL (2005) Metabolism and disposition kinetics of nicotine. Pharmacol Rev 57:79–115

Hurd YL, Manzoni OJ, Pletnikov MV, Lee FS, Bhattacharyya S, Melis M (2019) Cannabis and the developing brain: insights into its long-lasting effects. J Neurosci 39:8250–8258

Jacobsen LK, Pugh KR, Constable RT, Westerveld M, Mencl WE (2007) Functional correlates of verbal memory deficits emerging during nicotine withdrawal in abstinent adolescent cannabis users. Biol Psychiatry 61:31–40

Jamal A, Phillips E, Gentzke AS, Homa DM, Babb SD, King BA, Neff LJ (2018) Current cigarette smoking among adults—United States, 2016. MMWR Morb Mortal Wkly Rep 67:53

Janes AC, Jensen JE, Farmer SL, Frederick BD, Pizzagalli DA, Lukas SE (2013) GABA levels in the dorsal anterior cingulate cortex associated with difficulty ignoring smoking-related cues in tobacco-dependent volunteers. Neuropsychopharmacology 38:1113–1120

Jasinska AJ, Zorick T, Brody AL, Stein EA (2014) Dual role of nicotine in addiction and cognition: a review of neuroimaging studies in humans. Neuropharmacology 84:111–122

Jeang K-T (1996) HIV-1 Tat: structure and function. Human Retroviruses and AIDS 1996: A compilation and analysis of nucleic acid and amino acid sequences. In: Myers G, Foley B, Korber B, Mellors JW, Jeang KT, Wain-Hobson S, (eds), pp III3-III18. United State: Los Alamos National Lab., NM (United States)

Kalk NJ, Guo Q, Owen D, Cherian R, Erritzoe D, Gilmour A, Ribeiro AS, McGonigle J, Waldman A, Matthews P, Cavanagh J, McInnes I, Dar K, Gunn R, Rabiner EA, Lingford-Hughes AR (2017) Decreased hippocampal translocator protein (18 kDa) expression in alcohol dependence: a [(11)C]PBR28 PET study. Transl Psychiatry 7:e996

Kaneko S, Maeda T, Kume T, Kochiyama H, Akaike A, Shimohama S, Kimura J (1997) Nicotine protects cultured cortical neurons against glutamate-induced cytotoxicity via alpha7-neuronal receptors and neuronal CNS receptors. Brain Res 765:135–140

Karama S, Ducharme S, Corley J, Chouinard-Decorte F, Starr JM, Wardlaw JM, Bastin ME, Deary IJ (2015) Cigarette smoking and thinning of the brain's cortex. Mol Psychiatry 20:778–785

Kesby JP, Markou A, Semenova S (2016) The effects of HIV-1 regulatory TAT protein expression on brain reward function, response to psychostimulants and delay-dependent memory in mice. Neuropharmacology 109:205–215

Khanna A, Guo M, Mehra M, Royal W III (2013) Inflammation and oxidative stress induced by cigarette smoke in Lewis rat brains. J Neuroimmunol 254:69–75

Kim BO, Liu Y, Ruan Y, Xu ZC, Schantz L, He JJ (2003) Neuropathologies in transgenic mice expressing human immunodeficiency virus type 1 Tat protein under the regulation of the astrocyte-specific glial fibrillary acidic protein promoter and doxycycline. Am J Pathol 162:1693–1707

Kim H-C, Jhoo W-K, Ko KH, Kim W-K, Bing G, Kwon M-S, Shin E-J, Suh J-H, Lee Y-G, Lee D-W (1999) Prolonged exposure to cigarette

smoke blocks the neurotoxicity induced by kainic acid in rats. Life Sci 66:317–326

Kim SW et al (2018) Influence of alcoholism and cholesterol on TSPO binding in brain: PET [(11)C]PBR28 studies in humans and rodents. Neuropsychopharmacology 43:1832–1839

Kuhn S, Romanowski A, Schilling C, Mobascher A, Warbrick T, Winterer G, Gallinat J (2012) Brain grey matter deficits in smokers: focus on the cerebellum. Brain Struct Funct 217:517–522

Kumari V, Gray JA, ffytche DH, Mitterschifffthaler MT, Das M, Zachariah E, Vythelingum GN, Williams SC, Simmons A, Sharma T (2003) Cognitive effects of nicotine in humans: an fMRI study. Neuroimage 19:1002–1013

Langford D, Oh Kim B, Zou W, Fan Y, Rahimain P, Liu Y, He JJ (2018) Doxycycline-inducible and astrocyte-specific HIV-1 Tat transgenic mice (iTat) as an HIV/neuroAIDS model. J Neurovirol 24:168–179

Ledgerwood DM, Yskes R (2016) Smoking cessation for people living with HIV/AIDS: A literature review and synthesis. Nicotine Tob Res 18:2177–2184

Lee MY, Chen L, Toborek M (2009) Nicotine attenuates iNOS expression and contributes to neuroprotection in a compressive model of spinal cord injury. J Neurosci Res 87:937–947

Lerman C, Gu H, Loughead J, Ruparel K, Yang Y, Stein EA (2014) Large-scale brain network coupling predicts acute nicotine abstinence effects on craving and cognitive function. JAMA Psychiatry 71:523–530

