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In-vivo imaging of neuroinflammation in Veterans with Gulf War Illness

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Abstract

Gulf War Illness (GWI) is a chronic disorder affecting approximately 30% of the veterans who served in the 1991 Gulf War. It is characterised by a constellation of symptoms including musculoskeletal pain, cognitive problems and fatigue. The cause of GWI is not definitively known but exposure to neurotoxicants, the prophylactic use of pyridostigmine bromide (PB) pills, and/or stressors during deployment have all been suspected to play some pathogenic role. Recent animal models of GWI have suggested neuroinflammatory mechanisms may be implicated, including a dysregulated activation of microglia and astrocytes. However, neuroinflammation has not previously been directly observed in veterans with GWI. To measure GWI-related neuroinflammation in GW veterans, we conducted a Positron Emission Tomography (PET) study using [^{11}C]PBR28, which binds to the 18 kDa translocator protein (TSPO), a protein upregulated in activated microglia/macrophages and astrocytes.

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GWI (n=15) and healthy controls (HC, n=33, including a subgroup of healthy Gulf War veterans, HC_{VET}, n=8), were examined using integrated [^{11}C]PBR28 PET/MRI. Standardized uptake values normalized by occipital cortex signal (SUVR) were compared across groups and against clinical variables and circulating inflammatory cytokines (TNF-α, IL-6 and IL-1β). SUVR were validated against volume of distribution ratio (n=13).

Whether compared to the whole HC group, or only the HC_{VET} subgroup, veterans with GWI demonstrated widespread cortical elevations in [^{11}C]PBR28 PET signal, in areas including precuneus, prefrontal, primary motor and somatosensory cortices. There were no significant group differences in the plasma levels of the inflammatory cytokines evaluated. There were also no significant correlations between [^{11}C]PBR28 PET signal and clinical variables or circulating inflammatory cytokines.

Our study provides the first direct evidence of brain upregulation of the neuroinflammatory marker TSPO in veterans with GWI and supports the exploration of neuroinflammation as a therapeutic target for this disorder.

1. Introduction

Gulf War Illness (GWI) is a chronic disorder affecting approximately 30% of the nearly 700,000 veterans who served in the 1991 Gulf War (Binns et al., 2008). It is characterised by a constellation of symptoms including musculoskeletal pain, fatigue, and cognitive/affective decrements. GWI has been suspected to be caused by exposure to neurotoxicants (including the nerve gas sarin and/or pesticides used to prevent insect-borne diseases), the prophylactic use of pyridostigmine bromide (PB) pills (an acetylcholinesterase inhibitor commonly used to protect troops from the harmful effects of nerve agents), and/or the experience of physical stressors (e.g., extreme temperature changes, sleep deprivation, physical exertion) during deployment (Binns et al., 2008; Fukuda et al., 1998; Janulewicz et al., 2018; Maule et al., 2018; Steele et al., 2012; Sullivan et al., 2018).

The wide range of symptoms of GWI is indicative of a complex underlying pathophysiology, for which the etiology has remained largely undetermined. Many of the symptoms reported by veterans with GWI are indicative of central nervous system (CNS) dysfunction, and indeed this has been corroborated by structural and functional neuroimaging and biomarker studies (Abou-Donia et al., 2017; White et al., 2016). Dysfunction of the CNS includes alterations in brain white matter [e.g., reduced volume and increased mean diffusivity; (Heaton et al., 2007; Rayhan et al., 2013b)], decreases in metabolite levels [e.g., lower NAA/creatine ratio; (Menon et al., 2004)], decreases in cerebral blood flow (Haley et al., 2009), reduced gray matter volume (Chao et al., 2010; Rayhan et al., 2013a) and altered gray matter activity in response to behavioral, sensory and chemical stimuli (Calley et al., 2010; Gopinath et al., 2012; Haley et al., 2009). There are also reports and reviews documenting neurobehavioral dysfunction such as slower motor function, poorer visual and verbal memory and worse attention in GWI, and studies have shown that these symptoms are associated with inflammatory cytokines and reduced hippocampal volume (Janulewicz et al., 2018; Jeffrey et al., 2019; O'Donovan et al., 2015; Sullivan et al., 2018).

While the exact pathophysiology of GWI remains unknown, recent studies suggest a possible role for neuroinflammation, and dysregulated activation of microglia and astrocytes (Madhu et al., 2019b; Parihar et al., 2013). Microglia are the resident macrophages of the CNS and rapidly activate in response to pathological danger signals (Kreutzberg, 1996). Acutely, this response is essential for survival, as it allows for the identification of a potentially harmful event, limiting its impact and favoring its resolution. However, overactivation of microglia can lead to production of excessive pro-inflammatory cytokines and excitotoxins, which can be deleterious (Mika, 2008). Similarly, astrocytes can enact responses to pathological events that are adaptive in the acute phase, but can sometimes become dysregulated and pathogenic (Pekny and Pekna, 2014; Pekny et al., 2014). It has been proposed that such aberrant activation can be the result of glial cells being 'primed' by prior pathological events including systemic infections, toxic environmental exposures, and trauma, making them more vulnerable to subsequent stressors, in a 'two-hit' model (Blaylock and Maroon, 2011; Perry et al., 1985; Watkins et al., 2007a). In GWI, multiple neurotoxicant chemical exposures (pesticides, PB, sarin), compounded with the experience of mental or physical stressors, has been suggested to be among potential triggering mechanisms for the chronic symptoms and neuroinflammation in GWI (Binns et al., 2008). For instance, in a recent mouse model investigation, the exposure to a sarin surrogate (DFP) induced widespread neuroinflammation in multiple brain areas such as the frontal cortex, hippocampus, cerebellum and the hypothalamus, and these effects were exacerbated by pre-exposure to corticosterone, the endogenous glucocorticoid typically released in conditions of high physiological stress (O'Callaghan et al., 2015). Despite these animal observations, to our knowledge, GWI-related neuroinflammation has never been demonstrated in humans.

