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This project is aimed at evaluating the contribution of brain glia to the pathophysiology of Gulf War Illness, as well as fibromyalgia (a functional pain disorder characterized by similar symptoms). At this point, we have closed the study to enrollment and are analyzing data. At the end of this study, 15 Fibromyalgia patients, 15 veterans with Gulf War, 8 patients who served in the Gulf War but did not have Gulf War Illness, and 23 healthy controls have completed all study procedures. We have published one paper with data that we have collected from this study and have also presented this work at various meetings. A second paper is currently being prepared.					
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Recruitment					
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1. INTRODUCTION:

In this project, we are using simultaneous magnetic resonance imaging (MR) and positron emission tomography (PET) with [¹¹C]PBR28 – a recently developed PET ligand which binds to activated microglia with unprecedented specific-to-nonspecific binding ratio – to test the hypothesis that patients with Fibromyalgia (FM) or Gulf War Illness (GWI) demonstrate over-activation of brain microglia. Microglia are a subpopulation of macrophages known to mediate the inflammatory response of the central nervous system. While under normal conditions these cells are involved in adaptive homeostatic defense responses, such as the destruction of invading microorganisms, animal models have also provided evidence for a role of microglial activation in the development of chronic pain. Recognizing the role of these chronically active microglial cells in FM and GWI might lead to the development of new and potentially more effective treatment approaches for both conditions. Furthermore, if disease-specific patterns of microglial activation can be identified, it would improve our ability to correctly diagnose the two conditions and treat them with specificity not possible to date.

2. KEYWORDS:

Fibromyalgia; Gulf War Illness; Chronic Pain; Microglia; Neuroinflammation; Positron Emission Tomography; Magnetic Resonance Imaging

3. ACCOMPLISHMENTS:

What were the major goals of the project?

As stated in approved Statement of Work, we anticipated the following accomplishments during the Year of the project:

- Task 2: Subject Recruitment
- Task 3: Study Visits
- Task 4: Data Analysis
- Task 5: Manuscript preparation

What was accomplished under these goals?

During year 4, we stated that we would accomplish tasks 2-5.

Subject Recruitment (Task 2) In 2018, we enrolled 4 veterans with GWI, 1 participant who served in the Gulf war but does not have GWI, and 1 healthy control in our study. We have closed the study to enrollment.

Study Visits (Task 3) At the end of the study, 28 participants with FM, 20 veterans with GWI, 11 veterans who served in the Gulf war but do not have GWI, and 19 healthy controls have completed the behavioral visit process. 15 FM patients, 15 GWI veterans, 8 patients who served in the Gulf War but did not have GWI, and 2 healthy controls have completed all study procedures. Of the 6 participants we enrolled in 2018, one veteran with GWI was unable to be scanned.

Data Analysis (Task 4) We have compared the brain scans from Fibromyalgia as well as GWI patients against controls. In both cases we have observed elevated [¹¹C]PBR28 signal in remarkably similar regions (precuneus, cingulate and prefrontal regions) supporting our hypothesis that neuroinflammation is involved in both disorders, and that the similarity in clinical presentation of both disorders may reflect similar neuroinflammatory signatures.

A manuscript (**Task 5**) examining brain glial activation in fibromyalgia patients has been accepted into the Journal of Brain, Behavior and Immunity in September 2018 (**Figure 1**). A second manuscript on glial activation in Gulf War Illness is currently in preparation (**Figures 2, 3**).

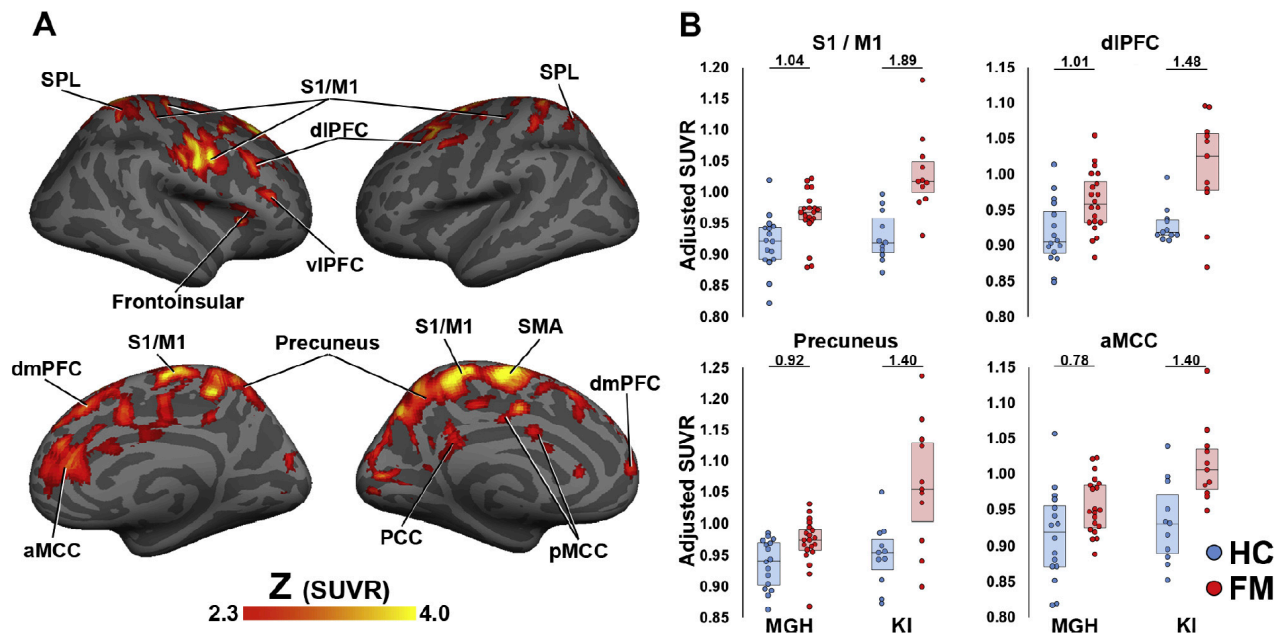


Figure 1. A. Voxelwise group differences in [11C]PBR28 signal between FM patients and healthy volunteers. Data from MGH and Karolinska Institutet (KI) were combined to enhance statistical power and generalizability. **B.** Data from individual research sites are displayed separately, showing that overall that the PET group differences, while larger for the KI dataset, can be observed at each site independently (Albrecht et al., Brain Behavior and Immunity, 2018)