Leroy C, Karila L, Martinot JL, Lukasiewicz M, Duchesnay E, Comtat C, Dolle F, Benyamina A, Artiges E, Ribeiro MJ, Reynaud M, Trichard C (2012) Striatal and extrastriatal dopamine transporter in cannabis and tobacco addiction: a high-resolution PET study. Addict Biol 17:981–990

Lesage E, Sutherland MT, Ross TJ, Salmeron BJ, Stein EA (2020) Nicotine dependence (trait) and acute nicotinic stimulation (state) modulate attention but not inhibitory control: converging fMRI evidence from Go-Nogo and Flanker tasks. Neuropsychopharmacology 45:857–865

Lesage E, Aronson SE, Sutherland MT, Ross TJ, Salmeron BJ, Stein EA (2017) Neural signatures of cognitive flexibility and reward sensitivity following nicotinic receptor stimulation in dependent smokers: a randomized trial. JAMA Psychiatry 74:632–640

Levitzky YS, Guo CY, Rong J, Larson MG, Walter RE, Keaney JF Jr, Sutherland PA, Vasan A, Lipinska I, Evans JC, Benjamin EJ (2008) Relation of smoking status to a panel of inflammatory markers: the framingham offspring. Atherosclerosis 201:217–224

Lewis AS, van Schalkwyk GI, Lopez MO, Volkmar FR, Picciotto MR, Sukhodolsky DG (2018) An exploratory trial of transdermal nicotine for aggression and irritability in adults with autism spectrum disorder. J Autism Dev Disord 48:2748–2757

Li MD, Cao J, Wang S, Wang J, Sarkar S, Vigorito M, Ma JZ, Chang SL (2013) Transcriptome sequencing of gene expression in the brain of the HIV-1 transgenic rat. PLoS One 8:e59582

Li W, Huang Y, Reid R, Steiner J, Malpica-Llanos T, Darden TA, Shankar SK, Mahadevan A, Satishchandra P, Nath A (2008) NMDA receptor activation by HIV-Tat protein is clade dependent. J Neurosci 28:12190–12198

Li Y, Yuan K, Cai C, Feng D, Yin J, Bi Y, Shi S, Yu D, Jin C, von Deneen KM, Qin W, Tian J (2015) Reduced frontal cortical thickness and increased caudate volume within fronto-striatal circuits in young adult smokers. Drug Alcohol Depend 151:211–219

Liang H, Chang L, Chen R, Oishi K, Ernst T (2018) Independent and combined effects of chronic HIV-infection and tobacco smoking on brain microstructure. J Neuroimmune Pharmacol 13:509–522

Liang HJ, O'Connor EE, Ernst T, Oishi K, Cunningham E, Chang L (2020) Greater sensorimotor deficits and abnormally lower globus pallidus fractional anisotropy in HIV + Women than in HIV + Men. J Neuroimmune Pharmacol

Liao Y, Tang J, Liu T, Chen X, Hao W (2012) Differences between smokers and non-smokers in regional gray matter volumes: a voxel-based morphometry study. Addict Biol 17:977–980

Liao Y, Tang J, Deng Q, Deng Y, Luo T, Wang X, Chen H, Liu T, Chen X, Brody AL, Hao W (2011) Bilateral fronto-parietal integrity in young chronic cigarette smokers: a diffusion tensor imaging study. PLoS One 6:e26460

Lin F, Wu G, Zhu L, Lei H (2013) Heavy smokers show abnormal microstructural integrity in the anterior corpus callosum: a diffusion tensor imaging study with tract-based spatial statistics. Drug Alcohol Depend 129:82–87

Liu L, Yu J, Li L, Zhang B, Liu L, Wu CH, Jong A, Mao DA, Huang SH (2017) Alpha7 nicotinic acetylcholine receptor is required for amyloid pathology in brain endothelial cells induced by Glycoprotein 120, methamphetamine and nicotine. Sci Rep 7:40467

Liu X, Shah A, Gangwani MR, Silverstein PS, Fu M, Kumar A (2014) HIV-1 Nef induces CCL5 production in astrocytes through p38-MAPK and PI3K/Akt pathway and utilizes NF-kB, CEBP and AP-1 transcription factors. Sci Rep 4:1–10

Liu Y, Jones M, Hingtgen CM, Bu G, Laribee N, Tanzi RE, Moir RD, Nath A, He JJ (2000) Uptake of HIV-1 tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. Nat Med 6:1380–1387

Lorenzetti V, Chye Y, Silva P, Solowij N, Roberts CA (2019) Does regular cannabis use affect neuroanatomy? An updated systematic review and meta-analysis of structural neuroimaging studies. Eur Arch Psychiatry Clin Neurosci 269:59–71

Louboutin JP, Strayer D (2014) Role of oxidative stress in HIV-1-associated neurocognitive disorder and protection by gene delivery of antioxidant enzymes. Antioxidants (Basel) 3:770–797

Luhar RB, Sawyer KS, Gravitz Z, Ruiz SM, Oscar-Berman M (2013) Brain volumes and neuropsychological performance are related to current smoking and alcoholism history. Neuropsychiatr Dis Treat 9:1767–1784

