HHS Public Access

Author manuscript

Mol Psychiatry. Author manuscript; available in PMC 2017 November 30.

Published in final edited form as:

Mol Psychiatry. 2017 December ; 22(12): 1759–1766. doi:10.1038/mp.2017.10.

In Vivo Imaging of Translocator Protein, a Marker of Activated Microglia, in Alcohol Dependence

AT Hillmer, Ph.D.[1,2], **CM Sandiego, Ph.D.**[2,3], **J Hannestad, M.D., Ph.D.**[4], **GA Angarita, M.D.**[3,5], **A Kumar, M.D.**[6], **EM McGovern, B.A.**[3], **Y Huang, Ph.D.**[1,2], **KC O'Connor, Ph.D.**[6], **RE Carson, Ph.D.**[1,2], **SS O'Malley, Ph.D.**[3], and **KP Cosgrove, Ph.D.**[1,2,3,7]

[1]Department of Diagnostic Radiology, Yale University School of Medicine, New Haven, CT

[2]Yale PET Center, Yale University School of Medicine, New Haven, CT

[3]Department of Psychiatry, Yale University School of Medicine, New Haven, CT

[4]UCB Pharma, Braine-l'Alleud, Belgium

[5]Connecticut Mental Health Center, New Haven, CT

[6]Department of Neurology, Yale University School of Medicine, New Haven, CT

[7]Department of Neuroscience, Yale University School of Medicine, New Haven, CT

Abstract

Neuroinflammation may be a critical component of the neurobiology of alcohol use disorders, yet the exact nature of this relationship is not well understood. This work compared the brain and peripheral immune profile of alcohol dependent subjects and controls. Brain levels of 18-kDa translocator protein (TSPO), a marker of microglial activation and neuroinflammation, were measured with [^{11}C]PBR28 positron emission tomography imaging in 15 healthy controls and 15 alcohol dependent subjects. Alcohol dependent subjects were imaged 1-4 days (n=14) or 24 days (n=1) after their last drink. Linear mixed modeling of partial volume corrected [^{11}C]PBR28 data revealed a main effect of alcohol dependence ($p=0.034$), corresponding to 10% lower TSPO levels in alcohol dependent subjects. Within this group, exploratory analyses found a negative association of TSPO levels in hippocampus and striatum with alcohol dependence severity ($p<0.035$). Peripheral immune response was assessed in a subset of subjects by measuring

Corresponding Author: Kelly P. Cosgrove, Ph.D., Yale University School of Medicine, 2 Church St. South, Suite 511, New Haven, CT 06519, Phone; 203-737-6969, kelly.cosgrove@yale.edu.
CM Sandiego's current affiliation is Molecular NeuroImaging, LLC, New Haven, CT
J Hannestad's current affiliation is Denali Therapeutics, South San Francisco, CA
EM McGovern's current affiliation is Memorial Sloan Kettering Cancer Center, New York City, NY

Conflict of Interest

Dr. Hannestad reports the following: He was a full-time employee of UCB Pharma at the time this study was planned and conducted.
Dr. O'Connor reports the following: Genentech, EMD Serono – speaking honoraria; NeuroPhage Pharmaceuticals, Scitemex – consulting.
Although not directly relevant to the reported work, Dr. O'Malley reports the following: donated study medications: AstraZeneca, Pfizer; consultant or advisory board member – Alkermes, Cerecor, Opiant; Scientific Review Group, Hazelden Betty Ford Foundation; member of the American Society of Clinical Pharmacology Alcohol Clinical Trials Initiative supported by Ethypharm, Lilly, Lundbeck, Otsuka, Pfizer, Arbor Pharmaceuticals, Indivior.
All other authors have no conflict of interest to report.

cytokine expression from monocytes cultured both in the presence and absence of lipopolysaccharide. Peripheral monocyte response to lipopolysaccharide stimulation was lower in alcohol dependent subjects compared to controls for *the pro-inflammatory cytokines IL-6 and IL-8.* Thus, alcohol dependent individuals exhibited less activated microglia in brain and a blunted peripheral pro-inflammatory response compared to controls. These findings suggest a role for pharmaceuticals tuning the neuroimmune system as therapeutics for alcohol dependence.

Introduction

Alcohol abuse is a leading cause of preventable death, and can cause or detrimentally affect over 200 health conditions[1]. Many of these conditions, including alcoholic liver disease, sepsis, and diminished immunity, can be classified as alcohol-attributed immunodeficiencies[2, 3]. Other changes to innate immune function caused by alcohol dependence include increased blood levels of peripheral immune signaling proteins such as cytokines. These observations provide clear evidence that alcohol dependence broadly alters the function of the peripheral immune system. Alcohol dependence also damages the brain, causing gray matter atrophy, neurodegeneration, and a variety of cognitive impairments[4]. There may be an important link between brain changes and immune changes induced by alcohol dependence. The effects of alcohol on the neuroimmune system have been a topic of much recent preclinical research (see [5] for review). Yet, possible associations between clinical alcohol dependence and the brain's neuroimmune system are poorly understood.

Microglia are brain cells that are resident macrophages of the central nervous system critical to the brain's immune function. Under normal conditions, microglia exist in a 'resting' state that is regulated closely by interactions with neurons[6]. However, microglia become activated by signals indicating the presence of tissue damage or pathogens. This microglial activation is a key process in the initiation and maintenance of neuroinflammation[7-9]. Although activated microglia serve necessary repair functions, excessive microglial activation may lead to the release of substances such as inflammatory cytokines, reactive oxygen species, nitric oxide, and glutamate that can cause neuronal dysfunction and death[10-13]. The type of microglial activation depends in part on the type of molecular signal: pro-inflammatory cytokines such as tissue necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) lead to a pro-inflammatory phenotype which can contribute to neuronal loss[11], while exposure to anti-inflammatory cytokines induces a neuroprotective phenotype in microglia[14, 15]. Preclinical studies indicate that an initial alcohol binge activates microglia into a neuroprotective state[16]. An initial activation may "prime" microglia for subsequent alcohol doses, resulting in a classically activated proinflammatory state[5]. When this proinflammatory state becomes chronic, such as during a sustained alcohol binge, neurodegeneration and diminished neural function may occur[17, 18]. Thus, microglial activation represents a potential mechanism through which alcohol interferes with neuronal function, ultimately leading to neurodegeneration[17, 18]. For this reason, therapies targeting microglia and neuroimmune function are of great interest for treating alcohol dependence[19].

Positron Emission Tomography (PET) imaging can be used to measure levels of activated microglia *in vivo* using radioligands, such as [^{11}C]PBR28, that bind to the 18-kDa

translocator protein (TSPO)[20]. TSPO is a protein expressed on the outer mitochondrial membrane of microglia[21]. Microglial activation increases TSPO levels, evidenced by multiple reports of a tight relationship between immunohistochemical measures of microglial activation and TSPO levels[22, 23]. TSPO imaging is therefore thought to provide an *in vivo* marker of activated microglia levels in brain[24]. PET imaging of TSPO has been used to investigate clinical neuropathologies such as Alzheimer's disease[25], multiple sclerosis[26, 27], and temporal lobe epilepsy[28]. To our knowledge, PET imaging of TSPO has not yet been used to study clinical alcohol dependence.