• **GWV group demonstrates elevated PET signal compared to the GWH+HC group**

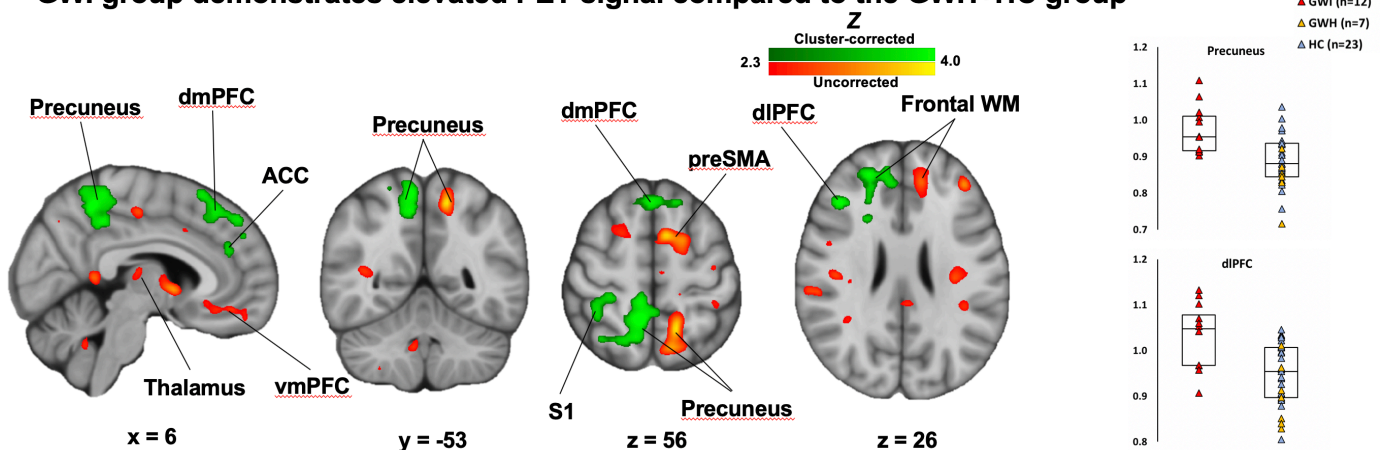


Figure 2. Voxel-wise comparison of veterans with GWV and controls (including GW healthy veterans and healthy civilians; N=30). **Left:** Cluster-corrected statistical maps are displayed in green colorscale. Uncorrected z-maps are underlaid in red-yellow colorscale for display purposes, in order to highlight the bilaterality of some effects. **Right:** Average SUVR from regions displaying group differences were extracted for each group (GWV, GWH, and HC) (Albrecht et al., in preparation).

- **GWV group demonstrates elevated PET signal compared to the GWH group**

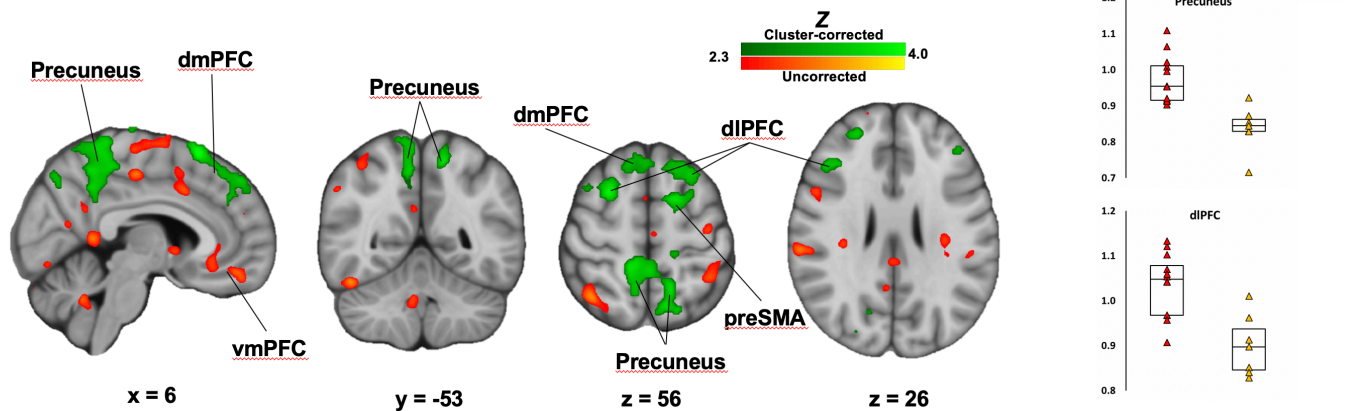


Figure 3. Voxel-wise comparison of veterans with GWV and GW healthy veterans. **Left:** Cluster-corrected statistical maps are displayed in green colorscale. Uncorrected z-maps are underlaid in red-yellow colorscale for display purposes, showing additional regional group differences below threshold for the main analysis. Veterans with GWV displayed increased regional SUVR in cortical gray matter and anterior white matter compared to healthy GW veterans. **Right:** Average SUVR from regions displaying group differences were extracted for each group (GWV and GWH). (Albrecht et al., in preparation).

What opportunities for training and professional development has the project provided?

This project has provided the opportunity for one postdoc and one research assistant to be trained in the clinical evaluation and imaging of GWV. Results from this project have provided the opportunity to present data at national and international meetings, therefore providing further opportunities for professional development.

How were the results disseminated to communities of interest?

Our fibromyalgia paper (Albrecht et al., Brain Behavior and Immunity 2018) is now an open-access publication, available to anyone. This paper received significant media attention, and featured on both the Mass General website and the Mass General Research Institute blog.