Madani A, Alack K, Richter MJ, Krüger K (2018) Immune-regulating effects of exercise on cigarette smoke-induced inflammation. J Inflamm Res 11:155–167

Manda VK, Mittapalli RK, Geldenhuys WJ, Lockman PR (2010) Chronic exposure to nicotine and saquinavir decreases endothelial Notch-4 expression and disrupts blood-brain barrier integrity. J Neurochem 115:515–525

Marks MJ, Grady SR, Collins AC (1993) Downregulation of nicotinic receptor function after chronic nicotine infusion. J Pharmacol Exp Ther 266:1268–1276

Marshall AM, Heffernan T, Hamilton C (2016) The synergistic impact of excessive alcohol drinking and cigarette smoking upon prospective memory. Front Psychiatry 7:75

Mason GF, Petrakis IL, de Graaf RA, Gueorguieva R, Guidone E, Coric V, Epperson CN, Rothman DL, Krystal JH (2006) Cortical gamma-aminobutyric acid levels and the recovery from ethanol dependence: preliminary evidence of modification by cigarette smoking. Biol Psychiatry 59:85–93

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561–564

Mazzone P, Tierney W, Hossain M, Puvenna V, Janigro D, Cucullo L (2010) Pathophysiological impact of cigarette smoke exposure on the cerebrovascular system with a focus on the blood-brain barrier: expanding the awareness of smoking toxicity in an underappreciated area. Int J Environ Res Public Health 7:4111–4126

McArthur JC, Steiner J, Sacktor N, Nath A (2010) Human immunodeficiency virus-associated neurocognitive disorders: Mind the gap. Ann Neurol 67:699–714

McCallum SE, Parameswaran N, Bordia T, Fan H, Tyndale RF, Langston JW, McIntosh JM, Quik M (2006) Increases in alpha4* but not alpha3*/alpha6* nicotinic receptor sites and function in the primate

striatum following chronic oral nicotine treatment. J Neurochem 96: 1028–1041

McCorkindale AN, Sheedy D, Kril JJ, Sutherland GT (2016) The effects of chronic smoking on the pathology of alcohol-related brain damage. Alcohol 53:35–44

McCorkindale AN, Sizemova A, Sheedy D, Kril JJ, Sutherland GT (2019) Re-investigating the effects of chronic smoking on the pathology of alcohol-related human brain damage. Alcohol 76:11–14

McQuail JA, Frazier CJ, Bizon JL (2015) Molecular aspects of age-related cognitive decline: the role of GABA signaling. Trends Mol Med 21:450–460

Mdodo R, Frazier EL, Dube SR, Mattson CL, Sutton MY, Brooks JT, Skarbinski J (2015) Cigarette smoking prevalence among adults with HIV compared with the general adult population in the United States: cross-sectional surveys. Ann Intern Med 162:335–344

Mediouni S, Jablonski J, Paris JJ, Clementz MA, Thenin-Houssier S, McLaughlin JP, Valente ST (2015) Didehydro-cortistatin A inhibits HIV-1 Tat mediated neuroinflammation and prevents potentiation of cocaine reward in Tat transgenic mice. Curr HIV Res 13:64–79

Meier MH, Caspi A, Danese A, Fisher HL, Houts R, Arseneault L, Moffitt TE (2018) Associations between adolescent cannabis use and neuropsychological decline: a longitudinal co-twin control study. Addiction 113:257–265

Mennecke A, Gossler A, Hammen T, Dörfler A, Stadlbauer A, Rösch J, Kornhuber J, Bleich S, Dölken M, Thürauf N (2014) Physiological effects of cigarette smoking in the limbic system revealed by 3 T magnetic resonance spectroscopy. J Neural Transm 121:1211–1219

Midde NM, Gomez AM, Harrod SB, Zhu J (2011) Genetically expressed HIV-1 viral proteins attenuate nicotine-induced behavioral sensitization and alter mesocorticolimbic ERK and CREB signaling in rats. Pharmacol Biochem Behavior 98:587–597

Mohamed M, Barker PB, Skolasky RL, Sacktor N (2018) 7T brain MRS in HIV infection: Correlation with cognitive impairment and performance on neuropsychological tests. AJNR Am J Neuroradiol 39:704–712

Mohamed M, Skolasky RL, Zhou Y, Ye W, Brasic JR, Brown A, Pardo CA, Barker PB, Wong DF, Sacktor N (2020) Beta-amyloid (Aβ) uptake by PET imaging in older HIV + and HIV-individuals. J Neurovirol 26:382–390

Mon A, Durazzo TC, Gazdzinski S, Meyerhoff DJ (2009) The impact of chronic cigarette smoking on recovery from cortical gray matter perfusion deficits in alcohol dependence: longitudinal arterial spin labeling MRI. Alcohol Clin Exp Res 33:1314–1321

Monnig MA, Kahler CW, Lee H, Pantalone DW, Mayer KH, Cohen RA, Monti PM (2016) Effects of smoking and alcohol use on neurocognitive functioning in heavy drinking, HIV-positive men who have sex with men. AIDS Care 28:300–305