The primary goal of this work was to measure TSPO levels in the brains of individuals with alcohol dependence compared to healthy controls. We hypothesized that alcohol dependence would be associated with higher TSPO levels in brain based on preclinical and post mortem data[29, 30]. In particular, the frontal-striatal and frontal-cerebellar circuits are heavily implicated in neurodegeneration associated with heavy alcohol use[31,32], while the hippocampus is implicated in dysregulated neuroimmune function[33]. Thus our analysis focused on these four regions of frontal cortex, striatum, cerebellum, and hippocampus. To accomplish this goal, [^{11}C]PBR28 PET scans were acquired in 15 alcohol dependent subjects and 15 healthy controls matched for age, sex, and rs6971 single nucleotide polymorphism (SNP) genotype, which affects the affinity of [^{11}C]PBR28 for TSPO. A secondary aim was to assess peripheral innate immune response in a subset of these subjects. To accomplish this goal, venous monocytes were stimulated with lipopolysaccharide (LPS, also known as endotoxin) to robustly trigger "classic" monocyte activation[34, 35], and changes in inflammatory cytokine levels in LPS-incubated samples were measured. While baseline cytokine levels in periphery are higher in alcohol dependence compared to healthy controls, previous studies reported a reduced cytokine response in periphery to inflammatory insult in alcohol dependence[36, 37]. *Thus* we hypothesized a similar blunted pro-inflammatory cytokine response *of peripheral monocytes to LPS for* alcohol dependent subjects relative to healthy controls. Taken together, these experiments provide an important *in vivo* assessment of innate immune function during clinical alcohol dependence in both brain and peripheral circulatory monocytes.

Methods

Subjects

Fifteen alcohol dependent subjects (11 men, 4 women) and fifteen healthy control subjects (11 men, 4 women) matched for age, sex, and single nucleotide polymorphism (SNP) *rs6971* genotype were recruited from the local population to participate in one [^{11}C]PBR28 PET scan including arterial blood sampling and one magnetic resonance imaging (MRI) scan. Prior to scanning, all subjects were genotyped for the *rs6971* SNP as previously described[38]. This polymorphism affects the affinity of [^{11}C]PBR28 for the TSPO site[39]. T/T homozygotes (Low Affinity Binders) were excluded from the study.

Alcohol dependent subjects met DSM-IV criteria for alcohol dependence with no axis I disorder other than nicotine dependence, with no current or past significant medical or neurological disorders and no psychotropic medication use over the previous month. *Cigarette use was evaluated by self report.* Subjects with prior alcohol detoxifications using

benzodiazepines were not eligible, since they would be more likely to require benzodiazepines during detoxification and these drugs are active at the TSPO site[40]. During screening, alcohol dependence severity was evaluated with the Alcohol Dependence Scale[41], alcohol craving was surveyed with the Alcohol Craving Questionnaire[42], and alcohol use over the previous month was collected with the Timeline Follow-Back (TLFB[43]). Alcohol dependent subjects were admitted to the Connecticut Mental Health Center Clinical Neuroscience Unit and imaged at 1-4 days (*n=14)* or 24 days (*n=1*) after their last drink. This time frame was selected to most closely examine microglia in alcohol dependence in the absence of alcohol but before major brain changes occur following alcohol abstinence. While on the unit, the Clinical Institute Withdrawal Assessment was administered every 6 hours until scores were stably 0. None of the subjects in this study required benzodiazepine treatment.

Healthy controls had no history of significant major medical disorders or head trauma and did not meet DSM-IV criteria for current or past psychiatric or substance abuse diagnosis other than nicotine use. In addition, control subjects reported drinking fewer than eight alcoholic drinks per week. For all female subjects, negative pregnancy tests were established during screening and prior to radiotracer administration on the day of the scans. All procedures were approved by the Yale University School of Medicine Human Investigation Committee and the Radiation Safety Committee. Written informed consent, approved by the Yale University School of Medicine Human Investigation Committee, was obtained from all subjects prior to participation in this study.

Imaging Data Acquisition

[^{11}C]PBR28 was produced as previously described[38], resulting in high specific activities of 294±188 MBq/nmol. PET data were acquired with a High Resolution Research Tomograph (HRRT; Siemens), and head motion data were acquired with an optical motion-tracking tool (Vicra, NDI Systems). PET imaging sessions began with acquisition of a 6 min ^{137}Cs transmission scan for attenuation correction of the emission data. PET data acquisition began simultaneously with administration of 551±174 MBq [^{11}C]PBR28 as a slow bolus over 1 min, with data acquisition lasting 120 min. The metabolite-corrected arterial input function was measured as previously described[27].

Magnetic resonance image (MRI) data were acquired for anatomical localization of [^{11}C]PBR28 uptake. MR data were acquired with a 3T Trio Scanner (Siemens Medical Systems, Erlangen, Germany) with a weighted gradient-echo (MPRAGE) sequence featuring the following parameters: (TE=3.3 ms; TI=1,100 ms, TF=2,500 ms, FA=7°) giving 1 mm^3 isotropic resolution.

Imaging Data Processing and Analysis

Dynamic list-mode PET data were histogrammed into discrete time frames up to 5 min and reconstructed with the MOLAR algorithm[44]. To transform PET data into MR space, a summed image of the first 10 min of PET data was registered to the subject-specific T1-weighted MRI using a mutual information algorithm with six degrees of freedom (FLIRT, FSL 3.2; Analysis Group; FMRIB, Oxford, UK). The native MRI was then co-registered to

the Montreal Neurological Institute (MNI) template space with a nonlinear transformation algorithm (BioImage Suite; http://www.bioimagesuite.com) for Region of Interest (ROI) definition. The dynamic PET data were corrected for partial volume effects with previously published methods[45] using SPM12-segmented tissue masks. The time-activity curves of radioactivity concentration in tissue were extracted from the regions of cerebellum, frontal cortex, hippocampus, and striatum, since these are important regions affected by alcohol-induced neurodegeneration[4] and dysregulated neuroimmune function[33].

For PET data, the primary outcome measure of this study was the total distribution volume (V_T), which is the ratio at equilibrium of [^{11}C]PBR28 concentration in tissue to [^{11}C]PBR28 concentration in arterial plasma[46]. The V_T is indirectly proportional to the number of TSPO sites available for [^{11}C]PBR28 binding. Multilinear analysis (MA1;[47]) with t*=30 min was used to estimate V_T as previously validated[27]. For visualization purposes, voxel-wise parametric images were also calculated with an algorithm constraining the MA1 parameters to reduce noise following spatial smoothing with an 8 mm FWHM Gaussian kernel.

Endotoxin Stimulation of Peripheral Monocytes

Briefly, venous blood samples were acquired on the day of the PET scan for 11 alcohol dependent subjects and 9 healthy controls. Peripheral blood mononuclear cells (PBMCs) were isolated and placed in culture medium RPMI-1640. After initial incubation over 1 h, cells were stimulated with either 10 ng/mL lipopolysaccharide (LPS) or 50 μL phosphate buffered saline (PBS), and further incubated for an additional 24 h. Cytokine concentrations were assayed with MILLIPLEX panel assay, and fold-change in cytokine expression for each cytokine within each subject was reported as the ratio of average cytokine concentration in LPS-stimulated samples to average cytokine concentration in PBS-added (non-stimulated) samples. A detailed description of these methods is available in **Supplementary Methods**.

Statistics

[^{11}C]PBR28 V_T values were statistically analyzed with a linear mixed modeling approach. To test the null hypothesis of no difference in [^{11}C]PBR28 V_T between the alcohol dependent and healthy control groups, a model was constructed with *rs6971* genotype as a fixed factor, group as a between-subjects factor and region as a within-subjects factor. Post-hoc linear contrasts were generated to examine regional differences in [^{11}C]PBR28 V_T between alcohol dependent subjects and healthy controls. A power analysis using preliminary data before the study began determined a sample size of 30 subjects (15 in each group) to provide 80% probability of detecting significant differences in [^{11}C]PBR28 V_T between the two groups.

As an exploratory analysis, possible associations between [^{11}C]PBR28 V_T were examined for all regions with reported drinks per day over the previous month, Alcohol Dependence Scale score, and Alcohol Craving Questionnaire Score in each brain region. For this analysis, correction for rs6971 genotype was implemented by separating the subjects by genotype, and then creating a z-score for each variable (regional [^{11}C]PBR28 V_T, drinks per day over the previous month, Alcohol Dependence Scale score, and craving score) within

each genotype group. Standard linear regression analysis techniques were then performed on these 'standardized' variables. This procedure is analogous to previous reports that 'correlated' each variable with genotype and ran regression analysis on standardized residuals of this correlation[25], which effectively created a z-score by genotype. The results of this analysis were not corrected for multiple comparisons due to the exploratory nature of these analyses.