Here we hypothesized that veterans with GWI would demonstrate neuroinflammation in the CNS compared to veterans without GWI and healthy civilians. More specifically, we hypothesized that the pattern of neuroinflammation would be similar to that observed in participants with fibromyalgia (Albrecht et al., 2019). Both fibromyalgia and GWI are in fact accompanied by chronic sickness behavior, with similar hallmarks such as widespread musculoskeletal pain, fatigue, and cognitive difficulties (Arnett and Clark, 2012; Maule et al., 2018; O'Callaghan and Miller, 2019; Wolfe et al., 1990), suggesting the possibility that these conditions present shared mechanisms.

In this study, we used positron emission tomography (PET) and the radioligand [^{11}C]PBR28 to evaluate and document the role of neuroinflammation in veterans with GWI. [^{11}C]PBR28 binds to the 18-kDa translocator protein (TSPO) (Briard et al., 2008; Brown et al., 2007), a mitochondrial protein that is expressed at very low levels in the healthy CNS but becomes dramatically upregulated by activated microglia/macrophages and reactive astrocytes, and is therefore considered a surrogate marker of neuroinflammation (Cagnin et al., 2007; Lacor et al., 1996; Lavisse et al., 2012; Rupprecht et al., 2010).

2. Materials and methods

2.1 Study design

The study was conducted at the Athinoula A. Martinos Center for Biomedical Imaging at Massachusetts General Hospital. The institutional review board and the Radioactive Drug Research Committee approved this study. All participants gave written informed consent.

2.2 Participants

Fifteen veterans with GWI (12 males, 51.1 ± 1.3 years old [mean ± SD]), and 33 healthy controls (17 males, 47.9 ± 12.6 years old; HC) were recruited for the study. The HC sample included 8 healthy veterans of the Gulf War (7 males, 51.4 ± 2.1 years old; HC_{VET}) and 25 healthy civilians (10 males, 46.8 ± 2.8 years old; HC_{CIV}). All GWI veterans met the Kansas diagnostic criteria for GWI which requires endorsing at least three out of six symptom domains (fatigue, pain, neurological, skin, gastrointestinal, and respiratory) and either moderate or multiple mild symptoms within each domain (Steele, 2000). Veterans not meeting Kansas GWI or other exclusionary criteria were considered healthy controls. Participants from all groups were excluded for the presence of a history of major psychiatric illness, neurological illness, cardiovascular disease, inability to communicate in English and for contraindication for PET/MR scanning (e.g., pacemaker, metallic implants, pregnancy, etc.).

2.3 Behavioral visit

All participants in the study were asked to complete the Beck Depression Inventory [BDI; (Beck et al., 1961)] and the Brief Pain Inventory (BPI; (Daut et al., 1983)]. In addition, all veterans were asked to complete the 2011 American College of Rheumatology (ACR) self-report survey for the assessment of fibromyalgia symptoms (Wolfe et al., 2011), the Kansas GWI Questionnaire to detect and score the severity of GWI (Steele, 2000) and Conner's Continuous Performance Test III (CPT3) for the assessment of attention (Conners et al., 2000). During the visit, venous blood was drawn from all participants in order to have all participants genotyped for the Ala147Thr *TSPO* polymorphism, which predicts binding affinity to the radioligand (Owen et al., 2010; Owen et al., 2012). Subjects exhibiting the Thr/Thr genotype, which predicts low affinity binding status, were excluded from the imaging procedures, whereas participants with the Ala/Ala or Ala/Thr polymorphisms, which are associated with high and mixed affinity binding, respectively, were allowed to proceed.

2.4 Imaging visit

At the beginning of the imaging visit, a subset of subjects (n=32) had venous blood collected to measure the level of circulating inflammatory cytokines, using the Meso Scale Discovery V-Plex Plus Proinflammatory Panel. Cytokine analyses were performed through a third-party vendor (Beantown Biotech, Natick MA). In this study we focused on IL-6, TNF-α and IL-1β because their level is commonly reported as altered in animal models and/or humans with pain disorders (Ji et al., 2013; Loggia et al., 2015) and/or GWI (Johnson et al., 2016; O'Donovan et al., 2015). Brain imaging was then performed with a Siemens PET/MRI

scanner, consisting of a dedicated brain avalanche photodiode-based PET scanner in the bore of a Siemens 3T Tim Trio MRI (Kolb et al., 2012). Up to 15 millicurie (mCi) of [^{11}C]PBR28, produced in-house using a procedure modified from the literature (Imaizumi et al., 2007), were injected as an intravenous bolus, and dynamic PET were acquired for 90 min as described previously (Albrecht et al., 2018; Loggia et al., 2015). Because the HC_{CIV} were recruited through a different study protocol, they happened to have a significantly lower injected dose compared to GWI (GWI: 14.307±1.07 mCi [529.4±39.5 Mbq]; HC_{VET}: 14.307±0.98 mCi [529.4±36.2 Mbq]; HC_{CIV}: 12.262±1.42 mCi [453.7±56.1 Mbq; mean ±SD]; GWI vs HC_{CIV}: $p = 0.002$). However, there was no difference in dose between GWI and HC_{VET} ($p = 0.99$). For anatomical localization, spatial normalization and generation of attenuation correction maps (Izquierdo-Garcia et al., 2014b), a multi-echo MPRAGE (T1-weighted structural MRI) volume was also acquired (TR/TE1/TE2/TE3/TE4 = 2530/1.64/3.5/5.36/7.22 ms, flip angle = 7°, voxel size = 1mm isotropic).