Moran ML, Aksenov YM, Booze MR, Webb MK, Mactutus FC (2012) Adolescent HIV-1 transgenic rats: evidence for dopaminergic alterations in behavior and neurochemistry revealed by methamphetamine challenge. Current HIV research 10:415–424

Moreno-Gonzalez I, Estrada LD, Sanchez-Mejias E, Soto C (2013) Smoking exacerbates amyloid pathology in a mouse model of Alzheimer's disease. Nat Commun 4:1495

Nesil T, Cao J, Yang Z, Chang SL, Li MD (2015) Nicotine attenuates the effect of HIV-1 proteins on the neural circuits of working and contextual memories. Mol Brain 8:43

Newhouse P, Kellar K, Aisen P, White H, Wesnes K, Oderre E, Pfaff A, Wilkins H, Howard D, Levin ED (2012) Nicotine treatment of mild cognitive impairement: A 6-month double-blind pilot clinical trial. Neurology. 2012 Jan 10;78(2):91–101

Nicholatos JW, Francisco AB, Bender CA, Yeh T, Lugay FJ, Salazar JE, Glorioso C, Libert S (2018) Nicotine promotes neuron survival and partially protects from Parkinson's disease by suppressing SIRT6. Acta Neuropathol Commun 6:1–18

Nizri E, Irony-Tur-Sinai M, Lory O, Orr-Urtreger A, Lavi E, Brenner T (2009) Activation of the cholinergic anti-inflammatory system by nicotine attenuates neuroinflammation via suppression of Th1 and Th17 responses. J Immunol 183:6681–6688

Nookala AR, Kumar A (2014) Molecular mechanisms involved in HIV-1 Tat-mediated induction of IL-6 and IL-8 in astrocytes. J Neuroinflammation 11:214

Nunez K, Kay J, Krotow A, Tong M, Agarwal AR, Cadenas E, de la Monte SM (2016) Cigarette smoke-induced alterations in frontal white matter lipid profiles demonstrated by MALDI-imaging mass spectrometry: Relevance to Alzheimer's Disease. J Alzheimers Dis 51:151–163

O'Connor EE, Jaillard A, Renard F, Zeffiro TA (2017) Reliability of white matter microstructural changes in HIV infection: Meta-analysis and confirmation. AJNR Am J Neuroradiol 38:1510–1519

O'Neill J, Tobias MC, Hudkins M, Oh EY, Hellemann GS, Nurmi EL, London ED (2014) Thalamic glutamate decreases with cigarette smoking. Psychopharmacology 231:2717–2724

Ortega M, Brier MR, Ances BM (2015) Effects of HIV and combination antiretroviral therapy on cortico-striatal functional connectivity. AIDS 29:703–712

Ott A, Slooter AJ, Hofman A, van Harskamp F, Witteman JC, Van Broeckhoven C, van Duijn CM, Breteler MM (1998) Smoking and risk of dementia and Alzheimer's disease in a population-based cohort study: the Rotterdam Study. Lancet 351:1840–1843

Papp-Peka A, Tong M, Kril JJ, De La Monte SM, Sutherland GT (2017) The differential effects of alcohol and nicotine-specific nitrosamine ketone on white matter ultrastructure. Alcohol Alcohol 52:165–171

Paris JJ, Carey AN, Shay CF, Gomes SM, He JJ, McLaughlin JP (2014) Effects of conditional central expression of HIV-1 tat protein to potentiate cocaine-mediated psychostimulation and reward among male mice. Neuropsychopharmacology 39:380–388

Paul RH, Ernst T, Brickman AM, Yiannoutsos CT, Tate DF, Cohen RA, Navia BA, team A, team A, Consortium HM (2008) Relative sensitivity of magnetic resonance spectroscopy and quantitative magnetic resonance imaging to cognitive function among nondemented individuals infected with HIV. J Int Neuropsychol Soc 14:725–733

Paul RH, Grieve SM, Niaura R, David SP, Laidlaw DH, Cohen R, Sweet L, Taylor G, Clark RC, Pogun S, Gordon E (2008b) Chronic cigarette smoking and the microstructural integrity of white matter in healthy adults: a diffusion tensor imaging study. Nicotine Tob Res 10:137–147

Pfefferbaum A, Rosenbloom MJ, Rohlfing T, Kemper CA, Deresinski S, Sullivan EV (2009) Frontostriatal fiber bundle compromise in HIV infection without dementia. AIDS 23:1977

Plessis SD, Vink M, Joska JA, Koutsilieri E, Stein DJ, Emsley R (2014) HIV infection and the fronto-striatal system: a systematic review and meta-analysis of fMRI studies. AIDS 28:803–811

Poe JA, Smithgall TE (2009) HIV-1 Nef dimerization is required for Nef-mediated receptor downregulation and viral replication. J Mol Biol 394:329–342

Power MC, Deal JA, Sharrett AR, Jack CR Jr, Knopman D, Mosley TH, Gottesman RF (2015) Smoking and white matter hyperintensity progression: the ARIC-MRI Study. Neurology 84:841–848