A linear mixed model approach was used to test the null hypothesis of no differences in fold-change of cytokine expression between alcohol dependent subjects and healthy controls. Since different effects were expected for pro-inflammatory and anti-inflammatory cytokines, a separate model was constructed for each class of cytokines using group as a between-subjects factor and cytokine as a within-subjects factor. Post-hoc linear contrasts were generated to examine individual cytokines for differences between alcohol dependent subjects and healthy controls. Statistical analyses were all performed with R 3.1.1.

Results

Individuals with alcohol dependence (n=15; duration of 22±10 years) reported drinking 6±3 drinks per day (range of 4-15 drinks per day) on 4-7 days a week. Healthy controls (n=15) were matched for age, sex, and single nucleotide polymorphism (SNP) *rs6971* genotype, which affects [^{11}C]PBR28 affinity for the TSPO site. The average score on the Alcohol Dependence Scale was 8.3±5.6, and average alcohol craving evaluated with the Tiffany Scale was 23.3±12.6. Five alcohol dependent subjects (5±2 cigarettes/day) and four healthy controls (13±4 cigarettes/day) were cigarette smokers. For [^{11}C]PBR28 scans, there were no significant group differences ($p<0.05$) in injected radioactivity dose, injected mass, or f_P (see **Table 1**).

In contrast to our hypothesis, partial-volume corrected levels of activated microglia in regions of brain, quantified by [^{11}C]PBR28 V_T, were significantly lower in alcohol dependent individuals compared to healthy controls ($F_{(1,26)}=4.983$, $p=0.034$; effect of genotype, $F_{(1,26)}=32.54$, $p<0.0001$; interaction effect, $F_{(1,26)}=0.094$, $p=0.762$). [^{11}C]PBR28 V_T values are shown in **Figure 1** adjusted by the modeled genotype coefficient for visualization, and were 10% lower in alcohol dependent subjects compared to healthy controls averaged across regions, illustrated in **Figure 2**. Post-hoc analysis revealed a significant effect of alcohol dependence in cerebellum ($F_{(1,27)}=5.000$, $p=0.034$), with similar trends in frontal cortex ($F_{(1,27)}=4.183$, $p=0.051$), striatum ($F_{(1,27)}=3.951$, $p=0.057$), and hippocampus (($F_{(1,27)}=2.941$, $p=0.098$), shown in ***Supplementary Figure 1***.

Partial volume correction (PVC) of PET data safeguards against tissue atrophy effects influencing PET outcome measures, but also introduces additional variability into the data. To ensure that PVC did not bias our result, analysis of [^{11}C]PBR28 data *without* this correction was also conducted. This analysis yielded a strong main effect of significantly lower [^{11}C]PBR28 V_T in alcohol dependent patients compared to healthy controls ($F_{(1,26)}=6.681$, $p=0.016$; effect of genotype, $F_{(1,26)}=44.98$, $p<0.0001$; interaction effect, $F_{(1,26)}=0.039$, $p=0.845$). Post-hoc analysis revealed a significant effect of alcohol dependence in all regions ($F_{(1,27)}>4.249$, $p<0.049$), *shown in* ***Supplementary Figure 2***.

Nonetheless, to be conservative, partial volume-corrected [^{11}C]PBR28 V_T is reported as the primary result to account for possible gray matter volume variations across subjects.

As an exploratory analysis, we tested for relationships of levels of activated microglia with dependence severity, reported drinks per day during the previous month, and alcohol craving within the alcohol dependent group. This analysis identified a significant negative relationship (uncorrected for multiple comparisons) between [^{11}C]PBR28 V_T and dependence severity, as assessed by the Alcohol Dependence Scale, in hippocampus (p=0.035) and striatum (p=0.035), with a similar trend in cerebellum (p=0.059; shown *as a representative region* in **Figure 3A**). These relationships indicated that more severe alcohol dependence was associated with lower levels of activated microglia. No significant relationships were found between reported drinks per day over the previous month (assessed with Timeline Followback; TLFB) or Alcohol Craving Questionnaire score with [^{11}C]PBR28 V_T using the entire alcohol dependent group. However, the heaviest drinker *was a statistical* outlier for this sample in reported drinks per day over the previous month (1.9 times the interquartile range greater than the third quartile). Analyses omitting this subject indicated significant negative relationships between reported drinks per day over the previous month and [^{11}C]PBR28 V_T. This relationship was significant in cerebellum and striatum (both p<0.01, uncorrected; see **Figure 3B** for example of cerebellum) and in hippocampus (p=0.015), with a similar trend in frontal cortex (p=0.098). A complete summary of these analyses is presented in ***Figure 3C***.

Monocytes successfully derived from 9 alcohol dependent individuals and 8 controls *were cultured and assayed* for cytokine expression. Stimulation of monocytes with LPS robustly increased measured cytokine expression relative to non-stimulated samples for all subjects, indicated by cytokine fold-change values significantly greater than 1 in **Figures 4**. For pro-inflammatory cytokines, which promote inflammatory signaling including microglial activation, there was a trend of blunted LPS-induced fold-increase of cytokine expression for alcohol dependent subjects relative to healthy controls ($F_{(1,14)}$=4.116, p=0.062). Post-hoc analysis revealed the effect to be significant for the cytokines IL-8 (p=0.031; **Fig 4A**) and IL-6 (p=0.043; **Fig. 4B**), while no significant differences were observed for IL-1β or TNF-α (**Fig. 4C-D**). For anti-inflammatory cytokines, which suppress inflammatory signaling including microglial activation, LPS-induced fold-changes of IL-10 and IL-4 were not significantly different between alcohol dependent individuals and healthy controls ($F_{(1,7)}$=2.355, p=0.169; **Figure 4E-F**). No significant relationships between LPS-induced fold-changes in cytokine expression and alcohol use characteristics or levels of activated microglia in brain were observed.

Discussion

The primary finding in this neuroimaging study is significantly lower levels of activated microglia in the brains of living alcohol dependent subjects compared to healthy controls as measured with [^{11}C]PBR28 PET. Importantly, exploratory analyses suggest that lower [^{11}C]PBR28 V_T values are associated with greater alcohol dependence severity and more reported drinks per day over the previous month. This PET imaging study fills a crucial gap in the literature by measuring an *in vivo* brain marker of activated microglia levels in alcohol

dependent subjects. Additionally, peripheral monocytes from alcohol dependent subjects exhibit a blunted response for the pro-inflammatory cytokines IL-6 and IL-8 to LPS stimulus compared to healthy controls. Taken together, these findings suggest that alcohol dependence is associated with lower levels of activated microglia in the brain and a blunted immune response in the periphery.

The main finding of lower activated microglia levels in alcohol dependent individuals compared to controls extends our current understanding of the influence of alcohol on the neuroimmune system. A large body of preclinical literature converges on a profile of microglial activation following models of binge alcohol treatment in rodents[48-52]. Specifically, recent studies provide evidence that an initial binge episode results in low or partial microglial activation, which 'primes' the microglia for greater activation following subsequent binge episodes[16, 53, 54]. 'Primed' microglia may be associated with increased cell death and neurodegenerative disorders[55]. However, rodent models of alcohol exposure have obvious limitations in modeling years of clinical alcohol dependence. The present finding of low levels of activated microglia in alcohol dependence extends these preclinical findings to the clinical case of chronic alcohol use over a period of many years, and is consistent with a preliminary report from a separate imaging center of reduced [^{11}C]PBR28 V_T *in the hippocampus of alcohol dependent patients*[56]. Indeed, the finding of negative associations of brain levels of activated microglia with both reported drinks per day over the previous month and alcohol dependence severity imply a close connection between chronic alcohol use and microglial levels. Whether lower levels of activated microglia in brain contributed to or were merely a result of alcohol dependence is not known, however, this finding is consistent with the observation that individuals with alcohol dependence have increased susceptibility to liver disease, sepsis, and reduced host repair, possibly due to an inability to mount sufficient responses to inflammatory challenges. Such a hypothesis parallels emerging evidence of dystrophic microglia associated with advanced neurodegeneration[57]. Thus, we hypothesize that chronic activation of microglia from alcohol abuse eventually diminishes, or 'burns out', levels of activated microglia in brain.

