Imaging Biomarkers of the Neuroimmune System among Substance Use Disorders: A Systematic Review

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Abstract

There is tremendous interest in the role of the neuroimmune system and inflammatory processes in substance use disorders (SUDs). Imaging biomarkers of the neuroimmune system in vivo provide a vital translational bridge between pre-clinical and clinical research. Herein, we examine two imaging techniques that measure putative indices of the neuroimmune system and review their application among SUDs. Positron emission tomography (PET) imaging of 18 kDa translocator protein availability is a marker associated with microglia. Proton magnetic resonance spectroscopy quantification of *myo*-inositol levels is a putative glial marker found in astrocytes. Neuroinflammatory responses are initiated and maintained by microglia and astrocytes, and thus represent important imaging markers. The goal of this re-

view is to summarize neuroimaging findings from the substance use literature that report data using these markers and discuss possible mechanisms of action. The extant literature indicates abused substances exert diverse and complex neuroimmune effects. Moreover, drug effects may change across addiction stages, i.e. the neuroimmune effects of acute drug administration may differ from chronic use. This burgeoning field has considerable potential to improve our understanding and treatment of SUDs. Future research is needed to determine how targeting the neuroimmune system may improve treatment outcomes.

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Introduction

There is increasing interest in the role of the brain's immune system, the “neuroimmune” system, in the pathophysiology of substance use disorders (SUDs) (Fig. 1). The neuroimmune system is related to disorder

Fig. 1. Results from a PubMed search on June 28, 2018, using the search terms: (1) “neuroimmune” or “neuroinflammation” and (2) “addiction” or “substance dependence” or “substance use disorder.”

pathogenesis [1], clinical severity [2, 3], and treatment trajectory (reviewed in [4–7]). Thus, a patient’s neuroimmune state may represent both a biomarker and treatment target. Treatment of chronic neuroimmune activation or disrupted neuroimmune signaling may significantly improve clinical outcomes for SUDs – hence the recent interest in better understanding these processes.

The immune system maintains homeostatic processes and is critical for survival. In the brain, the neuroimmune system protects neurons from damage caused by irritants or immunogenic stimuli. However, the neuroimmune system is also known to prune underutilized or damaged synapses, and chronic neuroinflammation can result in neuronal cell death. The neuroimmune system is complex and can exert tremendous influence on neuronal health and survival. In addition, neuroimmune signaling is integral in neural communication and cognitive function. One example to highlight this point is that experimental manipulation of interleukin (IL)-1 cytokine signaling modulates learning and memory function in preclinical studies [8–10]. Thus, disrupted neuroimmune signaling may contribute to, or exacerbate, underlying cognitive dysfunction in individuals with SUDs. Further, disrupted neuroimmune signaling may also contribute to psychiatric symptoms [7, 11], alter medication response [12, 13], and/or increase likelihood of SUD relapse [14, 15]. For example, a rigorous inpatient study among opioid-dependent subjects found ibudilast, a glial modulator that suppresses proinflammatory glial responses [16], reduced opioid-seeking behavior and self-reported opioid craving, relative to placebo levels [15]. These studies illustrate the complex and important role of the neuroimmune system in SUDs.

Hallmarks of a neuroimmune response include: (1) microglia and astrocyte activation; (2) elevated cytokine and chemokine levels; and (3) recruitment of leukocytes [17, 18]. Microglia are the brain’s resident macrophages and surveil their local environment for pathogens and other irritants [17, 18]. Upon detection of immunogenic stimuli, microglia become activated, which can include conformational changes, proliferation, and release of cytokines and chemokines [19]. Microglia activation occurs on a spectrum from proinflammatory and potentially neurotoxic (i.e., “M1” or “classical activation”) to anti-inflammatory and neuroprotective (“M2” or “alternative activation”) [19]. Under normal conditions, the initial response is typically dominated by proinflammatory processes until the irritant is removed, at which time, anti-inflammatory processes take precedence [17]. These states are not binary or static; rather, microglia dynamically change along a continuum between pro- and anti-inflammatory states in response to environmental signals [20, 21]. Astrocytes are also activated by immunogenic stimuli, albeit more slowly than microglia [22]. In addition to initiating a neuroimmune response, astrocytes can amplify microglia signals [23], and thus, play an important role in neuroimmune signaling. Acute neuroinflammatory responses are adaptive, promote neuronal survival, and restore homeostasis [24–26]. However, chronic proinflammatory signaling (often demarcated as longer than 6 weeks [27]) can be pathological and lead to significant glia and neuron cell death [28–33]. In vivo imaging can provide valuable insight into the neuroimmune state of patients and the role of the neuroimmune system in SUDs. In this review, we describe the current state of

imaging approaches to measure putative neuroimmune markers in SUDs and summarize findings across drugs of abuse.

Using Imaging to Measure Neuroimmune Biomarkers

Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy (MRS) is an imaging technique that facilitates quantitative measurement of metabolites in localized brain volumes [34]. With MRS, the neurometabolite *myo*-inositol is thought to be a biomarker of the neuroimmune system [35]. *Myo*-inositol is a putative glial marker located in cultured astrocytes [36–39] and is associated with microglia [40]. *Myo*-inositol functions as a secondary messenger [41, 42], osmolyte [36, 43–47], and precursor of membrane phospholipids [42]. *Myo*-inositol is synthesized from glucose and can be produced in significant quantities within minutes [48]. During neuroimmune activation, *myo*-inositol levels have been shown to increase in association with hypertrophic and proliferated glial cells [39]. Patients with clinical disorders known to be associated with a chronic proinflammatory state (e.g., Alzheimer's disease and multiple sclerosis [MS]) often exhibit higher *myo*-inositol levels [39, 49–51] (reviewed in [35]). In MS patients, higher *myo*-inositol levels were colocalized with brain lesions [39, 51]. Also, acute challenges with substances known to have proinflammatory effects (e.g., methylenedioxy-methamphetamine [MDMA]) increase *myo*-inositol levels in the brain [52]. However, *myo*-inositol has not been thoroughly validated as a neuroimmune marker and is not without caveats. Clinical studies have reported elevated *myo*-inositol levels in conditions not known to be associated with inflammation (reviewed in [53]). Also, *myo*-inositol is sensitive to osmotic changes [54–56] and edema [36, 43–47] which may confound neuroimmune investigations in SUDs (e.g., alcohol is known to exert osmotic effects [57]). Thus, *myo*-inositol is thought to be sensitive to neuroimmune signaling but exhibits only modest specificity. Finally, expression of *myo*-inositol levels is another important consideration. *Myo*-inositol levels are often normalized to either the unsuppressed water signal, i.e. “absolute” concentrations, or other metabolites, typically creatine or N-acetyl-aspartate (NAA). *Myo*-inositol normalized to unsuppressed water is susceptible to bias introduced by edema, which may accompany substance dependence and/or neuroinflammation [44, 58, 59]. As stated above, *myo*-inositol is involved in cell volume regulation, and thus, may be di-

rectly influenced by edema and/or osmotic changes [36, 43–47, 54–56]. Further, as described below, normalization to NAA may be susceptible to bias due to neurodegeneration [60], which may or may not be attributed to chronic neuroinflammation [35]. Conversely, normalization to creatine, often considered a stable metabolite, may be less susceptible to bias than NAA. However, there is evidence creatine levels are elevated in neuroinflammatory conditions [61, 62] and that normalization to creatine may increase measurement variability [63]. Thus, it is recommended to evaluate absolute *myo*-inositol concentration and *myo*-inositol normalized to creatine, especially in studies that contrast psychiatric patients with healthy volunteers. Convergent *myo*-inositol findings across normalization approaches will improve confidence that group differences are “real” and not due to a confound.

Other MRS metabolites may provide sensitive measures of neuroimmune signaling but exhibit nominally worse specificity. Lactate and choline levels are altered in chronic pro-neuroinflammatory conditions [39, 64], but also conditions not necessarily associated with neuroinflammation. For example, lactate levels are altered by phasic changes in neural activity [65, 66], metabolic processes [67–69], and impaired blood supply (ischemia/hypoxia; i.e., stroke [70, 71]). Similarly, brain choline levels are influenced by dietary choline consumption [72] and psychiatric comorbidities [73, 74]. NAA is a putative neuronal marker [35], and abnormally low levels may reflect neuronal degeneration [60], but it is not necessarily specific to neuroimmune signaling. In addition to MRS, several other imaging markers exist. Blood-brain barrier (BBB) permeability is associated with neuroimmune activation [75, 76] and can be imaged with MR and PET methods. However, abused substances that exert neuroimmune effects via mechanisms unrelated to BBB permeability, i.e. drugs that directly bind receptors on immune cells (e.g., opioids [77, 78]), may not be detected by BBB markers. Finally, imaging morphology can provide valuable metrics for tracking structural changes, but are not specific to neuroimmune signaling per se.