In 17 participants (6 veterans), a radial artery catheter was inserted and blood samples were collected at 6-10s intervals for the first three minutes, followed by samples collected at 5, 10, 20, 30, 50, 70 and 90 minutes post [^{11}C]PBR28 injection. These data were collected for the purpose of creating a radiometabolite-corrected arterial input function to perform full kinetic modelling, in order to validate the semiquantitative ratio metric used in the study (see below). Technical issues were encountered for 4 participants, thus the blood data from these participants were excluded from further analyses. Arterial blood processing was performed as previously described by Albrecht et al. (2018).

2.5 Imaging data preprocessing

From the [^{11}C]PBR28 PET data, standard uptake volume ratio (SUVR) images, from 60-90 min post-injection data, were generated as described previously (Albrecht et al., 2018; Loggia et al., 2015; Zurcher et al., 2015). Essentially, standard uptake volume (SUV) images, computed by normalizing radioactivity by injected dose/body weight, were attenuation corrected using a published MR-based method (Izquierdo-Garcia et al., 2014a). These SUV maps were then nonlinearly transformed to MNI space, smoothed with an 8mm full width half-maximum Gaussian kernel, and then intensity-normalised by dividing them by the mean SUV extracted from the occipital cortex [identified using a label from the AAL atlas available in PMOD (Tzourio-Mazoyer et al., 2002)] to obtain SUVR maps. We have previously utilized this approach for quantification of [^{11}C]PBR28 PET data in patients with chronic low back pain, amyotrophic lateral sclerosis (Albrecht et al., 2018) and, more relevant for the current study, fibromyalgia (Albrecht et al., 2019), a condition with a clinical presentation similar to that of GWI. The lack of significant group differences in [^{11}C]PBR28 SUV signal in the occipital cortex was confirmed in this study when the GWI veterans were compared to all HCs ($p = 0.81$) and when they were compared with the subset of HC_{VET} ($p = 0.87$). In order to further support the validity of SUVR as an outcome metric, we compared SUVR against distribution volume (V_T) ratio (DVR), in a subset of participants for whom arterial plasma data were available [n = 13; detailed methods described in (Albrecht et al., 2018)]. To this end, V_T was computed from “target regions” (ie. regions identified as statistically significant across groups in the voxel-wise analyses in this study; see below) as well as the occipital cortex, using radiometabolite-corrected arterial input

function (AIF) and traditional 2-tissue-compartmental modelling. Each target region was divided by occipital cortex V_T to obtain DVR. In all evaluated regions, SUVR were strongly correlated with DVR ($8.7 \times 10^{-7} \leq p \leq 8.9 \times 10^{-3}$, $0.69 \leq r \leq 0.95$; Supplementary Fig. 1). These results provide support for the use of SUVR as a viable PET metric in our study.

2.6 Statistical analysis

Group differences were assessed with Student's *t*-tests for continuous variables (age, clinical and cytokine variables) and chi-square tests for categorical variables (sex and genotype), using Statistica (TIBCO Software Inc., v.13). The main group analyses compared the GWI group with the whole HC group, taking advantage of the relatively large sample of controls. While differences in age between these groups did not meet our threshold for statistical significance ($p = 0.34$), the differences in sex distribution approached significance ($p = 0.061$). For this reason, comparisons between these groups included sex as a covariate. In addition, because *TSPO* genotype affects binding affinity (Owen et al., 2010; Owen et al., 2012), all PET analyses were also corrected for genotype. In addition to these main analyses, we compared the GWI group against the subset of healthy controls who were GW veterans ($n = 8$). These secondary, exploratory analyses were performed to evaluate whether the effects observed in the main analyses could be observed when contrasting groups that were better demographically matched and had comparable GW combat exposure. Because neither sex ($p = 0.65$) nor age ($p = 0.67$) were significantly different across these groups, analyses between GWI and HC_{VET} were only corrected for genotype.

Group analyses were performed using two strategies. First, because one of the hypotheses of this study was that GWI patients would demonstrate similar neuroinflammatory patterns as those observed in fibromyalgia patients, we performed ROI analyses using, as our a priori ROIs, statistically significant clusters from our previous study demonstrating increased [^{11}C]PBR28 signal in that patient group: primary motor/somatosensory cortex (M1/S1), dorsolateral prefrontal cortex (dlPFC), precuneus and anterior mid cingulate cortex (aMCC) (Albrecht et al., 2019). Next, a whole brain voxel-wise analysis was performed, in order to evaluate the possible presence of group differences in the [^{11}C]PBR28 signal beyond the boundaries of the a priori ROIs, as well as to localize any effects observed in the ROI analyses with higher spatial accuracy. Because the injected dose was significantly different between GWI and HC, these analyses were repeated including injected dose as a covariate in the analyses. These analyses were performed with FSL's FEAT GLM tool (www.fmrib.ox.ac.uk/fsl, version 5.0.10). For ease of visualization of the cortical effects and for better comparison with the results of the fibromyalgia study, imaging results are visualized on a surface (FreeSurfer's fsaverage) in the main manuscript. In addition, results were also overlaid onto MNI volumetric standard brain for visualisation of white matter and subcortical structures (Supplementary Figure 3).