Both our FM and Gulf War results have been disseminated at various national and international meetings:

Albrecht DS, Protsenko E, Lee Y, Edwards RR, Catana C, Akeju O, Cohen G, Hooker JM, Napadow V, Loggia ML. Brain glial activation in fibromyalgia: a [¹¹C]PBR28 pilot study. 11th International Symposium on Functional NeuroReceptor Mapping, Boston, MA, 2016.

Albrecht DS, Bergan C, Akeju O, Clauw DJ, Conboy L, Edwards RR, Kim M, Lee YL, Napadow V, Protsenko E, Sullivan K, Loggia ML. Glial activation in Gulf War Illness: Preliminary investigation. 47th Annual Scientific Meeting of the Society for Neuroscience (2016), Washington, DC.

Albrecht DS, Protsenko E, Mawla I, Lee Y, Edwards RR, Catana C, Akeju O, Cohen G, Hooker JM, Napadow V, Loggia ML. Does brain glial activation have a role in fibromyalgia? A [¹¹C]PBR28 PET study. 16th World Congress on Pain, the International Association for the Study of Pain (2016), Yokohama, Japan.

Albrecht DS, Forsberg A, Sandstrom A, Bergan C, Kadetoff D, Protsenko E, Lampa J, Lee YC, Höglund CO, Catana C, Cervenka S, Akeju O, Lekander M, Cohen G, Halldin C, Taylor N, Kim M, Hooker JM, Edwards RR, Napadow V,

Kosek E*, Loggia ML*. In vivo imaging of neuroinflammation in fibromyalgia: a multi-site [¹¹C]PBR28 Positron Emission Tomography study. 17th World Congress on Pain (2018), Boston MA.

What do you plan to do during the next reporting period to accomplish the goals?

At this time, the study is closed to enrollment and open to data analysis only.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

The results of this study suggest that glial activation may play a significant role in the neurobiological mechanisms underlying fibromyalgia symptoms. This may point to novel therapeutic targets for treating fibromyalgia and it may also help to improve diagnostic accuracy for this debilitating disorder.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

If glial activation does play a significant role in fibromyalgia, and other chronic pain disorders, then this could potentially impact drug discovery efforts for these conditions.

What was the impact on society beyond science and technology?

People with fibromyalgia often experience a significant amount of stigma surrounding their diagnosis, since many professionals still believe symptoms of fibromyalgia are more psychological than physiological in nature. It is our hope, that by beginning to identify the underlying pathophysiology of fibromyalgia, stigma surrounding the disorder will be reduced.

5. CHANGES/PROBLEMS:

All of the changes described below were reported to the DoD HRPO at the time of their approval by the Partners IRB. None of the changes listed required full meeting review by either the Partners IRB or the DoD HRPO. All changes have also been reported in the quarterly technical progress reports.

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

To date, the only major delays faced were issues presented by subject recruitment. Specifically, there were delays in the recruitment of Gulf War veterans, both healthy and ill, as well as healthy controls. In order to bolster our recruitment efforts with both groups, we created separate advertisements to be posted on the Partners Clinical Trials website. These additional avenues allowed us to supplement our recruitment efforts and substantially increase enrollment in the study.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

6. PRODUCTS:

Publications, conference papers, and presentations

Albrecht, D.S., Forsberg, A., Sandström, A., Bergan, C., Kadetoff, D., Protsenko, E., Lampa, J., Lee, Y.C., Höglund, C.O., Catana, C. and Cervenka, S., 2018. Brain glial activation in fibromyalgia—A multi-site positron emission tomography investigation. *Brain, behavior, and immunity*.

Website(s) or other Internet site(s)

scholar.harvard.edu/loggia

This is the lab's official website, hosted within the Harvard University network. The website provides an introduction to the lab's research, recent news, and a complete list of publications.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Personnel who have devoted at least 1 calendar month to the project are listed below:

Name:	Marco Loggia, PhD
Project Role:	Principal Investigator
Researcher Identifier (ORCID ID):	0000-0002-8026-5265
Nearest Person Month Worked:	12
Contribution to Project:	Directed the project, hired and supervised personnel, collected data
Funding Support:	

Name:	Courtney Bergan
Project Role:	Research Assistant
Researcher Identifier (ORCID ID):	N/A
Nearest Person Month Worked:	4
Contribution to Project:	Recruited and screened participants, collected data
Funding Support:	

Name:	Ciprian Catana
Project Role:	Co-investigator
Researcher Identifier (ORCID ID):	N/A
Nearest Person Month Worked:	<1
Contribution to Project:	Data analysis, consultation
Funding Support:	

Name:	Robert Edwards
Project Role:	Co-investigator
Researcher Identifier (ORCID ID):	N/A
Nearest Person Month Worked:	<1
Contribution to Project:	Data analysis, consultation
Funding Support:	

Name:	Jacob Hooker
Project Role:	Co-investigator
Researcher Identifier (ORCID ID):	N/A
Nearest Person Month Worked:	<1
Contribution to Project:	Data analysis, consultation
Funding Support:	

Name:	Vitaly Napadow
Project Role:	Co-investigator
Researcher Identifier (ORCID ID):	N/A
Nearest Person Month Worked:	<1
Contribution to Project:	Data analysis, consultation

Funding Support:	
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Name:	Daniel Albrecht
Project Role:	Post-doctoral Investigator
Researcher Identifier (ORCID ID):	0000-0002-0881-9913
Nearest Person Month Worked:	12
Contribution to Project:	Data analysis, collection, consultation, dissemination
Funding Support:	

Name:	Angel Torrado-Carvajal
Project Role:	Post-doctoral Investigator
Researcher Identifier (ORCID ID):	0000-0002-1540-2809
Nearest Person Month Worked:	5
Contribution to Project:	Data analysis and collection
Funding Support:	

Name:	Minhae Kim
Project Role:	Research Assistant
Researcher Identifier (ORCID ID):	N/A
Nearest Person Month Worked:	7
Contribution to Project:	Recruited and screened participants, collected data
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

In 2017-2018, we have no changes in active support since the last reporting period. Previous changes are listed below:

- 1R21NS087472 (PI: Loggia), The Role of Neuroimmune Activation in Chronic Pain and Negative Affect (2.79 cal; previously pending)
- R01 AT007550 (PI: Harris/Napadow) Neuroimaging Approaches to Deconstructing Acupuncture for Chronic Pain (1.4 cal).