Positron Emission Tomography

PET imaging of the 18-kDa translocator protein (TSPO) provides a quantitative measure of a biomarker expressed in neuroimmune cells. TSPO is expressed on the outer mitochondrial membrane [18] and is highly colocalized with microglia [79]. In the brain, TSPO is also expressed in astrocytes and endothelial cells, but the signal predominantly comes from microglia [79–83]. The

classical immune stimulus, lipopolysaccharide (LPS; endotoxin), which is known to “activate” microglia [80], robustly increased in vivo TSPO levels in humans within hours of stimulus challenge [84]. Also, pharmacological depletion of microglia significantly reduced TSPO availability, which recovered 14 days after discontinuation of the challenge [83]. Neurological conditions characterized by chronic neuroinflammation (e.g., MS) exhibit higher TSPO levels compared to controls (e.g., [85, 86]). Thus, TSPO PET is sensitive to changes in neuroinflammatory state. However, TSPO findings are complicated by several factors [87]. First, TSPO levels do not differentiate pro- versus anti-inflammatory processes [87]. Elevated TSPO levels in this context may reflect an upregulation in either pro- or anti-inflammatory processes or both, or leukocyte infiltration. Conversely, diminished TSPO levels likely reflect a suppressed/impaired neuroimmune system and/or loss of cells expressing TSPO. Second, TSPO is not functionally involved in neuroimmune signaling (reviewed in [87]). Third, microglia can exhibit “activated” phenotypes in the absence of neuroimmune activation [88, 89], which may lead to false positives. Despite these caveats, TSPO PET imaging is widely used as an imaging marker of the in vivo neuroimmune system.

First-generation TSPO radiotracers, such as [^{11}C]PK11195, exhibit poor extraction and lower signal-to-noise than second-generation tracers (e.g., [^{11}C]PBR28 and [^{11}C]DPA-713) [90]. Second-generation radiotracers exhibit moderate specific binding as indicated by saturation studies among rhesus monkeys [91] and healthy human subjects [92]. However, since there is no brain region devoid of specific binding, arterial blood sampling is required for full quantification of TSPO radioligand distribution volumes (V_T), the “gold standard” outcome measurement [93]. While correction for the plasma free fraction (f_p) can improve quantification accuracy, f_p is very low (~2–3%) for second-generation radioligands. This limitation results in excessive variability in outcome measurements, which may contribute to discrepancies in the TSPO literature. An additional caveat is that the rs6971 genotype alters TSPO ligand binding affinity; therefore, genotype must be considered in subject recruitment and data analyses [94, 95]. Recessive homozygotes, i.e. “low-affinity binders,” exhibit minimal specific binding of TSPO ligands and should be excluded from analyses. Low-affinity binders are estimated to compose 10% of the population.

Finally, multimodal neuroimaging approaches that measure both *myo*-inositol and TSPO levels could be more powerful and may reduce false positives in studies

that contrast groups by psychiatric diagnosis. TSPO PET and *myo*-inositol MRS levels were measured in tandem among amyotrophic lateral sclerosis (ALS) [96], hepatitis C [97], and severe MS patients [98]. In the hepatitis C and MS studies, *myo*-inositol and TSPO levels were elevated in patients relative to controls, but *myo*-inositol and TSPO levels were not correlated in either study [97, 98]. Among ALS patients, *myo*-inositol and TSPO levels were positively correlated, albeit TSPO levels were quantified using an unconventional semi-quantitative approach [96]. Thus, *myo*-inositol and TSPO may measure related, but distinct, neuroimmune processes that converge in specific phenotypes. Elevated *myo*-inositol levels may primarily reflect astrocyte activation [37, 38], while elevated TSPO levels may be associated with microglia activation [80] which may explain their statistical divergence.

Imaging the Neuroimmune System in SUDs

The primary goal of this review is to summarize what is known about the neuroimmune consequences in people with long-term substance use across six commonly abused substances: tobacco, alcohol, cocaine, opioids, cannabis, and MDMA/methamphetamine. It is important to note that imaging neuroimmune biomarkers in SUDs are complicated by numerous factors, including heterogeneous patterns of substance use, diverse mechanisms of action, and several routes of administration. The pharmacological state of subjects at time of the scan (i.e., satiation vs. acute withdrawal vs. prolonged abstinence) may influence neuroimmune findings independent of long-term substance use. Psychiatric disorders, such as major depression, are often comorbid with SUDs, which may also influence neuroimmune state [99]. Finally, as studies often contrast healthy controls versus SUDs, it is essential that groups are well-matched for immune-related confounds, such as ancillary substance use and subject age, to isolate the effect of the primary substance used.

In this review, we conducted PubMed searches prior to June 28, 2018. See Figure 2 for search terms and results. For MRS studies, each search was repeated excluding terms in step 1 to expand study inclusion. Only studies of humans and non-human primates reporting either TSPO PET data (Table 1) or *myo*-inositol MRS data (Tables 2, 3) were included. Moreover, only studies that contrasted SUDs versus healthy controls or examined acute pharmacological effects were considered. Rodent studies were consulted for mechanistic interpretation but are not sum-

Fig. 2. The PubMed search terms and results for each drug category are depicted. Search terms from boxes 1, 2, and 3 were used to identify articles. Only studies that investigated neuroimmune effects in humans or non-human primates and reported comparisons with healthy control subjects or acute pharmacological effects were included and summarized in this article. For MRS, studies

were only included if *myo*-inositol results were reported. Also, numerous MRS studies did not specifically investigate neuroimmune signaling. Thus, for each drug category, MRS searches were repeated only using terms from boxes 2 and 3 to expand study inclusion. Note: commas indicate “OR” in PubMed searches.

marized in tables. Finally, it should be noted that few MRS studies specifically investigated *myo*-inositol as a marker of neuroimmune signaling. As such, MRS experimental designs and acquisition sequences may not have been optimized to detect group differences in *myo*-inositol. Conversely, every PET study that was included specifically investigated TSPO as a neuroimmune marker, and thus, TSPO PET findings receive greater emphasis than *myo*-inositol findings throughout this review.

Results

Tobacco Smoking

The following section is focused on smoked tobacco and not solely nicotine. Nicotine, in isolation, is immunosuppressive in preclinical studies and in the human periphery [100–102]. However, nicotine is only one of > 3,000 constituents found in tobacco smoke. The preponderance of existing evidence suggests acute or short-term tobacco smoke exposure is pro-neuroinflammatory, while long-term tobacco smoking may suppress or impair the neuroimmune system. This is a working hypothesis with numerous caveats and one that attempts to uni-

fy data from two neuroimaging markers, peripheral human markers, and preclinical findings.

Three MRS studies of tobacco smokers were identified (Table 2). Each study imaged individuals without requiring smoking abstinence prior to scanning [103–105]. Two MRS studies found no difference in *myo*-inositol levels between tobacco smokers versus healthy controls across brain regions [103, 104], while the third found *myo*-inositol levels were significantly lower by ~12% among tobacco smokers relative to healthy controls in the dorsolateral prefrontal cortex, but not the anterior cingulate cortex (ACC) [105]. However, in that study, smokers reported more frequent alcohol use than controls, which may have contributed to these findings [105]. Gutzeit et al. [103] examined *myo*-inositol levels in the insula of tobacco smokers following 24 h abstinence from smoking and after acute Nicorette® (2 mg) administration and found *myo*-inositol levels were unchanged.