Quik M, Campos C, Parameswaran N, Langston JW, McIntosh JM, Yeluashvili M (2010) Chronic nicotine treatment increases nAChRs and microglial expression in monkey substantia nigra after nigrostriatal damage. J Mol Neurosci 40:105–113

Quik M, Parameswaran N, McCallum SE, Bordia T, Bao S, McCormack A, Kim A, Tyndale RF, Langston JW, Di Monte DA (2006) Chronic oral nicotine treatment protects against striatal degeneration in MPTP-treated primates. J Neurochem 98:1866–1875

Ragin AB, Wu Y, Gao Y, Keating S, Du H, Sammet C, Kettering CS, Epstein LG (2015) Brain alterations within the first 100 days of HIV infection. Ann Clin Transl Neurol 2:12–21

Rahimian P, He JJ (2016a) Exosome-associated release, uptake, and neurotoxicity of HIV-1 Tat protein. J Neurovirol 22:774–788

Rahimian P, He JJ (2016b) HIV-1 Tat-shortened neurite outgrowth through regulation of microRNA-132 and its target gene expression. J Neuroinflammation 13:247

Ranki A, Nyberg M, Ovod V, Haltia M, Elovaara I, Raininko R, Haapasalo H, Krohn K (1995) Abundant expression of HIV Nef and Rev proteins in brain astrocytes in vivo is associated with dementia. AIDS 9:1001–1008

Ransohoff RM (2016) How neuroinflammation contributes to neurodegeneration. Science 353:777–783

Reid W et al (2001) An HIV-1 transgenic rat that develops HIV-related pathology and immunologic dysfunction. Proc Natl Acad Sci U S A 98:9271–9276

Revathikumar P, Bergqvist F, Gopalakrishnan S, Korotkova M, Jakobsson PJ, Lampa J, Le Maitre E (2016) Immunomodulatory effects of nicotine on interleukin 1beta activated human astrocytes and the role of cyclooxygenase 2 in the underlying mechanism. J Neuroinflammation 13:256

Rivera J, Isidro RA, Loucil-Alicea RY, Cruz ML, Appleyard CB, Isidro AA, Chompre G, Colon-Rivera K, Noel RJ (2019) Infusion of HIV-1 Nef-expressing astrocytes into the rat hippocampus induces enteropathy and interstitial pneumonitis and increases blood-brain-barrier permeability. PLoS One 14:e0225760

Rosa A, Chande A, Ziglio S, De Sanctis V, Bertorelli R, Goh SL, McCauley SM, Nowosielska A, Antonarakis SE, Luban J, Santoni FA, Pizzato M (2015) HIV-1 Nef promotes infection by excluding SERINC5 from virion incorporation. Nature 526:212–217

Royal W III, Can A, Gould TD, Guo M, Huse J, Jackson M, Davis H, Bryant J (2018) Cigarette smoke and nicotine effects on brain proinflammatory responses and behavioral and motor function in HIV-1 transgenic rats. J Neurovirol 24:246–253

Rubin LH, Sacktor N, Creighton J, Du Y, Endres CJ, Pomper MG, Coughlin JM (2018) Microglial activation is inversely associated with cognition in individuals living with HIV on effective antiretroviral therapy. AIDS 32:1661–1667

Ryan RE, Ross SA, Drago J, Loiacono RE (2001) Dose-related neuroprotective effects of chronic nicotine in 6-hydroxydopamine treated rats, and loss of neuroprotection in alpha4 nicotinic receptor subunit knockout mice. Br J Pharmacol 132:1650–1656

Saito Y, Sharer LR, Epstein LG, Michaels J, Mintz M, Louder M, Golding K, Cvetkovich TA, Blumberg BM (1994) Overexpression of nef as a marker for restricted HIV-1 infection of astrocytes in postmortem pediatric central nervous tissues. Neurology 44:474–481

Sami Saribas A, Cicalese S, Ahooyi TM, Khalili K, Amini S, Sariyer IK (2017) HIV-1 Nef is released in extracellular vesicles derived from astrocytes: evidence for Nef-mediated neurotoxicity. Cell Death Dis 8:e2542

Sanford R, Cruz ALF, Scott SC, Mayo NE, Fellows LK, Ances BM, Collins DL (2017) Regionally specific brain volumetric and cortical thickness changes in HIV-infected patients in the HAART era. J Acquir Immune Defic Syndr 74:563

Savjani RR, Velasquez KM, Thompson-Lake DG, Baldwin PR, Eagleman DM, De La Garza R II, Salas R (2014) Characterizing white matter changes in cigarette smokers via diffusion tensor imaging. Drug Alcohol Depend 145:134–142

Saylor D, Dickens AM, Sacktor N, Haughey N, Slusher B, Pletnikov M, Mankowski JL, Brown A, Volsky DJ, McArthur JC (2016) HIV-associated neurocognitive disorder - pathogenesis and prospects for treatment. Nat Rev Neurol 12:309

Schauer GL, King BA, McAfee TA (2017) Prevalence, correlates, and trends in tobacco use and cessation among current, former, and never adult marijuana users with a history of tobacco use, 2005–2014. Addict Behav 73:165–171