Reduced effort

- 1R21NS082548-01A1 (PI: Zhang/Hooker), PET/MRI Imaging of Neuroaxial Inflammation in Sciatica Patients
- (from 2.4 cal to 0.34 cal)
- NCMIC Foundation (PI: Loggia), Neural Correlates of Spinal Manipulative Therapy (from 1.92 cal to 0.6 cal)

Terminated

- IASP Early Career Award (PI: Loggia), An in-vivo investigation of brain inflammation in fibromyalgia with integrated PET/MR imaging.

- 1R01AG03498-01 (PI: Edwards), Biobehavioral Risk Factors for Persistent Pain following Total Knee Arthroplasty.

What other organizations were involved as partners?

Organization Name:	Brigham and Women’s Hospital
Location of Organization:	Boston, MA
Partner’s Contribution to the Project: Financial Support In-kind Support Facilities Collaboration Personnel Exchange Other	Dr. Lee has been working with project staff on the project.

Organization Name:	Boston University School of Public Health – Gulf War Illness Consortium
Location of Organization:	Boston, MA
Partner’s Contribution to the Project: Financial Support In-kind Support Facilities Collaboration Personnel Exchange Other	Dr. Sullivan has been working with project staff on the project

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Nothing to report

QUAD CHARTS:

Nothing to report

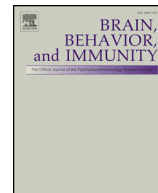
9. APPENDICES:

A. Albrecht, D.S., Forsberg, A., Sandström, A., Bergan, C., Kadetoff, D., Protsenko, E., Lampa, J., Lee, Y.C., Höglund, C.O., Catana, C. and Cervenka, S., 2018. Brain glial activation in fibromyalgia—A multi-site positron emission tomography investigation. *Brain, behavior, and immunity*.



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Brain glial activation in fibromyalgia – A multi-site positron emission tomography investigation

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MRI/PET
Deprenyl-D2

ABSTRACT

Fibromyalgia (FM) is a poorly understood chronic condition characterized by widespread musculoskeletal pain, fatigue, and cognitive difficulties. While mounting evidence suggests a role for neuroinflammation, no study has directly provided evidence of brain glial activation in FM. In this study, we conducted a Positron Emission Tomography (PET) study using [¹¹C]PBR28, which binds to the translocator protein (TSPO), a protein upregulated in activated microglia and astrocytes. To enhance statistical power and generalizability, we combined datasets collected independently at two separate institutions (Massachusetts General Hospital [MGH] and Karolinska Institutet [KI]). In an attempt to disentangle the contributions of different glial cell types to FM, a smaller sample was scanned at KI with [¹¹C]-L-deprenyl-D₂ PET, thought to primarily reflect astrocytic (but not microglial) signal.

Thirty-one FM patients and 27 healthy controls (HC) were examined using [¹¹C]PBR28 PET. 11 FM patients and 11 HC were scanned using [¹¹C]-L-deprenyl-D₂ PET. Standardized uptake values normalized by occipital cortex signal (SUVR) and distribution volume (V_T) were computed from the [¹¹C]PBR28 data. [¹¹C]-L-deprenyl-D₂ was quantified using λ_{k3}. PET imaging metrics were compared across groups, and when differing across groups, against clinical variables.

Compared to HC, FM patients demonstrated widespread cortical elevations, and no decreases, in [¹¹C]PBR28 V_T and SUVR, most pronounced in the medial and lateral walls of the frontal and parietal lobes. No regions

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showed significant group differences in [^{11}C]-L-deprenyl- D_2 signal, including those demonstrating elevated [^{11}C]PBR28 signal in patients (p 's ≥ 0.53 , uncorrected). The elevations in [^{11}C]PBR28 V_T and SUVR were correlated both spatially (i.e., were observed in overlapping regions) and, in several areas, also in terms of magnitude. In exploratory, uncorrected analyses, higher subjective ratings of fatigue in FM patients were associated with higher [^{11}C]PBR28 SUVR in the anterior and posterior middle cingulate cortices (p 's < 0.03). SUVR was not significantly associated with any other clinical variable.

Our work provides the first *in vivo* evidence supporting a role for glial activation in FM pathophysiology. Given that the elevations in [^{11}C]PBR28 signal were not also accompanied by increased [^{11}C]-L-deprenyl- D_2 signal, our data suggests that microglia, but not astrocytes, may be driving the TSPO elevation in these regions. Although [^{11}C]-L-deprenyl- D_2 signal was not found to be increased in FM patients, larger studies are needed to further assess the role of possible astrocytic contributions in FM. Overall, our data support glial modulation as a potential therapeutic strategy for FM.

1. Introduction

Fibromyalgia (FM) is a poorly understood chronic condition characterized by widespread musculoskeletal pain, fatigue, unrefreshing sleep, memory and attention difficulties, among other symptoms (Clauw, 2014). While the etiology of FM is unknown, central mechanisms are strongly implicated, including evidence of abnormalities in structure, function, and molecular chemistry of the central nervous system (CNS) (Albrecht et al., 2016; Clauw, 2014; Dehghan et al., 2016; Flodin et al., 2014; Gracely et al., 2002; Harris et al., 2007; Jensen et al., 2009; Jensen et al., 2010; Jensen et al., 2013; Kuchinad et al., 2007; Loggia et al., 2014; Loggia et al., 2015a; Napadow and Harris, 2014; Schreiber et al., 2017; Schrepf et al., 2016; Wood, 2008), though some evidence points to peripheral alterations as well (Oaklander et al., 2013; Uceyler and Sommer, 2013).