For visualization purposes, as well as for correlation analyses (see below), data were extracted from the significant clusters identified in the voxel-wise analyses comparing GWI and HC, anatomically split using labels from the Harvard-Oxford Cortical Structural Atlas (Centre for Morphometric Analyses, http://www.cma.mgh.harvard.edu/fsl_atlas.html).

In GWI patients, the [^{11}C]PBR28 signal from these ROIs was correlated with clinical variables (Kansas GWI score, fibromyalgia score, score on the fatigue item of the ACR self-report survey for the assessment of fibromyalgia symptoms, CPT3 hit reaction time, BPI pain and BDI), in order to evaluate potential association between neuroinflammation and GWI symptom severity, as well as with levels of circulating cytokines (IL-6, TNF-α and IL-1β), to explore the relationship between central and peripheral inflammation (correcting for genotype). Because these analyses were exploratory, the results in this case were not corrected for multiple comparisons.

3. Results

3.1 Participant characteristics

Demographic and other key characteristics for all participants are displayed in Table 1. As briefly mentioned in the methods section, there was no significant difference in sex or age between the GWI and HC groups, ($p = 0.061$ and 0.339, respectively). Differences in genotype distributions between these groups approached but did not reach statistical significance ($p = 0.051$). There was also no significant difference between GWI and HC_{VET} groups in sex, genotype, or age ($p = 0.651$, 0.435 and 0.919, respectively).

3.2 Behavioral measures and blood cytokine levels

There was a significant difference between groups in all behavioral measures, with the GWI participants demonstrating higher Kansas GWI and fibromyalgia scores, fatigue, pain and depression except for the Conner's CPT3 HRT test (Table 2). There was no significant elevation in levels of IL-6, IL-1β and TNF-α in veterans with GWI when compared to HC (p's > 0.05; Table 2).

3.3 Imaging results: a priori ROI analyses

Compared to the HC group, GWI participants demonstrated significantly elevated [^{11}C]PBR28 PET signal in the dlPFC, precuneus and aMCC, i.e., three out of four of the a priori ROIs. The PET signal elevations in the dlPFC and precuneus remained statistically significant when GWI were compared with HC_{VET}, and additional signal elevations were observed in M1-S1, (Figure 1).

3.4 Imaging results: voxel-wise group differences

The voxel-wise comparison between GWI and HC revealed widespread cortical [^{11}C]PBR28 PET signal elevations (and no regions of PET signal reduction) in GWI. These were observed both within the regions used as a priori ROIs in the analysis previously described (S1, M1, dlPFC, aMCC and precuneus) as well as additional regions (dorsomedial prefrontal cortex [dmPFC], paracingulate cortex, anterior cingulate cortex [ACC], ventral medial PFC [vmPFC] and posterior cingulate cortex [PCC]; Figure 2A). Several of these regions [S1, M1, dlPFC, dmPFC, precuneus and the superior parietal lobule (SPL; Figure 2B)] survived statistical significance when GWI were compared with the HC_{VET} subgroup. For display purposes, the mean SUVR values from a subset of regions are displayed in Figure 2C. In addition, as injected dose was significantly different between groups when veterans with

GWI were compared with HC, we ran an exploratory voxel-wise analysis, including injected dose as a covariate. This analysis yielded similar results (Supplementary Figure 2).

3.5 Imaging results: regression analyses

Regions that showed elevations in SUVR in GWI compared to HC were selected as an ROI for correlations with clinical variables and circulating cytokine levels in the GWI group only, in exploratory analyses. In no region was there a significant correlation between SUVR signal in GWI and clinical variables or circulating inflammatory cytokines (Table 3).

4. Discussion

The current study provides in-vivo evidence of neuroinflammation in veterans with GWI. When compared to GW veterans without GWI and healthy civilians, veterans with GWI demonstrated elevated TSPO binding, as measured with [^{11}C]PBR28 PET. This marker of neuroinflammation demonstrated elevated levels throughout cortical areas such as precuneus, prefrontal cortex, and primary motor and somatosensory areas, as well as underlying white matter and the putamen. The neuroinflammatory signal elevations observed in GWI demonstrated spatial similarities to that observed in fibromyalgia (Albrecht et al., 2019), as we had hypothesized given the overlap in the clinical presentation of the two conditions. Fatigue, musculoskeletal pain, disturbed sleep, memory and attention deficits are some of the symptoms that affect both conditions (Binns et al., 2008; Clauw, 2014). In fact, GWI veterans are often diagnosed with fibromyalgia (Blanchard et al., 2019).

While this study represents the first report of in-vivo neuroinflammation imaging in veterans with GWI, these results conform to a body of preclinical research that has shown neuroinflammation in animal models of GWI (White et al., 2016). Such models have shown that the exposure to neurotoxicant chemicals such as irreversible acetylcholinesterase inhibitor (AChEi), organophosphate pesticides, nerve agents and prophylactic treatment with pyridostigmine bromide pills (PB; a reversible AChEi), i.e., the same compounds the veterans had been exposed to during the GW, induces chronic neuroinflammation (Banks and Lein, 2012; Koo et al., 2018; O'Callaghan et al., 2015; Ojo et al., 2014; Parihar et al., 2013). Additionally, pyrethroid pesticides, which were also widely used during the GW, mediate their action by opening voltage gated sodium channels which results in excessive neuronal firing (Hue and Mony, 1987), possibly inducing neurogenic inflammation both centrally (Xanthos and Sandkühler, 2013), as well as peripherally (Chiu et al., 2012; Roosterman et al., 2006). While our study showed that veterans with GWI demonstrated elevations of central (TSPO PET signal) markers of inflammation, we found no evidence of elevations in peripheral (pro inflammatory cytokines) markers of inflammation.