[^{11}C]PBR28 binds specifically to TSPO[58], which are overexpressed during microglial activation[59]. TSPO are also expressed on astrocytes and with astrocyte activation[60], however, immunohistochemistry work from our lab indicated LPS-induced TSPO increases to result almost exclusively in activated microglia[61]. Therefore observed [^{11}C]PBR28 signal is generally attributed to levels of activated microglia[22]. While this is a simple interpretation for increased TSPO levels, the data here indicating lower TSPO levels have a more difficult interpretation. TSPO expression is low in healthy brain[22], but specific [^{11}C]PBR28 binding is still displaceable in normal humans[60]. Thus some baseline level of TSPO expression is present in the healthy brain. Additionally, pharmacological interventions can deplete microglia levels in healthy rodents[62] and reduce TSPO levels in humans[63], raising the possibility of a dynamic range in baseline microglial levels. Therefore, in this study we interpret lower TSPO levels as either diminished levels of activated microglia or possibly lower levels of TSPO in each cell. A further limitation in interpreting the source of [^{11}C]PBR28 binding is the radioligand's insensitivity to microglial phenotype (neuroprotective vs. neurodegenerative). There is strong evidence supporting an important role for microglial phenotype influencing alcohol associated neurodegeneration[16, 53], and

use of neuroimmune agents as interventions for alcohol use disorders has been postulated based on findings of microglial activation following alcohol exposure in rodent models[5]. The present finding of diminished levels of activated microglia in alcohol dependence does not support the use of treatments suppressing inflammation for alcohol dependence, but provides evidence supporting the use of therapeutics *promoting* innate immune function as possible treatment options for alcohol dependence and promoting recovery. Moreover, this study conclusively identifies TSPO-specific PET imaging as an important tool to aid in the development and evaluation of potential neuroimmune therapeutics.

Lower [^{11}C]PBR28 V_T values were significantly associated with more reported drinks per day over the previous month and greater alcohol dependence severity. This supports the primary finding that lower levels of activated microglia are associated with alcohol dependence. Given this observation, possible relationships between [^{11}C]PBR28 V_T and time of last drink were also investigated. There were no significant relationships ($F_{(1,27)}$<0.083, p>0.778). Analysis excluding the subject imaged 24 days after their last drink yielded a trend of reduced [^{11}C]PBR28 V_T in alcohol dependence ($F_{(1,25)}$=3.558, p=0.071; effect of genotype, $F_{(1,25)}$=72.30, p<0.0001; interaction effect, $F_{(1,25)}$=0.062, p=0.805), mirroring the findings incorporating the entire data set. The modest average Alcohol Dependence Scale score (8.3±5.6) and exclusion from benzodiazepine treatment indicates that this population is most representative of moderate alcohol dependence. Potential differences in levels of activated microglia after extended alcohol abstinence, the extent to which levels of activated microglia longitudinally may recover following abstinence, and the generalizability of this study to heavy alcohol dependence remain important lines of future research.

Previous imaging studies measuring TSPO levels in substance use disorders reported mixed findings. One study imaging methamphetamine abusers with [^{11}C]PK11195, a TSPO radiotracer less specific than [^{11}C]PBR28, reported higher TSPO levels in midbrain, striatum, and orbitofrontal cortex compared to healthy controls[64], however, unconventional kinetic analysis methods limit interpretation of this work[65]. In contrast, no differences in TSPO levels measured with [^{11}C]PBR28 were reported between cocaine abusers and healthy controls[66]. Thus the effects of substance use disorders on microglial activation may be drug specific. With this in mind, smoking status was an important consideration in this work, as 5 alcohol dependent subjects and 4 healthy controls were tobacco smokers. While there is evidence that nicotine itself may suppress microglial activation[67], tobacco smoke may lead to increased microglial activation[68]. It is not known currently how tobacco smoking may affect brain TSPO levels in vivo, as human imaging studies of neuroinflammation in tobacco smoking have not been reported. Thus, potential effects of tobacco smoking on measured TSPO levels were controlled for by matching for smoking status in the two study groups.

The kinetic and statistical analysis methods of [^{11}C]PBR28 PET data used here represent a major strength of this work. Recent studies using TSPO-specific PET imaging have reported outcome measures of regional radioactivity concentrations normalized to whole brain radioactivity levels[69-71]. These approaches ignore the consensus use of V_T as primary outcome measure for radioligands lacking a reference region that has been carefully validated to have no group differences in specific binding for a given pathology[46]. Using

normalized data severely limits the interpretation of whole brain normalization results to only test for differences in *relative* regional TSPO levels – effectively ignoring potential differences in absolute TSPO levels. Further drawbacks of this method have been detailed elsewhere[65]. In addition, the use of V_T/f_P as an alternative primary outcome measure to V_T was investigated, but statistical analysis with this outcome yielded no significant effect of *rs6971* genotype (effect of diagnosis, $F_{(1,26)}$=1367, p=0.253; effect of genotype, $F_{(1,26)}$=4.098, p=0.054; interaction effect, $F_{(1,26)}$=0.234, p=0.633). This finding provided evidence that the [^{11}C]PBR28 f_P measurement introduces excessive variability into the data, consistent with our center's previous report of increased test-retest variability with V_T/f_P as an outcome measure for [^{11}C]PBR28 PET imaging[27]. We contend that the analysis of PET data using conventional kinetic modeling techniques with full arterial sampling and metabolite analysis represents the most appropriate method for direct measurement of activated microglia levels *in vivo*.

The selection of regions of interest and corrections for possible gray matter atrophy were critical considerations to this work. Four large, non-adjacent regions of interest heavily implicated in the biology of alcohol neurodegeneration were selected for analysis. The frontal-striatal-cerebellar network and limbic system are brain regions affected the earliest and most severely in alcohol-related neurodegeneration[4,72,73], providing strong support motivating the selection of the a priori ROIs of frontal cortex, striatum, cerebellum, and hippocampus. This approach was chosen to provide some regional specificity without excessively increasing variance in the linear mixed model by introducing many regions of different sizes with different variance. For the interested reader, [^{11}C]PBR28 V_T values for additional brain regions, including whole brain average, are included in **Supplementary Table 1.** Notably, the 10% reduction in [^{11}C]PBR28 V_T for alcohol dependent patients compared to healthy controls is consistent across all regions. Additionally, a partial volume correction[45] was implemented to adjust for possible individual differences in normalized gray matter volume[74]. For the regions analyzed, alcohol dependent subjects exhibited 9.6±20.8% less regional gray matter volume than healthy controls as estimated by our center's automated segmentation and registration techniques. These differences were greatest in frontal cortex and cerebellum, the regions most associated with tissue atrophy in alcohol dependence[4], thus we believe possible gray matter atrophy to be a critical consideration for this work. However, analysis of PET data *without* partial volume correction yielded a stronger main effect of diagnosis ($F_{(1,26)}$=6.681) than the partial volume-corrected data ($F_{(1,26)}$=4.983). We hypothesize that the partial volume correction likely introduced additional variability into the brain data, yet, this correction was considered critical to account for possible tissue atrophy in alcohol dependence. Therefore, partial volume-corrected [^{11}C]PBR28 V_T was reported as the preferred outcome measure.