One PET study of tobacco smokers was identified (Table 1). In a [¹¹C]DAA1106 PET study, TSPO availability was measured in 30 daily tobacco smokers versus 15 age-matched nonsmokers [106]. Smokers smoked to satiety (2–3 cigarettes; preferred brand) 30 min prior to the 90-min PET scan. The authors reported standardized uptake

Table 1. PET studies of TSPO levels among SUDs

Drug	Citation	Tracer	Subjects	Methods	Analyses	Findings	Comments
Tobacco	Brody et al. [106], 2017	[¹¹ C]DAA1106; 90 min	30 ND (14 cigs/d; FTND = 4) vs. 15 HC; MAB/HAB	Scanned: smoked to satiety (2–3 cigs) 30 m prior	SUV, t = 20–40 min; covariate: rs6971 genotype	Global TSPO: ND <HC (17%)	Lower TSPO among ND; higher cigs/day ~ lower TSPO; well-matched HCs; SUV, not V _T
Alcohol	Kalk et al. [146], 2017	[¹¹ C]PBR28; 90 min	9 AD (5.6 kg alcohol/past mo) vs. 20 HC; MAB/HAB	Scanned: 3 wk BZD-assisted withdrawal; ad lib smoking	V _T ; covariates: age, rs6971 genotype	HPC TSPO: AD <HC (19%)	Lower TSPO among AD; HCs not well-matched (sex, genotype, psychiatric symptoms, smoking)
Alcohol	Hillmer et al. [142], 2017	[¹¹ C]PBR28; 120 min	15 AD (6 drinks/d; 22 yrs) vs. 15 HC; MAB/HAB	Scanned: 1–4 d abstinent; ad lib smoking	V _T ; covariate: rs6971 genotype	Global TSPO: AD <HC (10%)	Lower TSPO among AD; more alcohol use and AD severity ~ lower TSPO; well-matched HCs
Alcohol	Kim et al. [143], 2018	[¹¹ C]PBR28; 120 min	19 AD (9 drinks/d; 30 yrs) vs. 17 HC; MAB/HAB	Scanned: 0–7 d abstinent; ad lib smoking	V _T ; rs6971 MABs and HABs evaluated separately	Global TSPO: AD <HC (MABs only)	Lower TSPO among AD (MABs only); higher cholesterol ~ lower TSPO; HCs not well matched
Alcohol	Saba et al. [147], 2017	[¹⁸ F]DPA-714; 55 min	3 alcohol-naïve baboons (3–4 yrs); not genotyped	Scanned: before, during, and 7–12 mo after alcohol binge (0.8 g/L; 3 h)	V _T	Global TSPO: during >after >before alcohol binge	TSPO increased acutely and 7–12 mo after single alcohol binge; within-subject design
Cocaine	Narendran et al. [183], 2014	[¹¹ C]PBR28; 90 min	15 CD (USD 346/wk, 17 yrs) vs. 17 HC; LAB/MAB/HAB	Scanned: 14 d abstinence (UDS); ad lib smoking	V _T ; covariate: rs6971 genotype	No global or ROI TSPO differences	TSPO did not differ by group; well-matched HCs; allowed ND
Opioids	Auvity et al. [223], 2017	[¹⁸ F]DPA-714; 120 min	5 M baboons (8–10 yrs); 2 MAB, 3 HAB	Scanned: before and 2 h after morphine (i.m. 1 mg/kg)	V _T	Global TSPO: morphine >baseline (30%)	TSPO increased after morphine (including MOR-deficient regions); driven by HAB
Meth	Sekine et al. [209], 2008	[¹¹ C]PK11195; 62 min	12 MA (6.8 yrs) vs. 12 HC; not genotyped	Scanned: 1.8 yrs abstinent; ND/AD not allowed	SRTM; BP (k ₃ /k ₄); input function = normalized mean HC TACs	TSPO BP: MA >HC (264–1,530%)	Higher TSPO among MA; unconventional analytic strategy and first generation radiotracer

AD, alcohol dependent (DSM-IV criteria); BP, binding potential; CD, cocaine dependent (DSM-IV criteria); HAB, rs6971 genotype high-affinity binder; HC, healthy control subject; HPC, hippocampus; LAB, rs6971 genotype low-affinity binder; MA, methamphetamine users (not necessarily dependent); MAB, rs6971 genotype moderate-affinity binder; ND, nicotine dependent (DSM-IV criteria); SRTM, simplified reference tissue model; SUV, standardized uptake values; UDS, urine drug screen; V_T, volume of distribution; ~, significant bivariate correlation.

values (SUV) from 20 to 40 min post-injection were 16.8% lower (range: 14.6–19.7%) among tobacco smokers versus controls. Lower [¹¹C]DAA1106 SUV was associated with more cigarettes smoked/day. However, SUV is a semi-quantitative outcome measure that does not account for possible differences in radioligand concentrations in plasma. Indeed, preliminary data from a separate group measuring [¹¹C]PBR28 distribution volumes (V_T), which account for radiotracer behavior in plasma, suggest no group differences between tobacco smokers and healthy controls [107].

Preclinical research indicates that <6 months of tobacco smoke exposure was associated with higher levels of proinflammatory cytokine mRNA expression levels in rodent brains [108, 109] and immunohistochemical markers of microglia and astrocyte activation [110], compared to control animals. Conversely, there is evi-

dence for lower levels of neuroimmune markers in long-term (>1 year) tobacco smoking compared to controls [105, 106]. Also, in vivo human neuroimaging data indicate long-term tobacco smokers exhibit lower gray matter volume compared to nonsmokers, perhaps indicative of cell loss [111, 112]. It is well-documented that tobacco smokers recover from illness and injury more slowly than nonsmokers, indicative of impaired immune function [101, 113]. A unifying hypothesis for the preclinical and human data is that acute or short-term tobacco smoke exposure is pro-neuroinflammatory, while long-term exposure may suppress or impair the neuroimmune system and may be neurotoxic. A possible cause of the long-term effects of tobacco smoke is disrupted pro-inflammatory cytokine production. Relative to healthy subjects, cultured macrophages from tobacco smokers exhibit diminished proinflammatory cytokine secretion