Schulte MH, Kaag AM, Wiers RW, Schmaal L, van den Brink W, Reneman L, Homberg JR, van Wingen GA, Goudriaan AE (2017) Prefrontal Glx and GABA concentrations and impulsivity in cigarette smokers and smoking polysubstance users. Drug Alcohol Depend 179:117–123

Schuster RM, Mermelstein RJ, Hedeker D (2016) Ecological momentary assessment of working memory under conditions of simultaneous marijuana and tobacco use. Addiction 111:1466–1476

Schuster RM, Crane NA, Mermelstein R, Gonzalez R (2015) Tobacco may mask poorer episodic memory among young adult cannabis users. Neuropsychology 29:759–766

Schwartz S, Felber BK, Benko DM, Fenyö EM, Pavlakis GN (1990) Cloning and functional analysis of multiply spliced mRNA species of human immunodeficiency virus type 1. J Virol 64:2519–2529

Shah A, Kumar A (2010) HIV-1 gp120-mediated increases in IL-8 production in astrocytes are mediated through the NF-kappaB pathway and can be silenced by gp120-specific siRNA. J Neuroinflammation 7:1–6

Shahrir S, Crothers K, McGinnis KA, Chan KCG, Baeten JM, Wilson SM, Butt AA, Pisani MA, Baldassarri SR, Justice A, Williams EC (2020) Receipt and predictors of smoking cessation pharmacotherapy among veterans with and without HIV. Prog Cardiovasc Dis 63:118–124

Shang X, Shang Y, Fu J, Zhang T (2017) Nicotine significantly improves chronic stress-induced impairments of cognition and synaptic plasticity in mice. Mol Neurobiol 54:4644–4658

Shaw S, Bencherif M, Marrero MB (2003) Angiotensin II blocks nicotine-mediated neuroprotection against beta-amyloid (1–42) via activation of the tyrosine phosphatase SHP-1. J Neurosci 23:11224–11228

Shin AH, Kim HJ, Thayer SA (2012) Subtype selective NMDA receptor antagonists induce recovery of synapses lost following exposure to HIV-1 Tat. Br J Pharmacol 166:1002–1017

Silva AC, Rodrigues BS, Micheletti AM, Tostes S Jr, Meneses AC, Silva-Vergara ML, Adad SJ (2012) Neuropathology of AIDS: An autopsy review of 284 cases from Brazil comparing the findings pre- and post-HAART (Highly Active Antiretroviral Therapy) and pre- and postmortem correlation. AIDS Res Treat 2012:186850

Slotkin TA, Skavicus S, Ko A, Levin ED, Seidler FJ (2019) The developmental neurotoxicity of tobacco smoke can be mimicked by a combination of nicotine and Benzo[a]Pyrene: Effects on cholinergic and serotonergic systems. Toxicol Sci 167(1):293–304

Slotkin TA, Skavicus S, Card J, Stadler A, Levin ED, Seidler FJ (2015) Developmental neurotoxicity of tobacco smoke directed toward cholinergic and serotonergic systems: More than just nicotine. Toxicol Sci Sep 147(1):178–189

Slotkin TA, Stadler A, Skavicus S, Card J, Ruff J, Levin ED (2017) Is there a critical period for the developmental neurotoxicity of low-level tobacco smoke exposure? Toxicol Sci 155(1):75–84

Smith DM, Miller C, O'Connor RJ, Kozlowski LT, Wadsworth E, Fix BV, Collins RL, Wei B, Goniewicz ML, Hyland AJ, Hammond D (2020) Modes of delivery in concurrent nicotine and cannabis use ("co-use") among youth: Findings from the International Tobacco Control (ITC) Survey. Subst Abus:1–9

Sofuoglu M, Herman AI, Nadim H, Jatlow P (2012) Rapid nicotine clearance is associated with greater reward and heart rate increases from intravenous nicotine. Neuropsychopharmacology 37:1509–1516

Steel HC, Venter WDF, Theron AJ, Anderson R, Feldman C, Kwofie L, Cronje T, Arullapan N, Rossouw TM (2018) Effects of tobacco usage and antiretroviral therapy on biomarkers of systemic immune activation in HIV-infected participants. Mediators Inflamm 2018:8357109

Stevens TR, Krueger SR, Fitzsimonds RM, Picciotto MR (2003) Neuroprotection by nicotine in mouse primary cortical cultures

involves activation of calcineurin and L-type calcium channel inactivation. J Neurosci 23:10093–10099

Stoeckel LE, Chai XJ, Zhang J, Whitfield-Gabrieli S, Evins AE (2016) Lower gray matter density and functional connectivity in the anterior insula in smokers compared with never smokers. Addict Biol 21: 972–981

Straiker A, Stella N, Piomelli D, Mackie K, Karten HJ, Maguire G (1999) Cannabinoid CB1 receptors and ligands in vertebrate retina: localization and function of an endogenous signaling system. Proc Natl Acad Sci U S A 96:14565–14570

Su T, Caan MW, Wit FW, Schouten J, Geurtsen GJ, Cole JH, Sharp DJ, Vos FM, Prins M, Portegies P, Reiss P, Majoie CB, Study AGC (2016) White matter structure alterations in HIV-1-infected men with sustained suppression of viraemia on treatment. AIDS 30: 311–322