Dysregulation of neuroimmune activation is one potential mechanism contributing to previously reported central aberrations and central sensitization in FM. For instance, FM patients demonstrate elevated levels of fractalkine and interleukin-8 (IL-8) in cerebrospinal fluid (CSF) (Backryd et al., 2017; Kadetoff et al., 2012; Kosek et al., 2015). Both chemokines are implicated in neuron-glial communication (Montague and Malcangio, 2017; Puma et al., 2001), and have been associated with central sensitization and pain (Kosek et al., 2015; Montague and Malcangio, 2017). However, no study to date has clearly demonstrated that glial activation occurs in the brain of FM patients. Acknowledging a role for neuroimmune dysfunction in FM would open the exploration of glial modulation as a therapeutic option for this condition.

In the human CNS, glial activation can be studied *in vivo* using positron emission tomography (PET) and radioligands that bind to the 18-kDa translocator protein (TSPO), such as [^{11}C]PBR28, which displays nanomolar affinity to this protein (~ 0.5 nM; Imaizumi et al., 2008). Located mainly on the outer mitochondrial membrane, TSPO expression is low in healthy CNS tissue, but is widely upregulated in microglia and astrocytes under inflammatory conditions (Lavissee et al., 2012; Rupprecht et al., 2010). Our group has recently used TSPO PET imaging to document neuroimmune activation in the central and peripheral nervous system of patients with chronic low back pain (CLBP) (Albrecht et al., 2018; Loggia et al., 2015b). While no TSPO PET studies of FM patients have been published yet, a possible link between TSPO and FM pathophysiology is provided by an association between the Ala147Thr polymorphism (rs6971) in the TSPO gene and FM symptom severity and cerebral pain processing (Kosek et al., 2016).

The aims of the current study were to evaluate the hypothesis that brain TSPO binding in FM patients, as assessed using the [^{11}C]PBR28 PET ligand, is 1) elevated compared to healthy controls (indicating the presence of glial activation) and 2) correlated with specific symptoms attributable to FM pathophysiology. An additional third aim was to tease out the most likely cellular sources of increased TSPO binding because, while TSPO upregulation in neuroinflammatory responses consistently colocalizes with microglia, an accompanying astrocytic

component has been observed in some (Liu et al., 2016; Rupprecht et al., 2010; Toth et al., 2016; Wei et al., 2013), but not all cases (Abourbeh et al., 2012; Mirzaei et al., 2016). To this end, a smaller sample of FM patients received a PET scan with [^{11}C]-L-deprenyl- D_2 , which binds to monoamine oxidase B (MAO-B) with high specificity in postmortem tissue (~ 30 fmol/mg tissue; Gulyas et al., 2011). Because the expression of MAO-B in glial cells is thought to be predominantly, if not exclusively, within astrocytes (Ekblom et al., 1994), we reasoned that the presence of elevations in [^{11}C]-L-deprenyl- D_2 signal within regions also demonstrating TSPO elevations would support the presence of an astrocytic contribution to the TSPO signal. Conversely, the absence of [^{11}C]-L-deprenyl- D_2 in regions showing elevated TSPO signal would suggest a predominantly microglial cellular source.

2. Materials and methods

2.1. Study design

This collaborative project combines data from two independent research centers to amass a [^{11}C]PBR28 PET imaging cohort of FM patients and matched healthy controls (HC): Massachusetts General Hospital (MGH) in Boston, MA, United States, and Karolinska Institutet (KI) in Stockholm, Sweden. Both studies were approved by ethical committees (MGH – Partners Human Research Committee; KI – Regional Ethical Review Board in Stockholm), and all subjects provided informed consent. The initial study design and data collection at each site were completed independently, and the decision to aggregate the data into a common analysis was made after completion of data collection. Potential confounds attributable to site-specific differences were taken into account in the statistical models of the combined dataset. Group differences identified in the primary analysis of the combined dataset were also assessed within each site separately. Because arterial plasma data were collected for all subjects scanned at Karolinska, we decided to perform two levels of analyses. The first analysis took advantage of the arterial blood data collected at Karolinska, in order to obtain quantitative distribution volume (V_T) metrics using kinetic modeling. The second analysis employed a blood-free ratio approach previously validated in separate cohorts (Albrecht et al., 2017), and utilized the enhanced statistical power of the combined sample (31 patients vs. 27 controls). An evaluation of the agreement between V_T computed in the smaller (KI only) sample and SUVR in the larger, combined sample (KI + MGH) was performed by assessing the extent of spatial overlap of the group differences, as well as the regional correlation of these metrics.

2.2. Subjects

In total, 31 FM patients (29 female, 50.7 ± 11 y/o) and 27 controls (25 female, 49.4 ± 11 y/o) received a [^{11}C]PBR28 PET brain scan. A total of 11 FM patients (11 female, 51.5 ± 8.2 y/o), and 11 controls (11 female, 51.0 ± 7.0 y/o) received a [^{11}C]-L-deprenyl- D_2 brain scan.

All FM patients had received a diagnosis of FM from a physician, and met the 2011 modifications of the American College of Rheumatology classification criteria for fibromyalgia (Wolfe et al., 2011). FM patients were excluded for the presence of any pain conditions other than FM. Exclusionary criteria for all subjects at either site included: history of major psychiatric illness, neurological illness, cardiovascular disease, inability to communicate in English (MGH) or Swedish (KI), and contraindication for PET/MR scanning (e.g., pacemaker, metallic implants, pregnancy, etc). Additionally, at MGH, the use of benzodiazepine medications was exclusionary, except for clonazepam, alprazolam, and lorazepam, that show negligible binding to TSPO *in vitro*, even at large clinical doses (Canat et al., 1993; Clow et al., 1985; Gehlert et al., 1985; Kalk et al., 2013; Wamsley et al., 1993). At KI, benzodiazepines were not exclusionary, however no patients reported benzodiazepine use (see Results). Please see [Supplementary Table 1](#) for a detailed list of exclusion criteria by site.