Table 2. MRS studies of *myo*-inositol levels among SUDs

Drug	Citation	Imaging	Voxel/s	Subjects	Methods	Findings	Comments
Tobacco	Gutzeit et al. [103], 2013	3T, PRESS, TE = 30 ms, TR = 2 s, 96 avgs	L and R insula (15 cm ³)	14 ND (28 cigs/d; 33 yrs) vs. 10 HC	Scanned: ad libitum, 24 h abstinence, and 40 min after 2 mg Nicorette®	Insula: Gln increased 14% w/24 h abstinence and Glu decreased 4% w/sublingual Nicorette®	Myo-I did not differ by group or w/abstinence or 2 mg Nicorette®; only M; HC not matched on age
Tobacco	Mennecke et al. [104], 2014	3T, PRESS, TE = 30 ms, TR = 1.7 s, 128 avgs	L, R HPC and L, R ACC (2 cm ³)	12 ND (≥20 cigs/d; ≥2 yrs) vs. 12 HC	Scanned: ad libitum smoking and 3d abstinence	ACC Glx/Cr: ND > HC (29%); ACC Cho/Cr: ND < HC (18%)	Myo-I did not differ by group or w/abstinence; well-matched HCs
Tobacco	Durazzo et al. [105], 2016	4T, STEAM, TE = 12 ms, TR = 2 s, TA = 2.5 min	R dlPFC (16 cm ³), ACC (17.5 cm ³)	35 ND (27 pack-years; 18 cigs/d) vs. 30 HC	Scanned: ad libitum smoking	dlPFC Myo-I, NAA, Cr, and Glu: ND < HC; ACC NAA: ND < HC*; ACC Cho: ND > HC*	Lower Myo-I (12%) among ND; HC not matched on alcohol use
Cannabis	Chang et al. [168], 2006	4T, PRESS, TE = 30 ms, TR = 3 s	BG, thalamus (3–5 cm ³)	21 MJ+HIV+ (13 d/mo) vs. 24 MJ (20 d/mo) vs. 21 HIV+ vs. 30 HC	Scanned: active MJ use and ad libitum smoking	BG Myo-I (27%): MJ < HC; BG NAA, Cho, Glu: MJ, MJ+HIV < HIV, HC; thalamus Cr: MJ, MJ+HIV > HIV, HC	Lower Myo-I among MJ (27%), independent of HIV; well-matched HC
Cannabis	Prescot et al. [170], 2011	3T, PRESS, TE = 30 ms, TR = 2 s, 128 avgs	ACC (15 cm ³)	17 adolescent MJ (100+ uses/past yr) vs. 17 HC	Scanned: active MJ use and ad libitum smoking	ACC Myo-I (10%), Glu (15%), NAA (13%), and Cr (10%): MJ < HC	Lower Myo-I among adolescent MJ (10%); HC mostly well matched
Cannabis	Silveri et al. [171], 2011	4T, PRESS, TE-averaged (30–330 ms), TR = 1.25 s	MRSI; nominal voxel (6.6 cm ³)	13 M MJ (6 uses/wk; 6 yrs; 13 drinks/wk) vs. 10 M HC	Scanned: active MJ use and ad libitum smoking	Global Myo-I/Cr: MJ < HC (20%)	Lower Myo-I/Cr among MJ (20%); HC not matched on drug use; M only
Cannabis	Muetzel et al. [167], 2013	3T, TE = 71 ms, TR = 3 s, 192 avgs	BG (8 cm ³)	27 MJ (25 d/mo; 9 uses/d) vs. 26 HC	Scanned: 12 h MJ abstinent	BG Myo-I sex × group interaction: M MJ < M HC, F MJ > F HC	Myo-I did not differ by group; interaction w/sex; HC not matched on alcohol use
Cannabis	Mashhoon et al. [169], 2013	4T, PRESS, TE-averaged (30–330 ms), TR = 1.25 s	MRSI; nominal voxel (6.6 cm ³)	13 M MJ (6 uses/wk; 5 yr; 17 drinks/wk) vs. 10 M HC	Scanned: active MJ use	Thal Myo-I/Cr: MJ < HC (36%)	Lower Myo-I/Cr among MJ (36%); HC not matched on alcohol use; M only
Cocaine	Chang et al. [179], 1997	1.5T, STEAM, TE = 30 ms, TR = 2 s, 128 avgs	Occipital GM and subcortical WM (7–15 cm ³)	19 CU (regular use 7 yrs) vs. 25 HC	Scanned: 47 mo cocaine abstinence; ad libitum smoking	Subcortical WM Myo-I: CD > HC (18%) and Cr: CD > HC (Occipital GM Cr p = 0.07)	Higher Myo-I levels among CD (18%)M; HC not well matched for drug use; only AA M
Cocaine	Chang et al. [182], 1999	1.5T, PRESS, TE = 30 ms, TR = 3 s, 128 avgs	Frontal GM and frontal WM (3–5 cm ³)	64 CD (6 d/wk; 9 yr) vs. 58 HC	Scanned: 10 mo cocaine abstinence; ad libitum smoking	Frontal GM and WM Myo-I and Myo-I/Cr: CD > HC (5–12%); frontal GM and WM NAA and NAA/Cr: CD < HC (3–4%)	Higher Myo-I among CD (5–12%); F more frequent cocaine use than M; unclear if groups well matched
Cocaine	Yang et al. [181], 2009	3T, PRESS, TE-averaged (35–355 ms), TR = 2 s, 128 avgs	ACC (8 cm ³)	14 CU (13 yrs; USD 213/wk) vs. 14 HC	Scanned: 44 h cocaine abstinence; ad libitum smoking	ACC Glu/Cr: CU < HC (16%)	Myo-I/Cr did not differ by group; unclear how many CU met DSM criteria for CD; well-matched HC
Cocaine	Abé et al. [125], 2013a	1.5T, MRSI, TE = 25 ms, TR = 1.8 s, TI = 165 ms	15-mm-thick slices; nominal voxel (1 cm ³)	18 CU (319 drinks/mo; 48 g cocaine/mo; 8 cigs/d) vs. 36 HC	Scanned: 1 mo alcohol and stimulants abstinence; ad libitum smoking	Temporal, cerebellar, subcortical GM Myo-I: CU > HC (12–17%); midbrain NAA: CU < HC (“trend”)	Higher Myo-I among CU (AD+CD; 12–17%); HC not well matched on drug use; CU only M
Cocaine	Hulka et al. [180], 2016	3T, PRESS, TE-averaged (26–224 ms), TR = 1.6 s, 800 avgs	ACC and dlPFC (9 cm ³)	18 CU (1.5 g/wk; 10 yrs) vs. 18 HC	Scanned: 1 wk cocaine and >24 h alcohol abstinence; ad libitum smoking	dlPFC Myo-I/Cr: CD > HC (23%; p = 0.06); dlPFC and ACC Cho/Cr: CU > HC (7–13%); ACC Glu/Cr: CD > HC	Myo-I levels “trended” higher among CU; only 50% CU met DSM-IV CD criteria; only M; HC not well matched for drug use
Opioids	Yucel et al. [217], 2007	3T, PRESS, TE = 30 ms, TR = 3 s, 128 avgs	L and R ACC (6.5 cm ³)	24 OD (opioids 9 yrs; 10 MMT/14 BMT) vs. 24 HC	Scanned: 24 h abstinence; ad libitum smoking	ACC NAA: OD < HC (9%); Glx: OD < HC (9%)	Myo-I did not differ by group; HC not well-matched for drug use
Opioids	Deng et al. [220], 2012	14.1T, ex vivo, TR = 7.66 s	PFC, HPC; tissue samples	12 OD rhesus monkeys (escalating s.c. M 3–15 mg/kg tid; 90 d) vs. 4 HC	Tissue analyzed after 90 d M + 7 d: MMT (n = 4), clonidine (n = 4), PLC (n = 4)	HPC Myo-I, Glu, Cho: OD < HC; HPC GABA, Gln, Lac: OD > HC; PFC GABA, Glu, Myo-I: OD < HC; PFC NAA, Cho, Lac, Cr: OD > HC	Lower Myo-I among OD (saline; withdrawal group), but not in MMT or clonidine; M and F included

Table 2. (continued)

Drug	Citation	Imaging	Voxel/s	Subjects	Methods	Findings	Comments
Opioids	Cloak et al. [219], 2011	9.4T, ex vivo, TR = 6 s, 128 avgs	Frontal WM, frontal GM, putamen, caudate	15 SIV+OD rhesus macaques (2 mg/kg inj M qid >2 yr) vs. 14 SIV+	Ex vivo tissue samples analyzed	Putamen Myo-I: SIV+OD >SIV+ (16%); frontal WM Cr: SIV+OD >SIV+ (6%; $p = 0.08$)	Higher Myo-I among OD (16%); variable AIDS (41%); CD4+ <200 μ L and survival time (AIDS < SIV); only M w/SIV+
Opioids	Verdejo-Garcia [218], 2013	3T, PRESS, TE = 30 ms, TR = 3 s, 128 avgs	L and R ACC (6.5 cm ³)	24 OD (10 MMT, 20 mo; 14 BMT, 47 mo) vs. 24 HC	Scanned: 24 h abstinent; ad libitum smoking	ACC Myo-I: BMT > HC (11%); NAA: MMT, BMT <HC; Glx: MMT, BMT <HC	Higher Myo-I among BMT (11%), not MMT; HC not well matched drug use
Opioids	Hansen et al. [221], 2014	3T, PRESS, TE = 30 ms, TR = 2 s, 128 avgs	ACC (8 cm ³)	20 HC (30 mg PO morphine vs. PLC)	Scanned: pre- and post-pain challenge; PLC vs. morphine	ACC Myo-I/Cr, Glu/Cr, and NAA/Cr: morphine+pain <pain; ACC NAA/Cr: pain >rest	Morphine decreased Myo-I/Cr during pain; within-subject, placebo-controlled, cross-over design HCs
Opioids	Hansen et al. [222], 2016	3T, PRESS, TR = 2 s, TE = 30 ms, 128 avgs	ACC (8 cm ³), Insula (15 cm ³), PFC (4.5 cm ³)	20 HC (PO oxycodone ER 10 mg/12 h; PO venlafaxine ER 37.5 mg/12 h; PLC)	Scanned: pre- post-5 d dosing each drug	Across ROIs, Glu/Cr decreased after 5d ox-yodone (8%)	Myo-I/Cr levels not altered by venlafaxine or oxycodone; within-subject, placebo-controlled, cross-over design w/HCs
Opioids	Murray et al. [138], 2016	4T, STEAM, TE = 12 ms, TR = 2 s, MEGA-PRESS, TE = 71 ms, TR = 2 s	ACC (17.5 cm ³), POC (16 cm ³), dlPFC (16 cm ³)	21 OD (16 g/mo, 11 yr; BMT >3 mo) vs. 28 HC	Scanned: 3 wks opioids abstinent; BMT >3 mo; ad libi-tum smoking	dlPFC Myo-I, NAA, and Cr: OD <HC; ACC Myo-I, NAA, and Cr: OD <HC	Lower Myo-I among BMT OD (30–48%); HC not well-matched on alcohol use; OD comorbid w/ND, SD, and CD