Sutherland MT, Stein EA (2018) Functional neurocircuits and neuroimaging biomarkers of tobacco use disorder. Trends Mol Med 24:129–143

Takarada T, Nakamichi N, Kawagoe H, Ogura M, Fukumori R, Nakazato R, Fujikawa K, Kou M, Yoneda Y (2012) Possible neuroprotective property of nicotinic acetylcholine receptors in association with predominant upregulation of glial cell line-derived neurotrophic factor in astrocytes. J Neurosci Res 90:2074–2085

Talhout R, Schulz T, Florek E, van Benthem J, Wester P, Opperhuizen A (2011) Hazardous compounds in tobacco smoke. Int J Environ Res Public Health 8:613–628

Tate DF et al (2011) Regional areas and widths of the midsagittal corpus callosum among HIV-infected patients on stable antiretroviral therapies. J Neurovirol 17:368–379

Thomas JB, Brier MR, Snyder AZ, Vaida FF, Ances BM (2013) Pathways to neurodegeneration: effects of HIV and aging on resting-state functional connectivity. Neurology 80:1186–1193

Toggas SM, Masliah E, Rockenstein EM, Rall GF, Abraham CR, Mucke L (1994) Central nervous system damage produced by expression of the HIV-1 coat protein gp120 in transgenic mice. Nature 367:188–193

Tong M, Yu R, Deochand C, de la Monte SM (2015) Differential contributions of alcohol and the nicotine-derived Nitrosamine Ketone (NNK) to insulin and insulin-like growth factor resistance in the adolescent rat brain. Alcohol Alcohol 50:670–679

Towgood KJ, Pitkanen M, Kulasegaram R, Fradera A, Kumar A, Soni S, Sibtain NA, Reed L, Bradbeer C, Barker GJ, Kopelman MD (2012) Mapping the brain in younger and older asymptomatic HIV-1 men: frontal volume changes in the absence of other cortical or diffusion tensor abnormalities. Cortex 48:230–241

Tsima B, Ratcliffe SJ, Schnoll R, Frank I, Kolson DL, Gross R (2018) Is tobacco use associated with neurocognitive dysfunction in individuals with HIV? J Int Assoc Provid AIDS Care 17: 2325958218768018

Usami Y, Wu Y, Göttlinger HG (2015) SERINC3 and SERINC5 restrict HIV-1 infectivity and are counteracted by Nef. Nature 526:218–223

Vago L, Bonetto S, Nebuloni M, Duca P, Carsana L, Zerbi P, D'Arminio-Monforte A (2002) Pathological findings in the central nervous system of AIDS patients on assumed antiretroviral therapeutic regimens: retrospective study of 1597 autopsies. AIDS 16:1925–1928

Vangkilde S, Bundesen C, Coull JT (2011) Prompt but inefficient: nicotine differentially modulates discrete components of attention. Psychopharmacology 218:667–680

Vera JH, Guo Q, Cole JH, Boasso A, Greathead L, Kelleher P, Rabiner EA, Kalk N, Bishop C, Gunn RN, Matthews PM, Winston A (2016) Neuroinflammation in treated HIV-positive individuals: A TSPO PET study. Neurology 86:1425–1432

Vigorito M, Cao J, Li MD, Chang SL (2013) Acquisition and long-term retention of spatial learning in the human immunodeficiency virus-1 transgenic rat: effects of repeated nicotine treatment. J Neurovirol 19:157–165

Viswanath H, Velasquez KM, Thompson-Lake DG, Savjani R, Carter AQ, Eagleman D, Baldwin PR, De La Garza R II, Salas R (2015) Alterations in interhemispheric functional and anatomical connectivity are associated with tobacco smoking in humans. Front Hum Neurosci 9:116

Volkow ND, Wise RA, Baler R (2017) The dopamine motive system: implications for drug and food addiction. Nat Rev Neurosci 18:741–752

Vossel S, Warbrick T, Mobascher A, Winterer G, Fink GR (2011) Spatial and sustained attention in relation to smoking status: behavioural performance and brain activation patterns. J Psychopharmacol 25: 1485–1495

Wang HA, Liang HJ, Ernst TM, Oishi K, Chang L (2020) Microstructural brain abnormalities in HIV + individuals with or without chronic marijuana use. J Neuroinflammation 17:230

Wang JJ, Durazzo TC, Gazdzinski S, Yeh PH, Mon A, Meyerhoff DJ (2009) MRSI and DTI: a multimodal approach for improved detection of white matter abnormalities in alcohol and nicotine dependence. NMR Biomed 22:516–522

Wang Z, Trillo-Pazos G, Kim S-Y, Canki M, Morgello S, Sharer LR, Gelbard HA, Su Z-z, Kang D-c, Brooks AI (2004) Effects of human immunodeficiency virus type 1 on astrocyte gene expression and function: potential role in neuropathogenesis. J Neurovirol 10:25–32

Waterhouse U, Brennan KA, Ellenbroek BA (2018) Nicotine self-administration reverses cognitive deficits in a rat model for schizophrenia. Addict Biol 23:620–630