The cytokine response of peripheral monocytes to LPS stimulation was examined to complement measurements of TSPO brain levels. These data suggest that LPS stimulation of monocytes elicits a significantly less pro-inflammatory response in alcohol dependent subjects compared to healthy controls. Given the high variability in both sample groups and small sample size which likely precluded detection of significant differences, post-hoc analyses were conducted on these data, revealing significantly lower LPS-simulated cytokine

response for IL-8 and IL-6 (see **Figure 4A-B**). Indeed, further assessment of the data indicates a pattern of lower pro-inflammatory IL-1β response in alcohol dependent subjects compared to healthy controls (see **Fig. 4C**). In contrast, the anti-inflammatory IL-10 cytokine expression suggests an opposite pattern of greater response to LPS stimulation in alcohol dependent subject compared to healthy controls (**Fig. 4E**). The finding of lower pro-inflammatory response to LPS challenge in alcohol dependence is consistent with previous reports of lower LPS-stimulated response of IL-1β, IL-12, and TNF-α in alcohol dependent subjects[75,76]. Interestingly, these previously reported findings were driven by significantly greater spontaneous (e.g. non-stimulated) cytokine levels in alcohol dependent subjects relative to controls, however, excessive experimental and physiological variability in this measurement limits the interpretation of this finding. Consistent with previous reports[20,77] , no direct relationship between activated microglia levels in brain and cytokine measures was observed. Therefore we are unable to comment on the extent to which the altered neuronal and peripheral immune profiles in alcohol dependence are directly related.

In conclusion, this work demonstrates an association of alcohol dependence with lower activated microglia levels in brain and blunted pro-inflammatory cytokine response from peripheral monocytes. These findings support a view of a dysregulated innate immune system in alcohol dependence, which could be a potential target for future therapeutic development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements and Funding

We thank the staff at the Yale PET center for their expertise and support of radiochemistry and imaging. We thank Jon Mikael Anderson and the staff at the Clinical Neuroscience Research Unit at the Connecticut Mental Health Center for assistance with subject monitoring and evaluation. Dr. Lesley Devine provided critical support for cytokine assay. We thank Drs. Betsy Bradshaw and Jean-Dominique Gallezot for insightful technical discussions. We gratefully acknowledge the funding support of the National Institute on Alcohol Abuse and Alcoholism (R21AA021866; K05AA014715), the National Institute on Drug Abuse (T32DA022975; K02DA031750), and the State of Connecticut Support for the Clinical Neuroscience Research Unit.

References

1. Organization WH. Global status report on alcohol and health. World Health Organization; 2014.
2. Nelson S, Kolls JK. Alcohol, host defence and society. Nat Rev Immunol. 2002; 2(3):205–209. [PubMed: 11913071]
3. Szabo G, Mandrekar P. A recent perspective on alcohol, immunity, and host defense. Alcohol Clin Exp Res. 2009; 33(2):220–232. [PubMed: 19053973]
4. Sullivan EV, Pfefferbaum A. Neurocircuitry in alcoholism: a substrate of disruption and repair. Psychopharmacology. 2005; 180(4):583–594. [PubMed: 15834536]
5. Crews FT, Vetreno RP. Mechanisms of neuroimmune gene induction in alcoholism. Psychopharmacology. 2016; 233(9):1543–1557. [PubMed: 25787746]
6. Ponomarev ED, Veremeyko T, Barteneva N, Krichevsky AM, Weiner HL. MicroRNA-124 promotes microglia quiescence and suppresses EAE by deactivating macrophages via the C/EBP-alpha-PU.1 pathway. Nat Med. 2011; 17(1):64–70. [PubMed: 21131957]
7. Chan WY, Kohsaka S, Rezaie P. The origin and cell lineage of microglia: new concepts. Brain Res Rev. 2007; 53(2):344–354. [PubMed: 17188751]

8. Tambuyzer BR, Ponsaerts P, Nouwen EJ. Microglia: gatekeepers of central nervous system immunology. J Leukoc Biol. 2009; 85(3):352–370. [PubMed: 19028958]
9. Chen SK, Tvrdik P, Peden E, Cho S, Wu S, Spangrude G, et al. Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. Cell. 2010; 141(5):775–785. [PubMed: 20510925]
10. van Gool WA, van de Beek D, Eikelenboom P. Systemic infection and delirium: when cytokines and acetylcholine collide. Lancet. 2010; 375(9716):773–775. [PubMed: 20189029]
11. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat Rev Neurosci. 2007; 8(1):57–69. [PubMed: 17180163]
12. McNally L, Bhagwagar Z, Hannestad J. Inflammation, glutamate, and glia in depression: a literature review. CNS Spectr. 2008; 13(6):501–510. [PubMed: 18567974]
13. Yakovleva T, Bazov I, Watanabe H, Hauser KF, Bakalkin G. Transcriptional control of maladaptive and protective responses in alcoholics: a role of the NF-kappaB system. Brain Behav Immun. 2011; 25(Suppl 1):S29–38. [PubMed: 21195164]
14. Li L, Lu J, Tay SS, Moochhala SM, He BP. The function of microglia, either neuroprotection or neurotoxicity, is determined by the equilibrium among factors released from activated microglia in vitro. Brain Res. 2007; 1159:8–17. [PubMed: 17572395]
15. Hanisch UK, Kettenmann H. Microglia: active sensor and versatile effector cells in the normal and pathologic brain. Nat Neurosci. 2007; 10(11):1387–1394. [PubMed: 17965659]
16. Marshall SA, McClain JA, Kelso ML, Hopkins DM, Pauly JR, Nixon K. Microglial activation is not equivalent to neuroinflammation in alcohol-induced neurodegeneration: The importance of microglia phenotype. Neurobiol Dis. 2013; 54:239–251. [PubMed: 23313316]
17. Hurley LL, Tizabi Y. Neuroinflammation, neurodegeneration, and depression. Neurotox Res. 2013; 23(2):131–144. [PubMed: 22895696]
18. Streit WJ, Mrak RE, Griffin WST. Microglia and neuroinflammation: a pathological perspective. J Neuroinflamm. 2004; 1(1):14.
19. Ray LA, Roche DJ, Heinzerling K, Shoptaw S. Opportunities for the development of neuroimmune therapies in addiction. Int Rev Neurobiol. 2014; 118:381–401. [PubMed: 25175870]
20. Sandiego CM, Gallezot J-D, Pittman B, Nabulsi N, Lim K, Lin S-F, et al. Imaging robust microglial activation after lipopolysaccharide administration in humans with PET. Proc Nat Acad Sci USA. 2015; 112(40):12468–12473. [PubMed: 26385967]
21. Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, et al. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. Nat Rev Drug Discov. 2010; 9(12):971–988. [PubMed: 21119734]
22. Cosenza-Nashat M, Zhao ML, Suh HS, Morgan J, Natividad R, Morgello S, et al. Expression of the translocator protein of 18 kDa by microglia, macrophages and astrocytes based on immunohistochemical localization in abnormal human brain. Neuropathol Appl Neurobiol. 2009; 35(3):306–328. [PubMed: 19077109]
23. Liu GJ, Middleton RJ, Hatty CR, Kam WWY, Chan R, Pham T, et al. The 18 kDa translocator protein, microglia and neuroinflammation. Brain Pathology. 2014; 24(6):631–653. [PubMed: 25345894]
24. Owen DR, Matthews PM. Imaging brain microglial activation using positron emission tomography and translocator protein-specific radioligands. Int Rev Neurobiol. 2011; 101:19–39. [PubMed: 22050847]
25. Kreisl WC, Lyoo CH, McGwier M, Snow J, Jenko KJ, Kimura N, et al. In vivo radioligand binding to translocator protein correlates with severity of Alzheimer's disease. Brain. 2013; 136:2228–2238. Pt 7. [PubMed: 23775979]
26. Oh U, Fujita M, Ikonomidou VN, Evangelou IE, Matsuura E, Harberts E, et al. Translocator Protein PET Imaging for Glial Activation in Multiple Sclerosis. J Neuroimmune Pharmacol. 2011; 6(3):354–361. [PubMed: 20872081]
27. Park E, Gallezot JD, Delgadillo A, Liu S, Planeta B, Lin SF, et al. ^{11}C-PBR28 imaging in multiple sclerosis patients and healthy controls: test-retest reproducibility and focal visualization of active white matter areas. Eur J Nucl Med Mol Imaging. 2015; 42(7):1081–1092. [PubMed: 25833352]