AA, African-American; ACC, anterior cingulate cortex; AD, alcohol dependent subjects (DSM criteria); AIDS, acquired immunodeficiency syndrome; avgs, # of spectra averaged for quantification; BG, basal ganglia; BMT, buprenorphine maintenance therapy; CD, cocaine-dependent subjects (DSM criteria); Cho, total choline (glycerophosphocholine + phosphocholine); Cr, total creatine (phosphocreatine + creatine); CR, corona radiata; CU, cocaine users (not necessarily CD); dlPFC, dorsolateral prefrontal cortex; DTL, diffusion tensor imaging; ER, extended release formulation; GABA, gamma-aminobutyric acid; Gln, glutamine; Glu, glutamate; Glx, glutamate + glutamine; GM, gray matter; HC, healthy control subjects; HD, heavy alcohol drinkers (not necessarily AD); HIV+, human immunodeficiency virus positive; HIV-, HIV negative; HPC, hippocampus; Lac, lactate; meth, methamphetamine; MJ, marijuana/cannabis users; MMT, methadone maintenance therapy; MRSI, proton magnetic resonance spectroscopy imaging; MTL, medial temporal lobe; Myo-I, *myo*-inositol; NAA, n-acetylaspartate; ND, nicotine-dependent subjects (DSM criteria); ns, non-smokers; OD, opioid-dependent subjects (DSM criteria); PFC, prefrontal cortex; PLC, placebo; PLIC, posterior limb of internal capsule; PO, per os (oral dosing); POC, parietal-occipital cortex; PRESS, point resolved spectroscopy; qid, 4 $\times$ daily; ROI, regions of interest; s, cigarette smokers (but not necessarily ND); s.c., subcutaneous injection; SIV+, simian immunodeficiency virus infected; STEAM, stimulate echo acquisition mode; T, tesla; TA, acquisition time; TE, echo time; TE-averaged, range of echo times; TI, inversion time; tid, 3 $\times$ daily; TR, repetition time; WM, white matter.

after incubation with LPS, while anti-inflammatory cytokine secretion was unaffected [114–116]. However, there is considerable evidence opposing this working hypothesis [103, 104, 107, 117, 118]. Future research is needed to parse these relationships.

Alcohol

A working hypothesis for alcohol's neuroimmune effects is that acute alcohol consumption, especially "binge" drinking, is pro-neuroinflammatory, while long-term use suppresses or impairs the neuroimmune system. There is reliable TSPO PET imaging evidence supporting this hypothesis, while the *myo*-inositol MRS literature is mixed and inconclusive.

Our search identified 20 MRS studies that contrasted *myo*-inositol levels between healthy controls and alcohol-abusing/-dependent individuals (Table 3). Subjects were imaged at various lengths of alcohol abstinence up to 3 months since last drink. Four of these studies reported higher *myo*-inositol levels in alcohol-dependent subjects

than healthy controls [119–122], while 15 reported no difference [123–138]. Gazdzinski et al. [139] reported no difference for alcohol-dependent individuals (DSM-IV criteria), but higher *myo*-inositol levels among "heavy" drinkers (100+ drinks/month; 96% met DSM-IV criteria for alcohol abuse or dependence), relative to controls. Across studies, elevated *myo*-inositol levels were observed throughout the brain: ACC, thalamus, frontoparietal gray and white matter, and the occipital cortex [119–122, 139]. Finally, Durazzo et al. [128], reported a significant increase in *myo*-inositol levels from 1 to 4 weeks of abstinence, but these levels did not differ from healthy controls at either time point. Abnormal liver function, which has been reported to be associated with lower *myo*-inositol levels [140, 141], could be a confound. Of the 15 alcohol studies that reported no *myo*-inositol alterations, 4 studies excluded subjects who did not exhibit normal liver function [126, 132, 133, 139], 5 studies *may have* excluded subjects without normal liver function [127–130, 135], and 6 studies did not exclude subjects based on, or

Table 3. MRS studies of *myo*-inositol levels among alcohol and stimulant users

Citations	Myo-I: AUD ~ HC Voxel/s	Details	Citations	Myo-I: AUD >HC voxel/s	Details
Seitz et al. [123], 1999	Cerebellum	Scanned: 3–6 days abstinence	Schweinsburg et al. [120], 2000	ACC, thalamus	Scanned: 3–5 wk abstinent
Parks et al. [133], 2002	Frontal WM, cerebellum	Scanned: 3–5 d, 3 wks, and 3 mo abstinence; Myo-I unchanged w/abstinence	Schweinsburg et al. [119], 2001	Frontal WM, parietal WM	Scanned: <28 d abstinent
Schweinsburg et al. [134], 2003	Frontal GM, frontal WM	Scanned: 1 mo abstinent	Meyerhoff et al. [2004], 2004	Parietal GM	Scanned: 12 h abstinence
Durazzo et al. [127], 2004	Frontal, parietal, subcortical GM, WM	Scanned: 1 wk abstinent	Gazdzinski et al. [129], 2010	Parietal GM and WM	Scanned: 12 h+ abstinent
Durazzo et al. [128], 2006	Frontal, parietal, subcortical GM, WM	Scanned: 1 wk and 4 wk abstinent; frontal WM Myo-I increased w/abstinence	Bagga et al. [122], 2014	Occipital	Scanned: 2–3 wk abstinent; Myo-I/Cr
Bartsch et al. [126], 2007	Frontal GM, cerebellum	Scanned: <7 d and 3–4 wks abstinent; Myo-I did not change w/abstinence			
Lee et al. [131], 2007	ACC, insula	Scanned: 2 wk abstinent; ACC Myo-I AD <HC, $p = 0.06$			
Gazdzinski et al. [129], 2010	Parietal GM and WM	Scanned: 1 wk abstinent			
Gazdzinski et al. [130], 2008	Medial temporal lobe	Scanned: 1 wk and 4–5 wk abstinent; Myo-I did not change w/abstinence			
Wang et al. [135], 2009	Frontal, parietal, subcortical WM	Scanned: 1 mo abstinent			
Gazdzinski et al. [129], 2010	Frontal, temporal, parietal, occipital WM	Scanned: 1 wk and 4–5 wk abstinent; Myo-I did not change w/abstinence			
Mon et al. [132], 2012	ACC, dlPFC, parietal/occipital	Scanned: 1 and 5 wk abstinent; Myo-I did not change w/abstinence			
Yeo et al. [136], 2013	ACC	Scanned: 24 h abstinent			
Zahr et al. [137], 2014	BG, cerebellum, pons	Scanned: BAC = 0.0			
Murray et al. [138], 2016	dlPFC, ACC, POC	Scanned: 21 d abstinent			
Citations	Myo-I: SD ~ HC voxel/s	Details	Citations	Myo-I: SD >HC voxel/s	Details
Taylor et al. [208], 2000	Frontal WM, ACC, caudate	SD; scanned: unclear	Chang et al. [182], 1999	Frontal GM, occipital, parietal WM	MDMA; scanned: 4 mo abstinent; parietal WM Myo-I and Myo-I/Cr
Reneman et al. [205], 2002	Frontal GM, occipital, parietal WM	MDMA; scanned: 12 wk abstinent; Myo-I/Cr	Ernst et al. [194], 2000	Frontal GM, frontal WM, BG	Meth; scanned: 4 mo abstinent; frontal GM Myo-I
Cowan et al. [207], 2007	Occipital	MDMA (mostly <40 uses); scanned: >3 wk abstinent	Chang et al. [193], 2005	Frontal GM, frontal WM, BG	Meth; scanned: 5 mo abstinent; frontal GM Myo-I
Taylor et al. [203], 2007	Frontal GM, frontal WM, BG	Meth; scanned: 2 wk abstinent	Sung et al. [195], 2007	Frontal WM, frontal GM	Meth; scanned: 2.1 yr abstinent; frontal WM Myo-I
de Win et al. [204], 2008	Parietal WM, frontal GM, occipital	Scanned before and after MDMA use (~6 tabs); 4–5 mo abstinent	Abulseoud et al. [192], 2009	Frontal WM; PCC	Meth; scanned: 1.9 yrs abstinent; frontal WM Myo-I
Ernst and Chang [199], 2008	Frontal GM, frontal WM, BG	Meth; scanned: 2 mo and 7 mo abstinent	Liu et al. [191], 2011	BG, frontal GM	MDMA; scanned: active use; BG Myo-I

Table 3. (continued)