In addition to neurotoxicant exposure, physical and mental stressors can induce neuroinflammation, perhaps compounding its effects. Indeed, some animal models of GWI are produced through the combination of exposure to GW-relevant neurotoxicant AChEi chemicals (sarin, pesticides, PB) and stress (White et al., 2016). In these models of GWI, astrogliosis and/or microglial activation have been reported within the prefrontal cortex, the hippocampus, striatum, hypothalamus, olfactory bulb and the cerebellum (O'Callaghan et al., 2015; Ojo et al., 2014; Parihar et al., 2013). Interestingly, O'Callaghan et al. (2015)

found that these neuroinflammatory responses were greatly exacerbated when animals were pre-treated with rodent stress hormone corticosterone (CORT), administered at levels compatible with those observed with high physiological stress (O'Callaghan et al., 2015), possibly at the level that the veterans might have experienced given the harsh conditions that were prevalent during the Gulf War [e.g., extreme heat or cold, or sleep deprivation (Gifford et al., 2006)]. Indeed, studies have shown that sleep disturbances and heat stress can induce neuroinflammation (Chauhan et al., 2017; Zhu et al., 2012). Furthermore, these stressors, along with other brain insults including a history of mild traumatic brain injury (mTBI) can 'prime' glial cells for aberrant activation which might lead to chronic neuroinflammation (Blaylock and Maroon, 2011; Burda et al., 2016; Chen et al., 2014; Kuhlmann and Guilarte, 2000; Perry et al., 1985; Watkins et al., 2007a).

In this investigation, we have used [^{11}C]PBR28 to image TSPO binding as a marker of neuroinflammation. Though TSPO is constitutively expressed by many cell types, within the CNS, this protein is upregulated primarily or exclusively in glial cells during neuroinflammatory responses, and hence can be used as a sensitive marker of glial activation (Wei et al., 2013). Indeed, animal models of neuropathic pain have shown increased TSPO expression co-localised with activated microglia and astrocytes (Liu et al., 2016; Wei et al., 2013). Similarly, TSPO expression has been localised with activated astrocyte and microglia in animal models and human investigations of multiple sclerosis, Alzheimer's disease and HIV encephalitis (Abourbeh et al., 2012; Chen and Guilarte, 2006; Cosenza-Nashat et al., 2009; Gulyas et al., 2009; James et al., 2017) and animal models and human post-mortem studies of ischemia (Cosenza-Nashat et al., 2009; Martin et al., 2010; Rojas et al., 2007). Further, TSPO PET imaging in amyotrophic and primary lateral sclerosis patients demonstrates increased TSPO signal in the primary motor cortex (Alshikho et al., 2016; Alshikho et al., 2018; Paganoni et al., 2018; Zurcher et al., 2015), a region where glial activation can be documented histologically (Hudson et al., 1993; Kawamata et al., 1992 ; Rothstein et al., 1995). Similarly, in Alzheimer's Disease, glial activation can be observed in amyloid positive regions (Araujo and Cotman, 1992; Rozemuller et al., 1989), and these regions have shown elevated TSPO expression (Kreisl et al., 2013; Parbo et al., 2017). Likewise, glial activation has been reported in the basal ganglia of Huntington's Disease patients (O'Kusky et al., 1999), who also have shown elevated TSPO expression (Lois et al., 2018). These observations support the use of TSPO as a marker of glial activation. Because in the CNS TSPO can be upregulated by microglia and/or astrocytes (Beckers et al., 2018; Lavisse et al., 2012), our study does not directly allow clarification of which glial cell subtype might contribute to the observed signal. For instance, several animal models have shown that initial upregulation of TSPO might be driven by microglia, whereas astrocytic TSPO upregulation might be maintained throughout the course of the disease (Chen et al., 2014; Chen and Guilarte, 2006; Kuhlmann and Guilarte, 2000; Liu et al., 2014; Martin et al., 2010). This phase-dependent activation of glial cells is supported by human post-mortem studies of multiple sclerosis, showing that in acute lesions, microglia and macrophages are the major cell contributors to TSPO expression, whereas in chronic lesions, astrocytes are the major contributors to TSPO expression (Cosenza-Nashat et al., 2009). However, because GWI is accompanied by sickness behavior (O'Callaghan and Miller, 2019), and given that microglia are largely the source of neuroinflammatory mediators that underlie sickness

behaviors (Dantzer et al., 2008; Konsman et al., 2002; Maier, 2003; Watkins et al., 2007b), it seems likely that microglial activation would account for a significant proportion of the neuroinflammatory signal observed. In addition, several preclinical GWI studies implicate microglia and not astrocytes in neuroinflammation (Carreras et al., 2018; Locker et al., 2017). Furthermore, a recent study employing a dual-ligand approach suggests that in fibromyalgia, a chronic condition that shares clinical features with GWI, the TSPO signal might be indeed driven by microglia rather than astrocytes. In that study, we reported significant elevations in TSPO signal (which may reflect microglial or astrocytic contributions), but not in MAO-B signal [which is thought to reflect mostly astrocytic, but not microglial, contributions; (Albrecht et al., 2019)]. Similar approaches will need to be implemented to understand whether a similar interpretation can apply to GWI as well.