Wei P, Liu Q, Li D, Zheng Q, Zhou J, Li J (2015) Acute nicotine treatment attenuates lipopolysaccharide-induced cognitive dysfunction by increasing BDNF expression and inhibiting neuroinflammation in the rat hippocampus. Neurosci Lett 604:161–166

Weiland BJ, Sabbineni A, Calhoun VD, Welsh RC, Hutchison KE (2015) Reduced executive and default network functional connectivity in cigarette smokers. Hum Brain Mapp 36:872–882

Weinberger AH, Pilver CE, Hoff RA, Mazure CM, McKee SA (2013) Changes in smoking for adults with and without alcohol and drug use disorders: longitudinal evaluation in the US population. Am J Drug Alcohol Abuse 39:186–193

Wenzel ED, Bachis A, Avdoshina V, Taraballi F, Tasciotti E, Mocchetti I (2017) Endocytic trafficking of HIV gp120 is mediated by dynamin and plays a role in gp120 neurotoxicity. J Neuroimmune Pharmacol 12:492–503

Wetherill RR, Jagannathan K, Hager N, Childress AR, Franklin TR (2015a) Sex differences in associations between cannabis craving and neural responses to cannabis cues: Implications for treatment. Exp Clin Psychopharmacol 23:238–246

Wetherill RR, Jagannathan K, Hager N, Childress AR, Rao H, Franklin TR (2015b) Cannabis, cigarettes, and their co-occurring use: Disentangling differences in gray matter volume. Int J Neuropsychopharmacol 18:pyv061

Wheelock MD, Reid MA, To H, White DM, Cropsey KL, Lahti AC (2014) Open label smoking cessation with varenicline is associated with decreased glutamate levels and functional changes in anterior cingulate cortex: preliminary findings. Front Pharmacol 5:158

Wingbermuhle R, Wen KX, Wolters FJ, Ikram MA, Bos D (2017) Smoking, APOE genotype, and cognitive decline: The Rotterdam study. J Alzheimers Dis 57:1191–1195

Wingo T, Nesil T, Choi J-S, Li MD (2016) Novelty seeking and drug addiction in humans and animals: From behavior to molecules. J Neuroimmune Pharmacol 11:456–470

Wojna V, Robles L, Skolasky RL, Mayo R, Selnes O, de la Torre T, Maldonado E, Nath A, Melendez LM, Lasalde-Dominicci J (2007) Associations of cigarette smoking with viral immune and cognitive function in human immunodeficiency virus-seropositive women. J Neurovirol 13:561–568

Yalcin EB, Nunez K, Tong M, de la Monte SM (2015) Differential sphingolipid and phospholipid profiles in alcohol and nicotine-

derived nitrosamine ketone-associated white matter degeneration. Alcohol Clin Exp Res 39:2324–2333

Yang Z, Nesil T, Connaghan KP, Li MD, Chang SL (2016) Modulation effect of HIV-1 viral proteins and nicotine on expression of the immune-related genes in brain of the HIV-1 transgenic rats. J Neuroimmune Pharmacol 11:562–571

Yang Z, Nesil T, Wingo T, Chang SL, Li MD (2017) HIV-1 proteins influence novelty-seeking behavior and alter region-specific transcriptional responses to chronic nicotine treatment in HIV-1Tg rats. Nicotine Tob Res 19:1024–1032

Yu R, Zhao L, Lu L (2011) Regional grey and white matter changes in heavy male smokers. PLoS One 6:e27440

Yu R, Deochand C, Krotow A, Leao R, Tong M, Agarwal AR, Cadenas E, de la Monte SM (2016) Tobacco smoke-induced brain white matter myelin dysfunction: Potential co-factor role of smoking in neurodegeneration. J Alzheimers Dis 50:133–148

Zhang X, Green MV, Thayer SA (2019) HIV gp120-induced neuroinflammation potentiates NMDA receptors to overcome basal suppression of inhibitory synapses by p38 MAPK. J Neurochem 148:499–515

Zhang X, Salmeron BJ, Ross TJ, Geng X, Yang Y, Stein EA (2011) Factors underlying prefrontal and insula structural alterations in smokers. Neuroimage 54:42–48

Zhou F, Liu X, Gao L, Zhou X, Cao Q, Niu L, Wang J, Zuo D, Li X, Yang Y, Hu M, Yu Y, Tang R, Lee BH, Choi BW, Wang Y, Izumiya Y, Xue M, Zheng K, Gao D (2019) HIV-1 Tat enhances purinergic P2Y4 receptor signaling to mediate inflammatory cytokine production and neuronal damage via PI3K/Akt and ERK MAPK pathways. J Neuroinflammation 16:71

Zou JY, Crews FT (2005) TNFα potentiates glutamate neurotoxicity by inhibiting glutamate uptake in organotypic brain slice cultures: neuroprotection by NFκB inhibition. Brain Res 1034:11–24

Zubieta J-K, Heitzeg MM, Xu Y, Koeppe RA, Ni L, Guthrie S, Domino EF (2005) Regional cerebral blood flow responses to smoking in tobacco smokers after overnight abstinence. Am J Psychiatry 162:567–577