Citations	Myo-I: AUD ~ HC Voxel/s	Details	Citations	Myo-I: AUD >HC voxel/s	Details
Cloak et al. [198], 2011	ACC, frontal WM, BG, thalamus	Meth; young adults; scanned: mostly abstinent ~8 mo	Abé et al. [124], 2013	ACC, POC, dlPFC	Meth; scanned: 3+ wks abstinent; dlPFC Myo-I
Sung et al. [202], 2013	Frontal GM	Meth; scanned: active use; adolescents; Myo-I/Cr	Yang et al. [196], 2015	ACC, BG	Rhesus macaques; Meth; scanned 1 wk, 1 mo, 3 mo, 6 mo, and 1 yr abstinent; ACC Myo-I/Cr higher at 1 wk
Howells et al. [200], 2014	Frontal GM, frontal WM, ACC	Meth; scanned: >24 h abstinent (~2 mo)	Burger et al. [197], 2018	dlPFC, frontal WM, ACC	Meth; scanned: 5 wks abstinent; ACC Myo-I/Cr; ACC Myo-I/Cr increased and frontal WM Myo-I/Cr decreased after 1 mo abstinence
Kim et al. [201], 2018	ACC	Meth; adolescents; scanned: active use			

Alcohol use disorder (AUD) studies are located in the upper panel and methamphetamine/MDMA (stimulant dependent: SD) studies in the lower panel. ACC, anterior cingulate cortex; CC, anterior cingulate cortex; BG, basal ganglia; Cr, total creatine; dlPFC, dorsolateral prefrontal cortex; GM, gray matter; MDMA, methylenedioxymethamphetamine ("ecstasy"); Meth, methamphetamine; Myo-I, *myo*-inositol; PCC, posterior cingulate cortex; POC, parietal-occipital region; WM, white matter; ~, groups not different.

did not describe testing, liver function [123, 131, 134, 136–138].

Three human PET studies were identified (Table 1). In the first study, Hillmer et al. [142], scanned 15 alcohol-dependent individuals using [^{11}C]PBR28, while inpatient (no benzodiazepines administered) and abstinent (93% within 1–4 days since last drink), matched with healthy controls for age, gender, cigarette smoking status, and rs6971 genotype. Global TSPO availability (partial-volume corrected V_T) was significantly lower by 10% in alcohol-dependent subjects than controls. In addition, more drinks/day and more severe alcohol dependence levels were associated with lower TSPO availability in the hippocampus and striatum. Peripheral monocytes from alcohol-dependent subjects exhibited blunted proinflammatory cytokine production (IL-6 and IL-8) following LPS incubation, compared to controls. In the second study, Kim et al. [143] imaged 19 alcohol-dependent individuals ~3 days since last drink (range: 0–7 days) and 17 healthy controls using [^{11}C]PBR28. Control subjects were well-matched for demographic characteristics, but not cigarette smoking. Results indicated global brain TSPO availability was lower by ~25–30% (estimated; no partial-volume correction) in alcohol-dependent individuals versus controls, but only among medium-affinity binders (rs6971 genotype) and not high-affinity binders. At present, it is unclear how rs6971 genotype and radiotracer binding affinity would influence alcohol's neuroimmune effects. Moreover, it should be noted that 5 alcohol-dependent subjects took benzodiazepines within 2 days of scanning in this study. Benzodiazepines are

known to bind TSPO [144] and thus could influence TSPO availability [145]. However, the author's reported [^{11}C]PBR28 binding was unaffected among those subjects [143]. In the third study, Kalk et al. [146] imaged 9 alcohol-dependent subjects approximately three weeks since their last drink (ad libitum cigarette smoking prior to scanning) and healthy controls. This [^{11}C]PBR28 study reported significantly lower TSPO availability (partial-volume corrected V_T) by 19% in the hippocampus relative to age-matched healthy controls, with similar trends in the midbrain, thalamus, ACC, and cerebellum. On average, subjects were imaged 14 days since last benzodiazepine dose, and thus, benzodiazepines were unlikely to alter TSPO availability in this study [146].

A preclinical PET study measured TSPO levels in 3 alcohol-naïve baboons before, during, and 7–12 months after an intravenous alcohol infusion titrated to blood ethanol levels of 0.7–1.0 g/L [147]. Alcohol infusion significantly increased global TSPO V_T by 58–138% above baseline levels. Global TSPO levels remained elevated for 7–12 months after the alcohol infusion (estimated: 30–90%). These data show an acute neuroimmune response to binge levels of alcohol, consistent with the preclinical literature [148–150].

A unifying hypothesis for these findings is that acute alcohol consumption, especially "binge" drinking, is proinflammatory, while long-term use is associated with a suppressed neuroimmune system, microglia cell loss, and neurodegeneration. Evidence supporting this working hypothesis is as follows. First, preclinical research indicates acute alcohol exposure increases proinflammatory

markers systemically [151, 152] and in the brain [153, 154]. Second, human studies of long-term alcohol consumption observe significantly lower TSPO levels [142, 143, 146], which remain lower than controls 3 weeks since last drink [146]. Third, in vivo gray matter volume is lower among alcohol-dependent subjects versus healthy controls [155]. Fourth, postmortem data indicate lower microglia, astrocyte, and oligodendrocyte levels in the hippocampi of alcohol-dependent individuals, compared to controls [156]. Alcohol may exert neuroimmune effects via several mechanisms: increased gut permeability and systemic exposure to resident gut bacteria [157]; interaction at Toll-like receptors 4s (TLR4s) on microglia and/or nuclear factor kappa-B [153, 158, 159]; BBB disruption [160]; or via adenosine's (byproduct of alcohol metabolism) inflammatory properties [161, 162].

There is evidence opposed to the working hypothesis related to long-term alcohol consumption: (1) 5 MRS studies that reported higher *myo*-inositol levels among alcohol-dependent individuals and "heavy" drinkers compared to controls [119–122, 139]; (2) none of the 20 MRS studies found significantly lower *myo*-inositol levels among alcohol-dependent individuals compared to controls; and (3) postmortem studies that reported higher microglia biomarker levels among alcohol-dependent individuals than controls [163, 164]. This contrarian evidence has caveats. First, MRS studies may be confounded by overlapping resonances of *myo*-inositol and alcohol [165]. However, this is unlikely to significantly influence findings as all MRS studies imaged subjects after 12+ h of alcohol abstinence. Second, *myo*-inositol is an osmolyte, and *myo*-inositol levels are influenced by changes in osmolality [54–56]. Alcohol consumption is known to acutely increase plasma osmolality [57], whereas prolonged abstinence is associated with persistently low plasma osmolality (≥ 5 days) among dependent individuals [166]. Thus, alcohol's osmotic effects may influence *myo*-inositol findings among active drinkers and recently abstinent individuals. Third, postmortem data may not reflect in vivo conditions. Postmortem findings are complicated by cause of death and other uncontrolled variables which may exert independent neuroimmune effects.

Cannabis

A working hypothesis unifying the existing neuroimaging and peripheral biomarker human literature is that long-term cannabis use is associated with a suppressed or impaired neuroimmune system. Little is known about the acute effects of cannabis on the neuroimmune system.

Five MRS studies met our inclusion criteria (Table 2) [167–171]. Four studies reported lower *myo*-inositol and *myo*-inositol/creatine ratio levels (10–36%) among regular cannabis users relative to controls [168–171]. In each study, subjects were not required to remain cannabis-abstinent prior to imaging [168–171]. Across studies, lower *myo*-inositol levels were observed in the basal ganglia, ACC, thalamus, and globally. In the fifth study, Muetzel et al. [167], observed a gender-by-group interaction in the basal ganglia: lower *myo*-inositol levels among male, but higher levels among female cannabis users, relative to same sex controls. Individuals in that study were scanned after 12+ h of cannabis abstinence.

There is consistent MRS evidence that long-term cannabis use is associated with lower in vivo *myo*-inositol levels, which may reflect a suppressed/impaired neuroimmune system or diminished glial levels. However, no TSPO PET studies have been published to date. The preclinical literature indicates cannabinoids exert anti-inflammatory effects in the periphery [172–174] and CNS (reviewed in [175–177]). Cannabinoids directly bind to immune cells via CB₂ receptors and modulate cytokine and chemokine production [175–177]. Cannabinoids also modulate T helper cells and exert neuroimmunosuppressive effects in preclinical models of MS [177]. Two caveats of these MRS findings are: (1) subjects were allowed to smoke tobacco cigarettes ad libitum prior to scans; and (2) cannabinoids may exert osmotic effects [178]; each of which may influence *myo*-inositol levels. Future TSPO PET studies may help to clarify long-term cannabis effects. It should be noted that cannabis plant material contains hundreds of chemicals which vary across strains and thus complicate neuroimmune investigations. Finally, sex effects may exist [167]. The majority of MRS studies conducted to date were underpowered to explore possible sex effects [168–171]; thus, future research is needed to investigate this possibility.