It is important to also stress that, in addition to being upregulated by glial cells within the CNS, TSPO is also highly expressed in activated macrophages and other peripheral immune cells (Lacor et al., 1996). For instance, a recent study has shown elevated TSPO expression in activated macrophages, fibroblast-like synoviocytes and CD4+ T lymphocytes in the synovial tissue of patients with rheumatoid arthritis (Narayan et al., 2018). In physiological conditions, the blood brain barrier (BBB) typically acts as a restriction to prevent easy recruitment into the CNS parenchyma of cells involved in the adaptive immunity response (with the exception of activated T cells) such as leukocytes (Ransohoff and Brown, 2012). However, several preclinical models of GWI document the presence of BBB disruptions [e.g., Abdel-Rahman et al., (2002)]. Indeed, a study in GW veterans detected CNS autoantibodies to glial fibrillary acidic protein, myelin basic protein, tau, tubulin and other neuro-glial proteins in the peripheral blood that would not be in circulation without at least prior BBB compromise at some point (Abou-Donia et al., 2017). Because the disruption in BBB permeability increases the likelihood of the CNS being infiltrated by activated macrophages or other cell types (Lopes Pinheiro et al., 2016), and given that TSPO is highly expressed in these cells, it is possible that the elevations in the brain levels of TSPO observed in this investigation might in part be due to this phenomenon. The recruitment of peripheral immune cells into the CNS was shown to be able to damage neuronal cells (Ransohoff and Brown, 2012), and thus might contribute to some of the symptoms of GWI such as cognitive/affective decrements and memory loss (Janulewicz et al., 2017; Jeffrey et al., 2019; Sullivan et al., 2018; Sullivan et al., 2003). However, future studies will need to directly measure BBB damage in veterans with GWI to assess the relevance of this mechanism to neuroinflammation.

In the present study, the brain TSPO PET signal did not correlate with circulating levels of proinflammatory cytokines, and when compared to HC, cytokine levels in GWI were not elevated. These results agree with several prior studies in patients with major depression (Richards et al., 2018; Setiawan et al., 2015), seasonal allergy (Tamm et al., 2018), schizophrenia (Coughlin et al., 2016) and in healthy participants imaged after administration of lipopolysaccharide (a potent immune activator) (Sandiego et al., 2015), which also reported no statistically significant correlations between brain TSPO signal and the majority of the peripheral markers of inflammation [although a prior study from our group did report a weak negative correlation between TSPO signal and IL-6 in chronic low back pain (Loggia et al., 2015)].

Why some veterans develop GWI while others do not is still yet to be answered. It is possible that the veterans with GWI have had other toxicant exposures or mTBIs that have 'primed' their glial cells for neuroinflammation at the exposure of further neurotoxicants and stressors (Blaylock and Maroon, 2011; Perry et al., 1985; Watkins et al., 2007a). Certainly, a larger sample size of veterans, ideally with detailed information about other brain insults including mTBIs, life stressors and the types of neurotoxicant exposures, would be required to begin answering some of these questions.

In conclusion, this study is the first to document an elevation of the neuroinflammatory glial marker, TSPO, in the brain of veterans with GWI. Further studies are required to validate and further refine these findings, and to determine whether glial modulation may be a viable therapy for GWI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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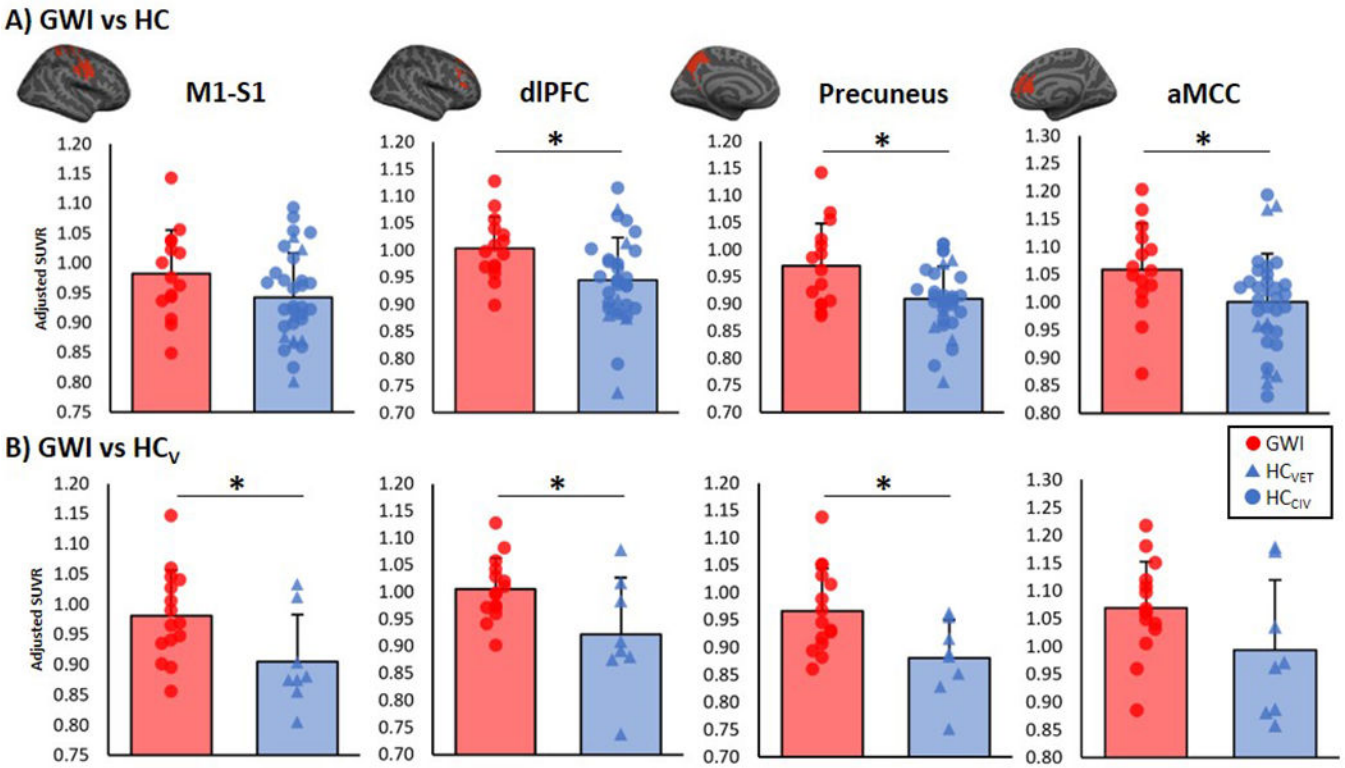

Figure 1: ROI analyses.