28. Hirvonen J, Kreisl WC, Fujita M, Dustin I, Khan O, Appel S, et al. Increased In Vivo Expression of an Inflammatory Marker in Temporal Lobe Epilepsy. J Nucl Med. 2012; 53(2):234–240. [PubMed: 22238156]
29. Crews FT, Qin L, Sheedy D, Vetreno RP, Zou J. High mobility group box 1/Toll-like receptor danger signaling increases brain neuroimmune activation in alcohol dependence. Biol Psychiatry. 2013; 73(7):602–612. [PubMed: 23206318]
30. He J, Crews FT. Increased MCP-1 and microglia in various regions of the human alcoholic brain. Exp Neurol. 2008; 210(2):349–358. [PubMed: 18190912]
31. Sullivan ED, Deshmukh A, De Rosa E, Rosenbloom MJ, Pfefferbaum A. Striatal and forebrain nuclei volumes: Contribution to motor function and working memory deficits in alcoholism. Biol. Psychiatry. 2005; 57(7):768–776. [PubMed: 15820234]
32. Makris N, Oscar-Berman M, Jaffin SK, Hodge SM, Kennedy DN, Caviness VS, et al. Decreased volume of the brain reward system in alcoholism. Biol. Psychiatry. 2008; 64(3):192–202. [PubMed: 18374900]
33. Geil CR, Hayes DM, McClain JA, Liput DJ, Marshall SA, Chen KY, et al. Alcohol and adult hippocampal neurogenesis: promiscuous drug, wanton effects. Prog Neuropsychopharm Biol Psychiatry. 2014; 54:103–113.
34. Cunningham C. Microglia and neurodegeneration: the role of systemic inflammation. Glia. 2013; 61(1):71–90. [PubMed: 22674585]
35. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol. 2008; 8(12):958–969. [PubMed: 19029990]
36. Mandrekar P, Bala S, Catalano D, Kodys K, Szabo G. The opposite effects of acute and chronic alcohol on lipopolysaccharide-induced inflammation are linked to IRAK-M in human monocytes. J Immunol. 2009; 183(2):1320–1327. [PubMed: 19561104]
37. Mandrekar P, Catalano D, White B, Szabo G. Moderate alcohol intake in humans attenuates monocyte inflammatory responses: inhibition of nuclear regulatory factor kappa B and induction of interleukin 10. Alcohol Clin Exp Res. 2006; 30(1):135–139. [PubMed: 16433741]
38. Hannestad J, DellaGioia N, Gallezot J-D, Lim K, Nabulsi N, Esterlis I, et al. The neuroinflammation marker translocator protein is not elevated in individuals with mild-to-moderate depression: A [^{11}C]PBR28 PET study. Brain Behav Immun. 2013; 33:131–138. [PubMed: 23850810]
39. Owen DR, Yeo AJ, Gunn RN, Song K, Wadsworth G, Lewis A, et al. An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. J Cereb Blood Flow Metab. 2012; 32(1):1–5. [PubMed: 22008728]
40. Chauveau F, Boutin H, Van Camp N, Dolle F, Tavitian B. Nuclear imaging of neuroinflammation: a comprehensive review of [^{11}C]PK11195 challengers. Eur J Nucl Med Mol Imaging. 2008; 35(12): 2304–2319. [PubMed: 18828015]
41. Skinner HA, Allen BA. Alcohol dependence syndrome: measurement and validation. J Abnorm Psychol. 1982; 91(3):199. [PubMed: 7096790]
42. Singleton E, Henningfield J, Tiffany S. Alcohol craving questionnaire: ACQ-Now: background and administration manual. Baltimore: NIDA Addiction Research Centre. 1994
43. Sobell, LC., Sobell, MB. Measuring alcohol consumption. Springer; 1992. Timeline follow-back; p. 41-72.
44. Carson RE, Barker WC, Liow J-S, Johnson CA. Design of a motion-compensation OSEM list-mode algorithm for resolution-recovery reconstruction for the HRRT. IEEE Nucl Sci Conf R. 2003; 5:3281–3285.
45. Giovacchini G, Lerner A, Toczek MT, Fraser C, Ma K, DeMar JC, et al. Brain incorporation of ^{11}C-arachidonic acid, blood volume, and blood flow in healthy aging: a study with partial-volume correction. J Nucl Med. 2004; 45(9):1471–1479. [PubMed: 15347713]
46. Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, et al. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J Cereb Blood Flow Metab. 2007; 27(9):1533–1539. [PubMed: 17519979]
47. Ichise M, Liow JS, Lu JQ, Takano A, Model K, Toyama H, et al. Linearized reference tissue parametric imaging methods: application to [^{11}C]DASB positron emission tomography studies of

the serotonin transporter in human brain. J Cereb Blood Flow Metab. 2003; 23(9):1096–1112. [PubMed: 12973026]

48. Alfonso-Loeches S, Pascual-Lucas M, Blanco AM, Sanchez-Vera I, Guerri C. Pivotal role of TLR4 receptors in alcohol-induced neuroinflammation and brain damage. J Neurosci. 2010; 30(24): 8285–8295. [PubMed: 20554880]
49. Pandey SC, Roy A, Mittal N. Effects of chronic ethanol intake and its withdrawal on the expression and phosphorylation of the CREB gene transcription factor in rat cortex. J Pharmacol Exp Ther. 2001; 296(3):857–868. [PubMed: 11181917]
50. Qin L, Crews FT. NADPH oxidase and reactive oxygen species contribute to alcohol-induced microglial activation and neurodegeneration. J Neuroinflamm. 2012; 9(5):5–9.
51. Vetreno RP, Qin L, Crews FT. Increased receptor for advanced glycation end product expression in the human alcoholic prefrontal cortex is linked to adolescent drinking. Neurobiol Dis. 2013; 59:52–62. [PubMed: 23867237]
52. Zhao Y-N, Wang F, Fan Y-X, Ping G-F, Yang J-Y, Wu C-F. Activated microglia are implicated in cognitive deficits, neuronal death, and successful recovery following intermittent ethanol exposure. Behav Brain Res. 2013; 236:270–282. [PubMed: 22985845]
53. Marshall SA, Geil CR, Nixon K. Prior Binge Ethanol Exposure Potentiates the Microglial Response in a Model of Alcohol-Induced Neurodegeneration. Brain Sci. 2016; 6(2)
54. Ward RJ, Colivicchi MA, Allen R, Schol F, Lallemand F, De Witte P, et al. Neuro-inflammation induced in the hippocampus of 'binge drinking'rats may be mediated by elevated extracellular glutamate content. J Neurochem. 2009; 111(5):1119–1128. [PubMed: 19765190]
55. Perry VH, Holmes C. Microglial priming in neurodegenerative disease. Nat Rev Neurol. 2014; 10(4):217–224. [PubMed: 24638131]
56. Kalk NJ, Guo Q, Owen DR, Waldman A, Dar K, Gunn RN, et al. Hippocampal microglial dysfunction in alcohol dependence: a [C-11]PBR28 positron emission tomography (PET) study. Alcohol Clin Exp Res. 38(s1):090A.
57. Streit WJ, Xue QS, Tischer J, Bechmann I. Microglial Pathology. Acta Neuropathologica Communications. 2014; 2:142. [PubMed: 25257319]
58. Owen DR, Guo Q, Kalk NJ, Colasanti A, Kalogiannopoulou D, Dimber R, et al. Determination of [^{11}C]PBR28 binding potential in vivo: a first human TSPO blocking study. J Cereb Blood Flow Metab. 2014; 34(6):989–994. [PubMed: 24643083]
59. Chen M-K, Guilarte TR. Translocator protein 18 kDa (TSPO): molecular sensor of brain injury and repair. Pharmacol Ther. 2008; 118(1):1–17. [PubMed: 18374421]
60. Lavisse S, Guillermier M, Hérard A-S, Petit F, Delahaye M, Van Camp N, et al. Reactive astrocytes overexpress TSPO and are detected by TSPO positron emission tomography imaging. J Neurosci. 2012; 32(32):10809–10818. [PubMed: 22875916]
61. Hannestad J, Gallezot J-D, Schafbauer T, Lim K, Kloczynski T, Morris ED, et al. Endotoxin-induced systemic inflammation activates microglia: [^{11}C]PBR28 positron emission tomography in nonhuman primates. Neuroimage. 2012; 63(1):232–239. [PubMed: 22776451]
62. Elmore Monica RP, Najafi Allison R, Koike Maya A, Dagher Nabil N, Spangenberg Elizabeth E, Rice Rachel A, et al. Colony-Stimulating Factor 1 Receptor Signaling Is Necessary for Microglia Viability, Unmasking a Microglia Progenitor Cell in the Adult Brain. Neuron. 2014; 82(2):380–397. [PubMed: 24742461]
63. Jucaite A, Svenningsson P, Rinne JO, Cselényi Z, Varnäs K, Johnström P, et al. Effect of the myeloperoxidase inhibitor AZD3241 on microglia: a PET study in Parkinson's disease. Brain. 2015; 138(9):2687–2700. [PubMed: 26137956]
64. Sekine Y, Ouchi Y, Sugihara G, Takei N, Yoshikawa E, et al. Methamphetamine causes microglial activation in the brains of human abusers. J Neurosci. 2008; 28(22):5756–5761. [PubMed: 18509037]
65. Narendran R, Frankle WG. Comment on Analyses and Conclusions of "Microglial Activity in People at Ultra High Risk of Psychosis and in Schizophrenia: An [^{11}C]PBR28 PET Brain Imaging Study". Am J Psychiatry. 2016; 173(5):536–537. [PubMed: 27133410]
66. Narendran R, Lopresti BJ, Mason NS, Deuitch L, Paris J, Himes ML, et al. Cocaine abuse in humans is not associated with increased microglial activation: an 18-kDa translocator protein