Cocaine

Cocaine's neuroimmune effects are unclear and may differ by preferred route of administration.

Five MRS studies of cocaine abusers were identified (Table 2) [125, 179–182]. Of these, 3 studies reported significantly higher *myo*-inositol levels by 5–23% in frontal gray and white matter and subcortical white matter among abstinent cocaine users relative to controls [125, 179, 182]. Hulka et al. [180] observed a trend ($p = 0.06$) of higher *myo*-inositol levels, scaled to total creatine, in the dorsolateral prefrontal cortex but no difference in the ACC. Yang et al. [181] also found no difference between

groups in the ACC, though it was unclear how many subjects met criteria for cocaine dependence, and *myo*-inositol was expressed as a ratio to creatine.

One [^{11}C]PBR28 PET study examined 15 abstinent (~14 days; urine screen verified) cocaine-dependent individuals and 17 healthy controls well matched for age, gender, ethnicity, and tobacco smoking status [183]. Results indicated no difference in TSPO V_T between groups across brain regions and no relationships between TSPO levels and cocaine use characteristics.

The majority of MRS studies observed elevated *myo*-inositol levels among cocaine users compared to controls, while one PET study reported no difference in TSPO levels between groups. Among the MRS studies cited, controls were generally not well matched with cocaine subjects for ancillary substance abuse (especially alcohol, nicotine, and other stimulant use), which may contribute to the discordant MRS and PET literature. As stated above, it is possible *myo*-inositol and TSPO reflect related, but distinct, neuroimmune signals, and cocaine may exert greater influence on *myo*-inositol levels than TSPO levels. Postmortem data indicate long-term cocaine use is associated with 2- to 4-fold higher activated microglia levels relative to controls [184], consistent with MRS findings. Conversely, clinical studies indicate cocaine users exhibit lower systemic proinflammatory cytokine levels than controls [185, 186]. Finally, it is intriguing to speculate that route of administration may modulate cocaine's effect on the immune system. Injected and intranasal cocaine may be especially pro-neuroinflammatory for different reasons: unclean needles introduce pathogens directly into the CNS versus snorted cocaine (and cutting agents/contaminants) may bypass the BBB to enter the brain [187–189]. Conversely, smoked cocaine enters the blood stream after filtration in the lungs – thus, possibly reflecting a less neuroinflammatory route of administration. This is speculative. However, 2 studies that reported null findings exclusively examined individuals who smoked cocaine [181, 183]. Future research is needed to investigate this speculation.

Methamphetamine/MDMA

Methamphetamine and MDMA findings were grouped due to overlapping subject populations and use patterns in the studies reviewed herein. Based on the existing pre-clinical and human periphery data, our working hypothesis is that methamphetamine/MDMA use activates the human neuroimmune system. However, the extant human imaging data are mixed. Future TSPO PET research is needed to confirm this hypothesis.

Our search identified 19 MRS studies of methamphetamine and/or MDMA abusers with mixed findings (Table 3). Across studies, subjects were imaged at various stages of substance use ranging from active use to ~2 years abstinent. Nine studies found *myo*-inositol levels were higher among methamphetamine and MDMA users relative to controls [124, 190–197], while 10 studies found no difference between groups [198–208]. Among studies that reported group differences, higher *myo*-inositol levels were most reliably observed in the frontal cortex [124, 190–197]. Among studies that did not report a difference, methamphetamine/MDMA subjects tended to use less frequently than those reporting group differences [198, 201, 202, 204, 207].

Relative to control animals, methamphetamine-treated (~5 years) rhesus macaques exhibited significantly higher *myo*-inositol relative to total creatine ratio levels (estimated: ~20%) at 1 week after methamphetamine discontinuation, but not after 1 month or longer of prolonged abstinence [196].

One PET study examined 12 methamphetamine users (~7 years of use) who did not meet criteria for nicotine or alcohol dependence versus 12 well-matched controls (Table 1) [209]. Methamphetamine users were abstinent nearly ~2 years at the time of the PET scan with first-generation TSPO radiotracer, [^{11}C]PK11195. Time-activity curves from both methamphetamine users and healthy controls were analyzed using the average of healthy control cortex tissue curves as reference – an unconventional analytic approach for TSPO which yielded implausible results. TSPO levels were 264–1,530% higher across brain regions among methamphetamine users versus controls. These findings should be reassessed using standard TSPO PET analysis techniques and a second-generation TSPO tracer.

The preponderance of prior preclinical and clinical research indicates methamphetamine is proinflammatory and neurotoxic (reviewed in [210]). Acute MDMA administration in rodents was associated with elevated *myo*-inositol levels [52]. In humans, MRS studies among long-term and heavy methamphetamine/MDMA users suggest *myo*-inositol levels are elevated, while less frequent users exhibited unaltered neurochemistry. The only human PET study had notable limitations but observed significantly higher TSPO levels among methamphetamine abusers [209]. Several mechanisms of action are possible, though microglia activation is likely central to methamphetamine's neuroimmune effects [211–213]. Microglial activation was found to precede methamphetamine's neurotoxic effects on striatal dopamine cells [214]. Also,

anti-inflammatory medications that target glial cells, e.g. minocycline, have been shown to reduce methamphetamine-induced microgliosis and neurotoxic effects [215, 216].

Opioids

There is reliable evidence indicating acute opioid administration activates the neuroimmune system. However, the effects of chronic opioid use are not well understood.

Our search revealed 3 clinical MRS studies that examined *myo*-inositol levels in the ACC of opioid-dependent subjects and healthy controls (Table 2). Yücel et al. [217], found no difference in *myo*-inositol levels among 24 opioid-dependent subjects maintained on methadone ($n = 10$) or buprenorphine ($n = 14$) scanned after 24 h of opioid abstinence (ad libitum cigarette smoking allowed) versus 24 healthy controls. Conversely, Verdejo-García et al. [218] found higher, while Murray et al. [138], found lower *myo*-inositol levels among buprenorphine-maintained opioid-dependent subjects versus healthy controls.

Cloak et al. [219], and Deng et al. [220], each used high-field ex vivo MRS to examine non-human primates after escalating doses of repeated morphine injections in separate studies (Table 2). Cloak et al. [219] found long-term morphine administration was associated with significantly higher *myo*-inositol levels by 16% in the putamen of morphine-treated versus control animals. However, in that study, all animals were infected with simian immunodeficiency virus, which complicates interpretation. Deng et al. [220] found monkeys maintained on morphine for 90 days exhibited significantly lower *myo*-inositol levels in the hippocampus and PFC than control animals.

Hansen et al. [221] examined the effect of 30 mg oral morphine versus placebo on acute pain in healthy controls. In vivo MRS indicated that morphine decreased *myo*-inositol/creatine ratio levels in the ACC following a pain challenge. In contrast, the same group found that neither 5 days of oral oxycodone (10 mg b.i.d) nor oral venlafaxine (37.5 mg b.i.d) altered ACC or PFC *myo*-inositol/creatine ratio levels in healthy controls [222].

No human TSPO PET studies of opioid-dependent subjects were identified. However, a preclinical [^{18}F] DPA-714 PET study examined in vivo TSPO availability in 5 baboons following a single intramuscular morphine injection (1 mg/kg) [223]. Results indicated acute morphine administration increased TSPO availability (V_T) by ~30% throughout the brain 2 h post-injection, even in

regions with few μ -opioid receptors (MOR), such as the occipital lobe.

The literature indicates a complex, bidirectional relationship between opioids and neuroimmune processes. First, opioids elicit an acute inflammatory response likely via TLR4s [77, 78] and/or MORs [224–226]. There is reliable preclinical [227–229] and non-human primate [223] evidence indicating acute opioid administration is proinflammatory in the periphery and brain. Second, microglia activation alters the pharmacological efficacy of opioids. Microglia activation diminishes, while anti-inflammatory medications (that attenuate inflammatory responses) enhance, morphine's analgesic efficacy [12, 13, 23, 230, 231]. Thus, acute opioid administration activates microglia, which can diminish opioid effects and may encourage higher doses or more frequent opioid use to achieve a consistent pharmacological effect. Third, evidence suggests long-term opioid abuse is immunosuppressive in the periphery [232–234]. Long-term opioid administration increases one's susceptibility to bacterial infection, viral progression, and likelihood of death following infection [235–237]. These findings highlight an alarming relationship between long-term opioid use, immunosuppression, and death. Fourth, the neuroimmune effects of long-term opioid use remain unclear. The extant human MRS literature reports mixed findings for *myo*-inositol levels [138, 217, 218], and no human PET studies were identified. The lack of PET studies is an especially glaring gap in the literature given the ongoing opioid epidemic [238]. As stated above, numerous preclinical findings indicate opioids influence the neuroimmune system. Thus, it seems likely to the authors that long-term opioid use will lead to allostatic neuroimmune changes in humans. Future neuroimaging research is needed to investigate this possibility.