2.2.1. Karolinska Institutet

Eleven patients diagnosed with FM (11 female, 51.8 ± 8.6 y/o) and 11 HC (11 female, 51.5 ± 9.0 y/o) were matched according to age, sex and genotype for the Ala147Thr *TSPO* polymorphism (rs6971) which affects binding of *TSPO* radioligands, including [^{11}C]PBR28, both *in vitro* and *in vivo* (Collste et al., 2016; Kreisl et al., 2013a; Owen et al., 2010; Owen et al., 2012). Sixteen subjects were Ala/Ala (i.e., high-affinity binders; HABs: FM $n = 8$; HC $n = 8$) and six were Ala/Thr (i.e., mixed-affinity binders; MABs: FM $n = 3$; HC $n = 3$). No Thr/Thr (i.e., low-affinity binders; LABs) were included. Eleven FM patients and 11 HC received a brain [^{11}C]PBR28 PET scan. Moreover, six of the 11 FM patients that completed the [^{11}C]PBR28 scan and agreed to participate in an additional PET scan, as well as five additional FM patients and 11 sex and age-matched healthy controls not previously examined, received a [^{11}C]-deprenyl- D_2 PET scan. In addition to meeting 2011 ACR diagnostic criteria, all patients in the KI cohort also met the ACR 1990 criteria (Wolfe et al., 1990).

2.2.2. Massachusetts general hospital

Twenty patients diagnosed with FM (18 female, 48.0 ± 1.2 y/o) were group matched with 16 HC subjects (14 female, 50.2 ± 13 y/o) according to age, sex, and *TSPO* polymorphism. Twenty-four subjects were HABs (FM $n = 14$; HC $n = 10$), and 12 were MABs (FM $n = 6$; HC $n = 6$). No LABs were included in the study. At the MGH site, one patient also had chronic hepatitis C, one had idiopathic CD8 lymphocytopenia, and one had Meniere's Disease and diabetes. These subjects were not outliers on any of the PET imaging measures, and results were unaffected if these subjects were excluded.

2.3. Clinical assessment

All FM patients completed the following clinical questionnaires: 2011 American College of Rheumatology self-report survey for the assessment of FM (ACR; Wolfe et al., 2011), FM Impact Questionnaire (FIQ; Bennett et al., 2009), Beck Depression Inventory (BDI; Beck et al., 1961), and Pain Catastrophizing Scale (PCS; Sullivan et al., 1995). All items were completed on the day of the scan, with the exception of the ACR survey for all KI patients and two MGH patients, and the BDI for MGH patients, which were completed only during the screening visit. Because 17 MGH patients completed the ACR survey on both the scan day and during the screening visit, we were able to confirm that this questionnaire has good temporal stability by correlating the scores across visits. All subscales of the ACR survey showed high to moderate intercorrelations (total: $r = 0.785$, $p < 0.001$; symptom severity: $r = 0.788$, $p < 0.001$; wide-spread pain index: $r = 0.728$, $p = 0.001$; fatigue: $r = 0.534$, $p = 0.027$; trouble thinking: $r = 0.581$, $p = 0.015$; waking up tired: $r = 0.810$, $p < 0.001$), supporting the temporal stability of these measures, and therefore the appropriateness of evaluating these variables in relation to imaging metrics collected at different timepoints.

Prior to tracer injection on the scan day, all patients also rated their pain on a visual analog scale, anchored by 0 ("No pain at all") and 100 ("Most intense pain tolerable").

2.4. Positron emission tomography (PET) and magnetic resonance (MR) imaging

2.4.1. Karolinska Institutet

PET imaging was performed using the High-Resolution Research Tomograph (Siemens Molecular Imaging, Knoxville, TN, USA) at the PET centre at Karolinska Institutet, Stockholm, Sweden. Structural MR images were collected using a 1.5-T Siemens Avanto scanner at Medicinsk Röntgen at Odenplan prior to the first PET scan (TR = 1790 or 1800 ms, TE = 3.53 or 2.8 ms, flip angle = 15° or 8° , voxel size = 1 mm isotropic). Prior to PET scanning, subjects received a cubital vein catheter for intravenous radioligand administration and a radial artery catheter in the contralateral arm for arterial blood sampling. To minimize motion during the PET data acquisition, each participant wore an individually-designed helmet, placed in a frame holder.

Preparation, injection and PET data acquisition for [^{11}C]PBR28 have been described previously (Collste et al., 2016; Kanegawa et al., 2016). Average administered radioactivity of [^{11}C]PBR28 (MBq) was – HC: 416 ± 40 (mean \pm SD), FM: 385 ± 71 ; average specific radioactivity (GBq/ μmol) – HC: 306 ± 195 , FM: 239 ± 74 ; average injected mass (μg) – HC: 0.60 ± 0.34 , FM: 0.61 ± 0.21 . Average administered radioactivity of [^{11}C]-deprenyl- D_2 was – HC: 364 ± 46 , FM: 366 ± 45 ; average specific radioactivity – HC: 193 ± 105 , FM: 213 ± 82 ; average injected mass – HC: 0.48 ± 0.30 , FM: 0.36 ± 0.13 . PET data were acquired for 63 min both for [^{11}C]PBR28 and [^{11}C]-deprenyl- D_2 . Manual samples were drawn at 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, and 60 min for [^{11}C]PBR28 and 1, 2, 4, 6, 8, 10, 16, 20, 30, 40, and 60 min for [^{11}C]-deprenyl- D_2 . For one control subject in the [^{11}C]-deprenyl- D_2 dataset, there was a technical issue with the arterial blood collection, and thus this subject was excluded from the analysis. Arterial blood data pre-processing was performed using Kaleidagraph 4.1 software (Synergy Software) as described previously (Collste et al., 2016). Radioligand metabolism correction was performed using the parent fraction in PMOD v3.3 (pixel-wise modelling software; PMOD Technologies Ltd., Zurich, Switzerland) where individual parent fraction data was fit with a 3-exponential model.