positron emission tomography imaging study with [^{11}C]PBR28. J Neurosci. 2014; 34(30):9945–9950. [PubMed: 25057196]

67. Guan YZ, Jin XD, Guan LX, Yan HC, Wang P, Gong Z, et al. Nicotine inhibits microglial proliferation and is neuroprotective in global ischemia rats. Mol. Neurobiol. 2015; 51(3):1480–1488. [PubMed: 25095782]
68. Khanna A, Guo M, Mehra M, Royal W. Inflammation and oxidative stress induced by cigarette smoke in Lewis Rats. J. Neuroimmunol. 2013; 254(1-2):69–75. [PubMed: 23031832]
69. Bloomfield PS, Selvaraj S, Veronese M, Rizzo G, Bertoldo A, Owen DR, et al. Microglial Activity in People at Ultra High Risk of Psychosis and in Schizophrenia: An [^{11}C]PBR28 PET Brain Imaging Study. Am J Psychiatry. 2016; 173(1):44–52. [PubMed: 26472628]
70. Coughlin JM, Wang Y, Ma S, Yue C, Kim PK, Adams AV, et al. Regional brain distribution of translocator protein using [^{11}C]DPA-713 PET in individuals infected with HIV. J Neurovirol. 2014; 20(3):219–232. [PubMed: 24567030]
71. Loggia ML, Chonde DB, Akeju O, Arabasz G, Catana C, Edwards RR, et al. Evidence for brain glial activation in chronic pain patients. Brain. 2015; 138(3):604–615. [PubMed: 25582579]
72. Oscar-Berman M, Marinkovic K. Alcohol: effects on neurobehavioral functions and the brain. Neuropsychol Rev. 2007; 17:239. [PubMed: 17874302]
73. Beck A, Wustenberg T, Genauck A, Wrase J, Schlagenhauf F, Smokla MN, et al. Effect of brain structure, brain function, and brain connectivity on relapse in alcohol-dependent patients. Arch Gen Psychiatry. 2012; 69(8):842–852. [PubMed: 22868938]
74. Fein G, Sclafani V, Cardenas V, Goldmann H, Tolou-Shams M, Meyerhoff DJ. Cortical gray matter loss in treatment-naive alcohol dependent individuals. Alcohol Clin Exp Res. 2002; 26(4):558–564. [PubMed: 11981133]
75. Laso FJ, Vaquero JM, Almeida J, Marcos M, Orfao A. Production of inflammatory cytokines by peripheral blood monocytes in chronic alcoholism: relationship with ethanol intake and liver disease. Cytom B: Clin Cytom. 2007; 72(5):408–415.
76. Leclercq S, De Saeger C, Delzenne N, de Timary P, Stärkel P. Role of inflammatory pathways, blood mononuclear cells, and gut-derived bacterial products in alcohol dependence. Biol Psychiatry. 2014; 76(9):725–733. [PubMed: 24629538]
77. Setiawan E, Wilson AA, Mizrahi R, Rusjan PM, Miler L, Rajkowska G, et al. Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. JAMA Psychiatry. 2015; 72(3):268–275. [PubMed: 25629589]

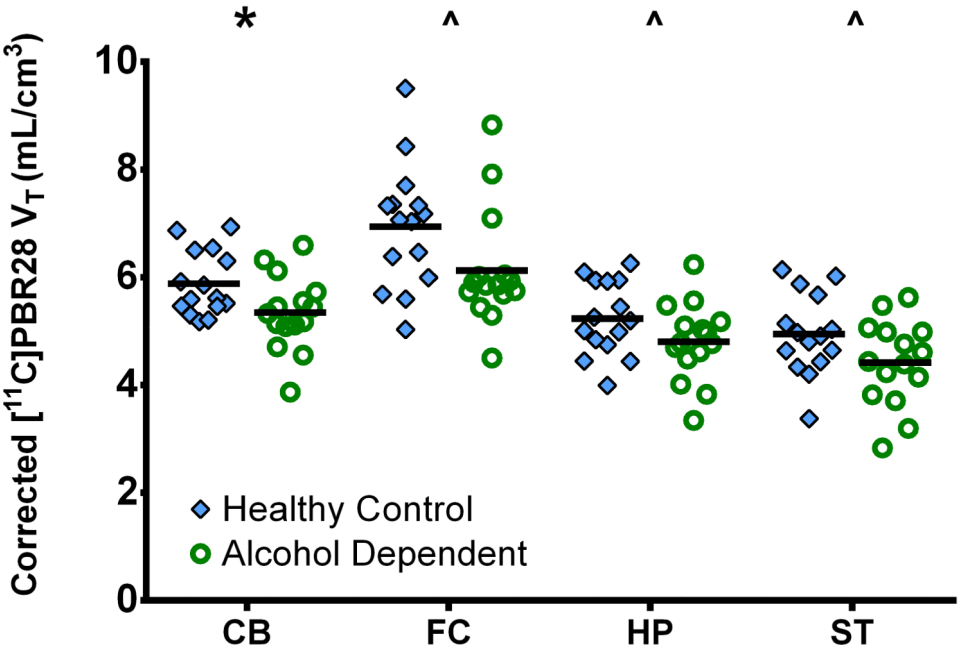


Figure 1.

Levels of activated microglia are lower in alcohol dependent subjects (n=15; open green circles) compared to healthy controls (n=15; closed blue diamonds). Partial-volume corrected [^{11}C]PBR28 V_T values were adjusted for rs6971 genotype for visualization purposes by adding the genotype coefficient determined by the mixed model to the measured [^{11}C]PBR28 V_T value for Medium-Affinity-Binders. Regions shown include cerebellum (CB), frontal cortex (FC), hippocampus (HP), and striatum (ST). Solid lines indicate group mean. * indicates $p<0.05$. ^ indicates $0.05<p<0.10$.