Group differences in [^{11}C]PBR28 standardized volume uptake (SUVR) in a priori ROIs. These regions were selected as they demonstrated [^{11}C]PBR28 PET signal elevations in fibromyalgia patients. Top panel: Average ± standard deviation SUVR extracted showing differences between GWI and HC (adjusted for genotype and sex). Bottom panel: Average ± standard deviation SUVR extracted showing differences between GWI and HC_{VET} (adjusted for genotype). Surface projections of regions are displayed in red above the plots. * significant difference between groups ($p < 0.05$). M1 = primary motor cortex; S1 = primary somatosensory cortex; dlPFC = dorsolateral prefrontal cortex; aMCC = anterior mid cingulate cortex.

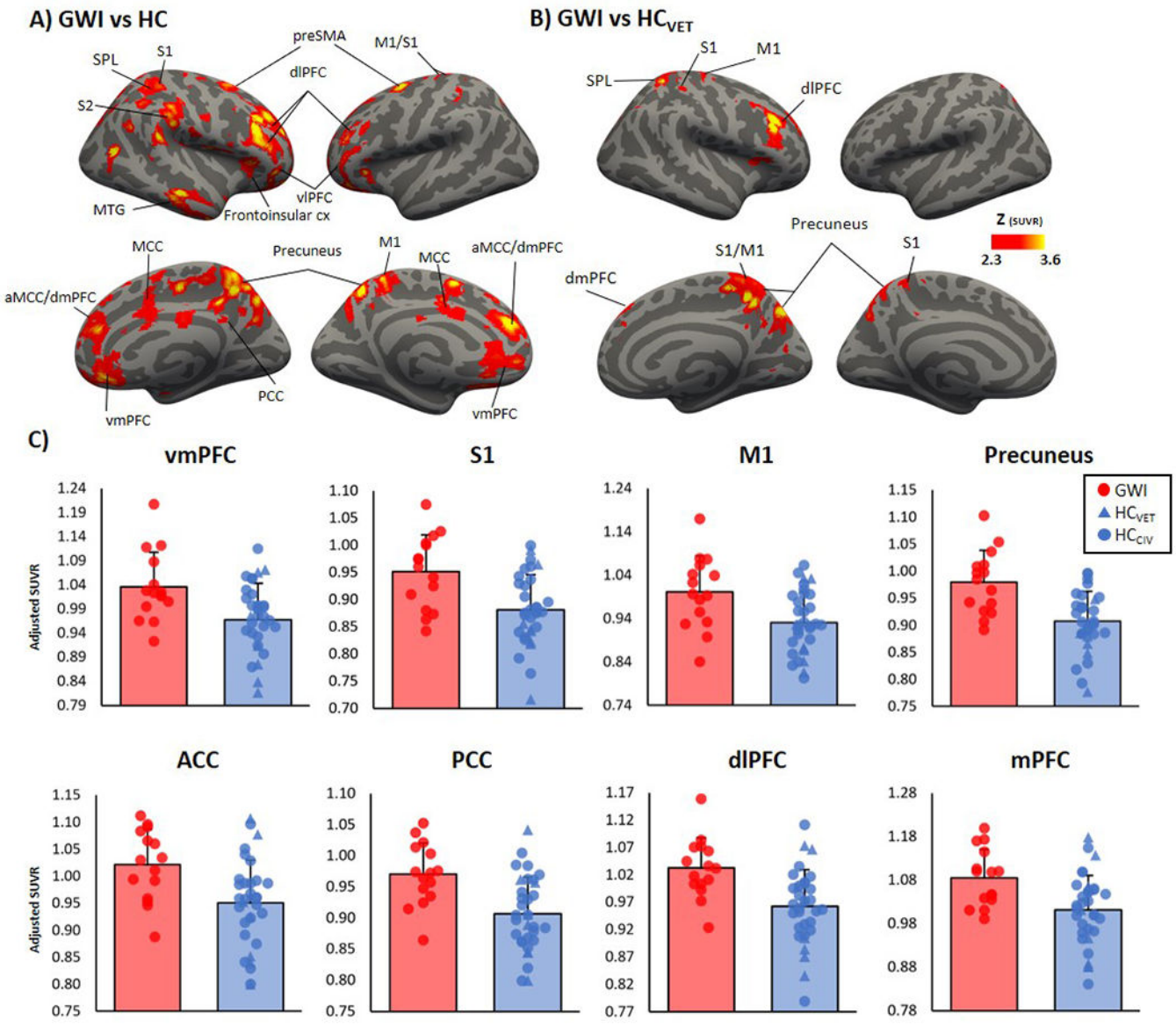

Figure 2:

Voxel-wise group difference in [^{11}C]PBR28 standardized volume uptake (SUVR). **A.** Surface projection maps displaying areas with significantly elevated [^{11}C]PBR28 SUVR in GWI (n=15) compared to HC (n=33), in voxel-wise analyses, data adjusted for sex and genotype. **B.** Surface projection maps displaying areas with significantly elevated [^{11}C]PBR28 SUVR in GWI (n=15) compared to HC$_{VET}$ (n=8), in voxel-wise analyses, data adjusted for genotype. **C.** Average ± standard deviation SUVR extracted from several clusters identifies as statistically significant in the voxel-wise SUVR analysis from A. Data plots have been adjusted for sex and genotype. vmPFC = ventral medial prefrontal cortex; S1 = primary somatosensory cortex; M1 = primary motor cortex; ACC = anterior cingulate cortex; PCC = posterior cingulate cortex; dlPFC = dorsolateral prefrontal cortex; dmPFC = dorsomedial prefrontal cortex; mPFC = medial prefrontal cortex; aMCC = anterior mid

cingulate cortex; SPL = superior parietal cortex; MCC = mid cingulate cortex; vlPFC = ventrolateral prefrontal cortex; S2 = secondary somatosensory cortex; cx = cortex.

Table 1:

Participant characteristics

Participant characteristics

	GWI	HC	GWI vs HC
N	15	33 (all)	-
		8 (veterans)	
Sex	12M; 3F	17M; 15F (all)	p = 0.06
		7M; 1F (veterans)	p = 0.65
Age (years: mean ± sd)	51.1 ± 5.0	47.9 ± 12.6 (all)	p = 0.34
		51.4 ± 6.1 (veterans)	p = 0.92
TSPO polymorphism	5H; 10M	21H; 12M (all)	p = 0.05
		4H; 4M (veterans)	p = 0.44

Table 2:

Participant clinical and cytokine variables

Group clinical and cytokine variables			
	GWI	**HC**	**GWI vs HC**
Kansas GWI score	45.2 ± 18.86	1.75 ± 2.96 (veterans)	$p = 5.49 \times 10^{-6}$
ACR Fibromyalgia score	18.4 ± 6.98	1.25 ± 2.12 (veterans)	$p = 2.12 \times 10^{-6}$
ACR Fatigue score	2.1 ± 0.80	0 (veterans)	$p = 9.69 \times 10^{-7}$
Conner's CPT3 Hit Reaction Time	54.4 ± 8.81	47.88 ± 4.88 (veterans)	p = 0.103
BPI Pain Intensity	5.6 ± 1.42	0.27 ± 0.58 (all)	$p < 0.0001$
		0.56 ± 0.95 (veterans)	$p = 2.57 \times 10^{-8}$
BDI	17.7 ± 9.92	1.62 ± 2.45 (all)	$p = 1.12 \times 10^{-9}$
		2.69 ± 3.65 (veterans)	$p = 0.0008$
IL-6	1.63 ± 1.05	0.76 ± 0.78 (all)	$p = 0.0344$
		1.26 ± 0.91 (veterans)	p = 0.428
TNF-α	6.16 ± 5.14	2.44 ± 1.97 (all)	$p = 0.0295$
		3.33 ± 2.68 (veterans)	p = 0.2195
IL-1β	0.93 ± 0.39	1.02 ± 0.69 (all)	p = 0.6862
		1.04 ± 0.53 (veterans)	p = 0.6104

Table 3:

GWI [^{11}C]PBR28 correlations with clinical and cytokine variables

GWI [^{11}C]PBR28 correlations with clinical variables

		vmPFC	S1	M1	Precuneus	ACC	PCC	dlPFC	mPFC
Kansas GWI score	r value	−0.3772	−0.1244	−0.3110	−0.1458	−0.1728	−0.2780	−0.3772	−0.2967
	p value	0.1840	0.6720	0.2790	0.6190	0.5550	0.3360	0.1900	0.3030
ACR Fibromyalgia score	r value	−0.3472	0.2181	−0.1311	0.1828	0.1263	0.0116	−0.0823	−0.0474
	p value	0.2240	0.4540	0.6550	0.5320	0.6670	0.9690	0.7800	0.8720
ACR Fatigue score	r value	−0.2340	0.2363	−0.0427	0.1678	0.1778	−0.1055	0.0398	0.0858
	p value	0.4210	0.4160	0.8850	0.5660	0.5430	0.7200	0.8930	0.7700
Conner's CPT3 Hit Reaction Time	r value	0.0180	−0.0412	−0.0782	−0.0529	0.0350	0.1562	−0.3173	0.0941
	p value	0.9510	0.8890	0.7910	0.8570	0.9050	0.5940	0.2690	0.7490
BPI Pain Intensity	r value	−0.4269	0.2825	−0.0211	0.2466	0.4259	−0.0009	0.3951	0.1422
	p value	0.1280	0.3280	0.9430	0.3950	0.1290	0.9980	0.1620	0.6280
BDI	r value	−0.2185	−0.0712	−0.1650	−0.0981	−0.2404	−0.2495	−0.2776	−0.3849
	p value	0.4530	0.8090	0.5730	0.7390	0.4080	0.3900	0.3370	0.1740
IL-6	r value	0.2803	−0.2696	−0.1165	−0.1851	−0.2864	−0.1407	−0.5564	0.0936
	p value	0.3320	0.3510	0.6920	0.5260	0.3210	0.6310	0.0750	0.7500
TNF-α	r value	0.7217	0.6942	0.6156	0.7709	0.7747	0.7562	0.7360	0.8292
	p value	*0.0120*	*0.0180*	*0.0440*	*0.0050*	*0.0050*	*0.0070*	*0.0100*	*0.0020*
IL-1β	r value	0.1616	0.2283	0.2511	0.1904	0.1601	0.4680	0.0134	0.3483
	p value	0.6350	0.5000	0.4560	0.5750	0.6380	0.1470	0.9690	0.2940