2.4.2. Massachusetts general hospital

Imaging was performed at the MGH/HST Athinoula A. Martinos Center for Biomedical Imaging in Charlestown, MA. [^{11}C]PBR28 was produced in-house using a procedure modified from the literature (Imaizumi et al., 2007). [^{11}C]PBR28 scans were performed for 90 min with an integrated PET/MR 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). A multi-echo MPRAGE volume was acquired prior to tracer injection (TR/TE1/TE2/TE3/TE4 = 2530/1.64/3.5/5.36/7.22 ms, flip angle = 7° , voxel size = 1 mm isotropic) for the purpose of anatomical localization, spatial normalization of the imaging data, as well as generation of attenuation correction maps (Izquierdo-Garcia et al., 2014). Average administered radioactivity of [^{11}C]PBR28 (MBq) was – HC: 457 ± 57 MBq, FM: 505 ± 40 ; average specific activity (GBq/ μmol) – HC: 77.4 ± 30 , FM: 71.8 ± 26 ; average injected mass (μg) – HC: 2.32 ± 0.8 , FM: 2.78 ± 1.1 .

2.5. PET data analysis and quantification

2.5.1. Kinetic analysis

Estimation of [^{11}C]PBR28 distribution volume (V_T) was performed using Logan graphical analysis with a metabolite corrected plasma input function (Logan et al., 1990), based on five frames from 33 to 63 min. For each PET scan, a parametric V_T image was generated using

the stationary wavelet aided parametric imaging (WAPI) approach (Cselenyi et al., 2002). WAPI analysis of TSPO binding has been previously shown to be sensitive to within-subject changes in V_T (Forsberg et al., 2017; Jucaite et al., 2015), and has shown both high correlation with V_T estimated with the two-tissue compartment model (2TCM), and good reliability for 63 min of data (Collste et al., 2016).

Quantification of [^{11}C]-deprenyl- D_2 data was performed as described previously, utilizing the 2TCM with three rate constants (K_1 , k_2 , k_3) with PMOD 3.3 (Sturm et al., 2017). The outcome measure λk_3 was calculated as $(K_1/k_2) * k_3$ which has been shown to reflect the regional enzyme concentration more accurately than k_3 alone (Fowler et al., 1995; Logan et al., 2000). There is presently no validated method to produce λk_3 parametric images with the WAPI methodology we used for the [^{11}C]PBR28 analysis. For this reason, [^{11}C]-deprenyl- D_2 data was analyzed only using a region-of-interest (ROI) approach. Because an aim of this project was to assess the presence of a possible astrocytic component to TSPO signal in FM, the regions identified as statistically different across groups in the [^{11}C]PBR28 voxel-wise SUVR analyses (see below) were selected as ROIs. Additionally, λk_3 values were computed for 23 anatomically defined ROIs from the AAL atlas, whole brain, and whole gray matter for exploratory analyses.

2.5.2. SUVR analysis

Static [^{11}C]PBR28 PET images were reconstructed from 33 to 63 min post-injection PET data, the latest 30-minute period available at both sites. Standardized uptake value (SUV) images were calculated by normalizing images by injected dose/body weight. SUV ratio images (SUVR) were obtained via normalization by PET signal from a pseudo-reference region (i.e., occipital cortex, identified using the occipital cortex label from the AAL atlas available in PMOD (Tzourio-Mazoyer et al., 2002)). We have previously utilized this approach for quantification of [^{11}C]PBR28 PET data in both chronic low back pain patients

and in patients with ALS (Albrecht et al., 2017), showing that group differences in SUVR (in the thalamus and motor cortex, in pain and ALS patients, respectively) can be similarly observed using V_T (or V_T ratio; DVR) estimated with 2TCM, and that SUVR and DVR are strongly correlated. However, since some medial portions of the occipital cortex exhibited group differences in the V_T analysis (see Results), these were excluded from the occipital pseudo-reference region. A general linear model (GLM) analysis with genotype and injected dose as regressors of no interest, revealed that the mean SUV extracted from the occipital region defined above did not show any significant effects of Group ($F_{1,52} = 4.38 \times 10^{-6}$, $p = 0.99$), Site ($F_{1,52} = 0.006$, $p = 0.72$), or a Group * Site interaction ($F_{1,52} = 0.08$, $p = 0.20$). These results support the appropriateness of using this region as a pseudo-reference in this particular study.

2.5.3. Image post-processing

For both SUVR and V_T images, FSL and Freesurfer tools were used for image processing. PET images were co-registered to individual structural T1 images, normalized to MNI standard space, and spatially smoothed with an 8 mm FWHM Gaussian kernel, as in Loggia et al., (2015b) and Albrecht et al., (2017).

2.6. Statistical analysis

Differences in continuous variables were assessed by performing a GLM analysis with Group and Site as fixed factors, and a Group * Site interaction term. Significant interaction terms were decomposed with post-hoc planned comparisons of least squares means. Differences in the distribution of categorical variables were assessed with Chi-Square tests. Between-site differences in FM questionnaire scores were assessed with two-sample t-tests.

As the primary analysis, voxelwise group comparisons of [^{11}C]