Discussion and Future Directions

Two putative imaging markers that target the neuroimmune system are PET TSPO and MRS *myo*-inositol. TSPO is a marker more consistently related to microglia than astrocytes and has been shown to detect neuroimmune challenges. *Myo*-inositol is a metabolite found in astrocytes and has been associated with microglia. Herein, we reviewed the literature describing neuroimmune effects of SUDs using these two neuroimaging approaches. Our examination revealed several working hypotheses. First, preclinical studies indicate that acute administration of tobacco smoke [108–110], alcohol [147, 153,

154], methamphetamine/MDMA [52, 210–212], and opioids [223, 227–229] activate the neuroimmune system. These findings have yet to be translated and confirmed in humans. Despite a lack of conclusive clinical neuroimaging evidence, robust preclinical findings and identification of neurobiological mechanisms suggest these relationships will translate to humans in future research.

Second, abused substances exert neuroimmune effects via diverse mechanisms. For example, opioids exert neuroimmune effects via directly binding receptors on microglia, e.g. TLR4s [77, 78] and MORs [224–226]. Other substances, e.g. tobacco smoke, likely exert neuroimmune effects indirectly. Tobacco smoke has numerous constituents known to induce oxidative damage and elicit proinflammatory responses [109]. Moreover, drugs such as alcohol likely have multiple mechanisms of action. Alcohol may interact directly with microglia via TLR4s [153, 158, 159] or via indirect effects such as increased gut permeability [157] or BBB disruption [160]. Despite diverse mechanisms of action, glial activation, especially microglial activation, is a critical intermediary of neuroimmune responses. This is important because microglial activation appears to precede neurotoxic effects for some drugs of abuse [214–216], and FDA-approved medications exist that block pro-neuroinflammatory microglial responses, e.g. ibudilast and minocycline (more on these medications below).

Third, there is some evidence that long-term cannabis and alcohol use may suppress or impair the neuroimmune system. MRS studies reliably indicate long-term cannabis use is associated with lower *myo*-inositol levels compared to controls [168–171]. However, no TSPO PET studies of cannabis users were identified. In the case of alcohol, the MRS literature is mixed, while 3 independent TSPO PET studies reported alcohol-dependent individuals reliably exhibited suppressed or impaired neuroimmune system relative to controls [142, 143, 146]. Alcohol's osmotic effects and/or overlapping resonance with *myo*-inositol may contribute to the discordant MRS and PET findings.

Finally, for other drugs of abuse, we can only speculate about the neuroimmune consequences of long-term use as too few studies exist to support a consensus working hypothesis. For example, 3 MRS studies found higher *myo*-inositol levels among cocaine users compared to controls [125, 179, 182], but groups were generally not well-matched for ancillary substance use. Conversely, the only TSPO PET study of cocaine users, which was well-controlled and groups well-matched, found TSPO levels did not differ from controls [183]. Similarly, the literature

is mixed for both MRS and TSPO PET investigations of long-term tobacco smoking [103–107]. The preponderance of prior research (reviewed [210]) suggests methamphetamine/MDMA use activates the neuroimmune system. However, the MRS literature for methamphetamine/MDMA use was mixed and the only TSPO PET study used a flawed analytic approach [209]. We speculate long-term opioid use may suppress the neuroimmune system based on extrapolations from preclinical findings [220, 235–237] and data from the human periphery [232–234], despite discordant MRS findings [138, 217, 218] and no published TSPO PET studies. As such, these tentative speculations highlight areas in need of further investigation.

Another important aspect of neuroimaging research is bridging valuable data from preclinical models, which carefully dissect mechanisms of action, to clinical studies. Translational findings are particularly important for studies of immune function because dramatic species differences exist between rodents and animals. For example, no rodent model of alcoholic liver disease effectively replicates human alcoholic hepatitis [239], and rodents are orders of magnitude more tolerant to LPS doses than people [240]. It is a nontrivial problem to recreate complex and varied psychiatric conditions such as alcohol dependence in rodents. Thus, clinical neuroimaging has a particularly vital role in testing preclinical hypotheses of chronic drug effects on the neuroimmune system in human studies.

Neurons and glia are known to exhibit tightly regulated symbiotic relationships. Accordingly, disrupted neuroimmune function may alter cognitive function, motivation, and mood – possibly mediating or contributing to the psychiatric comorbidities that often accompany SUDs. Acute neuroimmune activation can temporarily induce “sickness behaviors” such as reward dysfunction, anxiety, and mild cognitive impairment [241, 242]. Notably, these sickness behaviors map onto symptoms of SUDs [243], particularly those of the withdrawal/negative affect phase of the addiction cycle [244]. Future studies are needed to investigate how experimental manipulation of neuroimmune processes can influence sickness behaviors. The aforementioned endotoxin challenges may be particularly useful for investigating the cognitive and behavioral consequences of acute neuroimmune response.

Importantly, disrupted neuroimmune function may be treatable. Glial modulators, e.g. minocycline and ibudilast, may be effective medications for blocking the neuroimmune effects of abused substances [15, 245]. Mino-

cycline is a tetracycline derivative that inhibits microglial activation and proliferation [246–248]. Ibudilast is a phosphodiesterase inhibitor that suppresses pro-neuro-inflammatory glial responses [16, 249]. In preclinical studies, minocycline and ibudilast have been shown to attenuate alcohol self-administration [250, 251], morphine-induced dopamine release in the nucleus accumbens [252], and morphine-conditioned place preference [253]. Recent studies have begun to investigate these medications in humans. A rigorous inpatient study among opioid-dependent subjects found ibudilast reduced opioid-seeking behavior and self-reported opioid craving, relative to placebo levels [15]. In separate studies, ibudilast reduced self-reported opioid withdrawal symptoms [254] and amplified oxycodone-induced analgesia [245] among opioid-dependent individuals. These studies highlight the promise of glial modulators as therapeutic agents for SUDs, possibly as adjunctive medications to supplement existing therapies.

As mentioned, both *myo*-inositol and TSPO have marked limitations for interpretation, resulting in a somewhat opaque extant literature. Major gaps in the literature still exist that must be addressed with alternative biomarkers. The development of new imaging approaches that examine diverse targets, such as PET radioligands targeting cyclooxygenase-1 (COX-1), COX-2, purinergic receptors (e.g., P2RX7), colony stimulating factor-1, and monoamine oxidase-B, promise dramatic expansion in understanding the neuroimmune system in SUDs. For MRS, many studies cited herein were not specifically investigating neuroimmune effects and thus, were not optimized to measure *myo*-inositol levels. Also, recent technological advances in data acquisition and metabolite quantification have dramatically improved MRS data quality. Future MRS research can leverage these advances to clarify discordant findings. In addition, studies provoking a neuroimmune response, e.g. systemic endotoxin challenges with LPS [84], provide valuable models for estimating an individual's immune fitness, analogous to a cardiovascular "stress" test. These challenges provide additional information beyond "basal" or unperturbed assessments and may be particularly valuable considering the dynamic nature of the brain's immune system. Finally, studies using multimodal neuroimaging approaches may be less susceptible to false-positive findings.

In conclusion, neuroimmune research among SUDs is an important and growing field that may inform a more complete neurobiological understanding of SUDs, identify novel pharmacotherapies, and guide personalized

treatment protocols. Given the complexity of SUDs, careful consideration must be given to controlling extraneous variables to isolate the neuroimmune consequences of long-term substance use. Leveraging immune challenge paradigms to examine neuroimmune fitness and evaluation of glial modulators as both experimental probes and adjunctive medications are important areas of future research. While numerous relationships remain unclear, there is clear importance for continued neuroimaging research of the neuroimmune system in SUD.

Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interests to declare.

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Author Contributions

E.A.W. conducted the literature search, authored the manuscript, and created the tables/figures. A.T.H., G.F.M., and K.P.C. guided literature interpretation and manuscript conceptualization, and edited the manuscript. All authors have read and approved the manuscript.

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