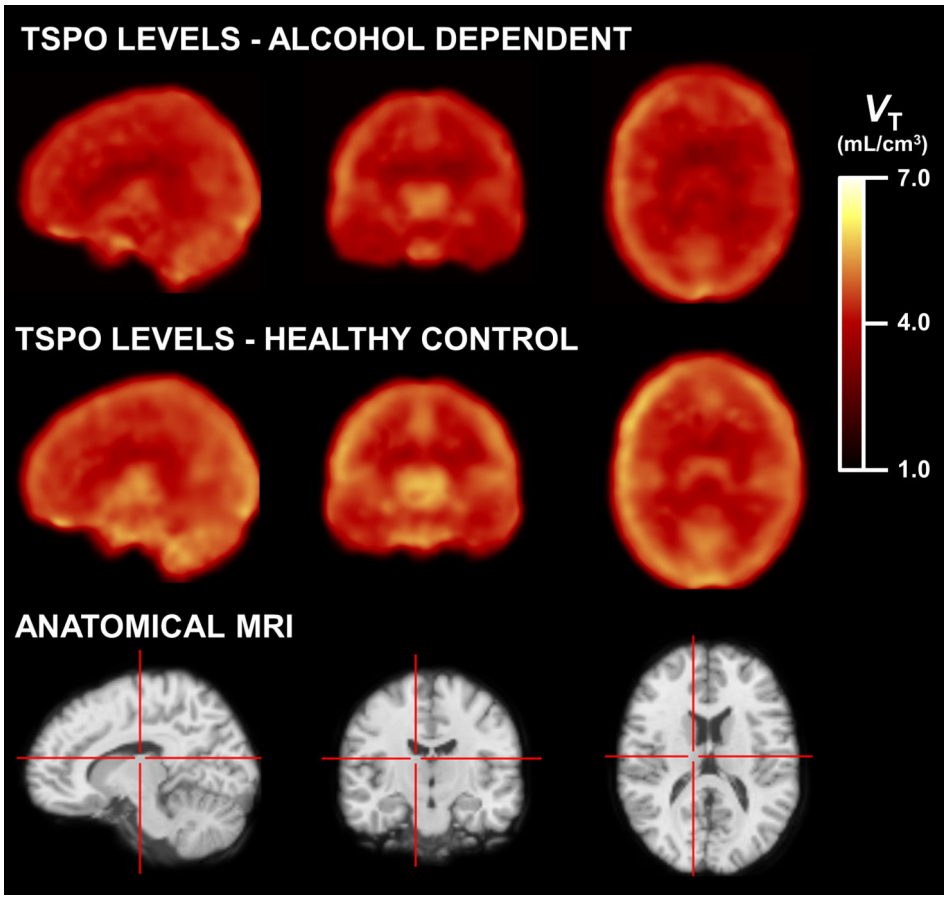
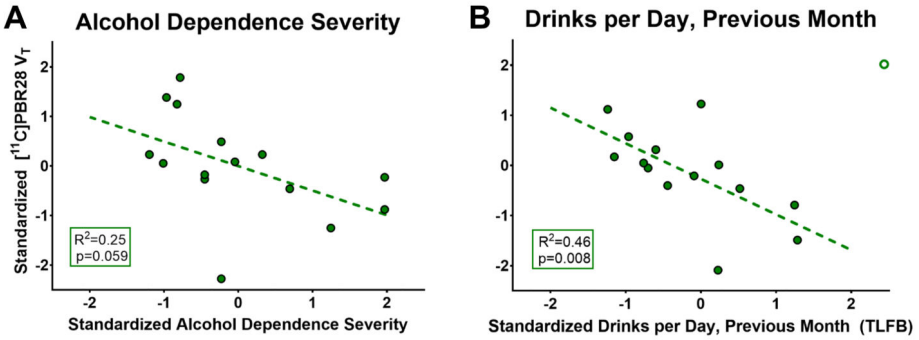


Figure 2.

Average levels of activated microglia are lower in alcohol dependent subjects (top) compared to healthy controls (middle) as illustrated by average parametric images of genotype-adjusted [^{11}C]PBR28 V_T values (scale shown on right in mL/cm^3). An MRI image is shown at bottom for anatomical reference.



C

	Drinks per Day, Last Month (Whole Patient Group)			Drinks per Day, Last Month (Outlier Excluded)			Alcohol Dependence Scale			Alcohol Craving Scale		
	Slope	R^2	p	Slope	R^2	p	Slope	R^2	p	Slope	R^2	p
Cerebellum	-0.10	0.01	0.719	***-0.68***	***0.46***	****0.008***	*-0.5*	*0.25*	*^0.059*	0.33	0.11	0.279
Frontal Cortex	0.21	0.05	0.446	*-0.46*	*0.21*	*^0.098*	-0.41	0.17	0.133	0.29	0.08	0.344
Hippocampus	-0.18	0.03	0.528	***-0.63***	***0.40***	****0.015***	***-0.55***	***0.3***	****0.035***	0.30	0.09	0.321
Striatum	-0.20	0.04	0.484	***-0.67***	***0.45***	****0.008***	***-0.55***	***0.3***	****0.035***	0.27	0.07	0.382

Figure 3.

Relationships between behavioral drinking characteristics and levels of activated microglia. Figures A and B show the illustrative region of cerebellum for significant negative relationships found with Alcohol Dependence Scale (A, p=0.035) including all subjects, and drinks per day over the previous month reported with TLFB (B, p=0.015) when the heaviest drinking subject was omitted (distinguished by open symbol). C summarizes the fitted slope, R^2 value, and p-value are shown for each relationship investigated. * indicates $p<0.05$. ^ indicates $0.05<p<0.10$.

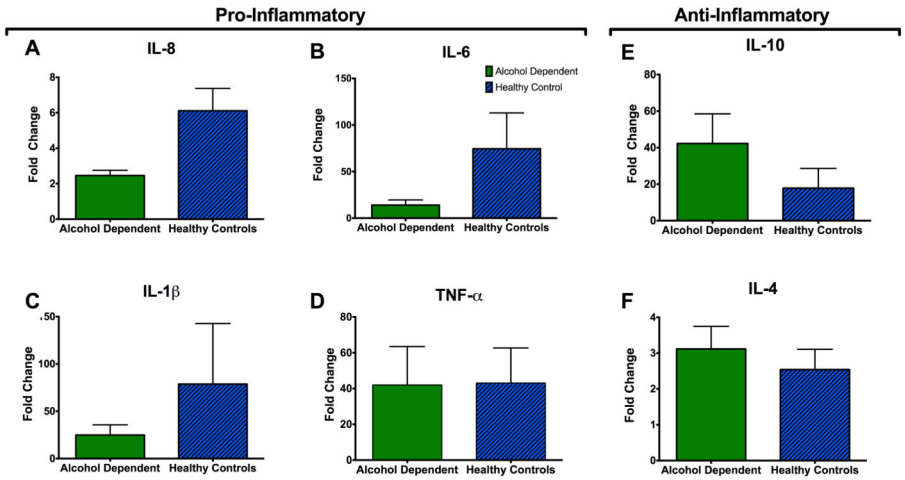


Figure 4.

Lipopolysaccharide (LPS) induced release of cytokines in alcohol dependent subjects (n=9; green solid bars) compared to healthy controls (n=8; blue hatched bars). The finding was significant for pro-inflammatory cytokines interleukin (IL)-8 (A; p=0.031) and IL-6 (B; p=0.043). Other pro-inflammatory cytokines examined include IL-1β (C) and tissue necrosis factor (TNF)-α (D). Anti-inflammatory cytokines examined include interleukin (IL)-10 (E) and IL-4 (F). Graphs show fold-change of cytokine expression of LPS-stimulated monocytes relative to non-stimulated monocytes. Error bars indicate standard error of the mean. * indicates $p<0.05$.

Table 1

Subject Demographics and Scanning Parameters

	Alcohol Dependent n=15	**Healthy Control n=15**
Age	39.9±9.7	35.0±11.6
Sex	11 M; 4F	11 M; 4F
Drinks per Day	6±3	1±1
Years Alcohol Dependence	22±10	N/A
rs6971 Genotype	8 HAB; 7 MAB	8 HAB; 7 MAB
Injected Dose (MBq)	496±197	609±123
Injected Mass (μg)	1.1±1.9	2.3±2.5
f_P (%)	2.7±1.1	2.4±0.8