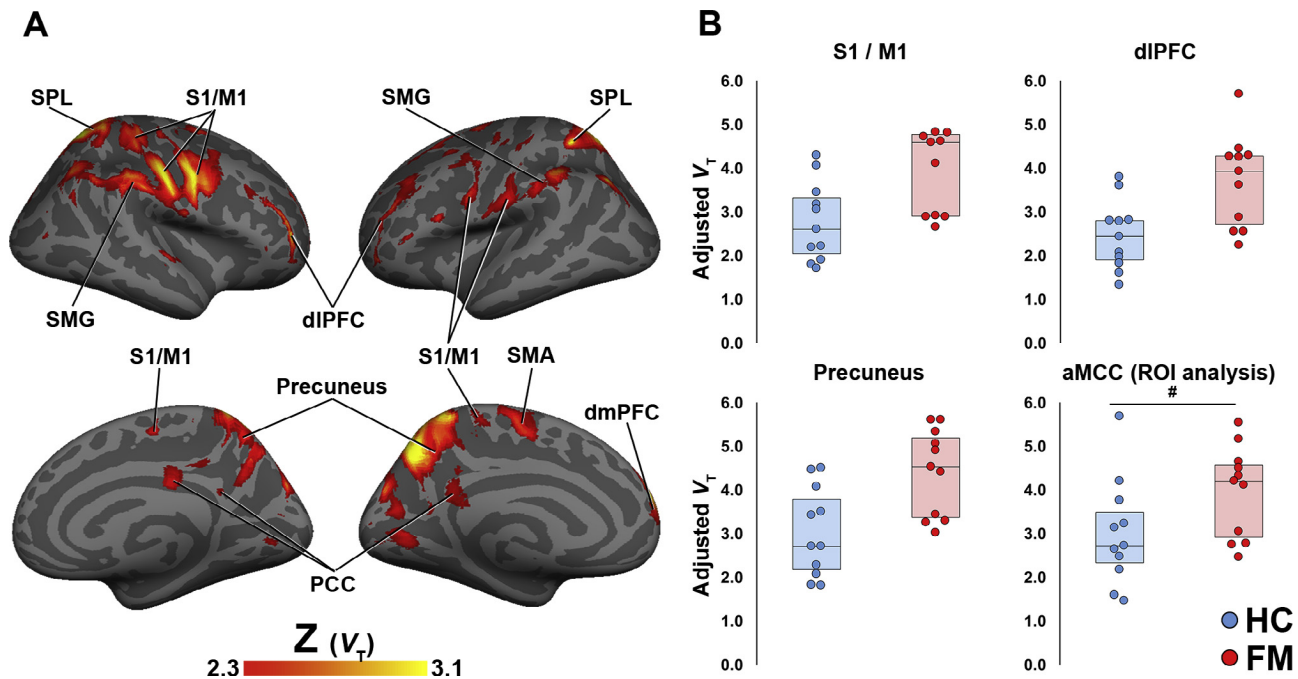


Fig. 1. Voxelwise group differences in [^{11}C]PBR28 V_T . **A:** Surface projection maps displaying areas with significantly elevated [^{11}C]PBR28 V_T in FM patients compared to controls (FM – $n = 11$; HC – $n = 11$) in voxelwise analyses (KI-only sample). **B:** average \pm standard deviation V_T extracted from several regions. The S1/M1, dIPFC and precuneus data were extracted from the clusters identified as statistically significant in the voxelwise V_T analysis. For these regions, the plots are displayed for illustrative purposes only, and the level of statistical significance noted for each plot reflects that of the voxelwise analyses. For the aMCC, the data was extracted from a region independently identified based on the results of the SUVR voxelwise analysis (see Fig. 2). The level of statistical significance noted for this region reflects the result of a region-of-interest analysis. SPL – superior parietal lobule, S1 – primary somatosensory cortex, M1 – primary motor cortex, SMG – supramarginal gyrus, dIPFC – dorsolateral prefrontal cortex, SMA – supplementary motor area, PCC – posterior cingulate cortex, dmPFC – dorsomedial prefrontal cortex. The box plots for S1/M1, dIPFC and Precuneus are for illustrative purposes. The box plot for aMCC illustrates an ROI analysis ($p = 0.071$).

PBR28 V_T and SUVR maps were performed with FSL's FEAT GLM tool (www.fmrib.ox.ac.uk/fsl, version 5.0.7), using a voxelwise cluster-forming threshold of $z > 2.3$ and a (corrected) cluster significance threshold of $p < 0.05$ to correct for multiple comparisons. *TSPO* polymorphism (Ala/Ala, Ala/Thr), was included as a regressor of no interest in both analyses. Study site (MGH, KI) was included as an additional regressor of no interest in SUVR analysis, as this was performed on the combined sample from both institutions. Because there was a statistically significant effect of Site ($F_{1,54} = 37.9$, $p < 0.001$) and a significant Group * Site interaction for injected dose ($F_{1,54} = 4.23$, $p = 0.044$), this variable was also added as a covariate of no interest in all group analyses using the combined dataset (KI + MGH). There were also significant effects of Site for specific activity and injected mass, but no significant Group * Site interactions. Therefore, we performed supplementary voxelwise analyses including these variables as regressors of no interest, in order to ensure that including them in the statistical model had negligible effects on the outcomes. Because no subcortical effects were detected, and for ease of visualization, imaging results were visualized on a surface (FreeSurfer's fsaverage).

For follow up analyses and illustration purposes, clusters of significant group differences from the SUVR or V_T analysis (Figs. 1–3) were parcellated into separate anatomically-constrained subregions, using the labels from the Harvard-Oxford probabilistic atlas (using an arbitrary threshold of 30). Average SUVR and/or V_T values were extracted from these regions, for the purposes of visualizing data, comparing group effects within each site independently, and assessing relationships between SUVR and clinical variables and between outcome metrics (SUVR and V_T). Although anterior middle cingulate (aMCC) did not exhibit significant group differences in the voxelwise V_T analysis, this region was significant in the SUVR analysis (see Results); therefore, average V_T was extracted from aMCC to test for potential V_T differences not detected in the voxelwise analysis, particularly because this region is highly relevant to pain processing (Kragel et al., 2018; Shackman et al., 2011). Partial correlation analyses were used to assess correlations between PET signal extracted from regions exhibiting significant

group differences in the voxelwise analyses and continuous clinical variables (i.e. ACR [total score, symptom severity score, widespread pain index], FIQR, BDI, PCS, and current VAS pain), correcting for *TSPO* polymorphism. These analyses were performed using SUVR, as these values were available for all patients at both sites. To assess the relationship between extracted PET signal and ordinal clinical variables (individual ACR symptom severity items: “fatigue”, “trouble thinking or remembering”, “waking up tired”), we performed GLM analyses with *TSPO* PET signal as the dependent variable, clinical score as a fixed factor (“slight or mild problem”, “moderate problem”, or “severe problem”), and *TSPO* polymorphism and study site as regressors of no interest. Post-hoc planned comparisons of least squares means were performed to decompose significant main effects. Additionally, partial correlation analysis was used to evaluate the association between [^{11}C]PBR28 V_T and SUVR in participants for whom both measures were available, correcting for *TSPO* polymorphism.

After identifying regions demonstrating group differences in the combined [^{11}C]PBR28 SUVR datasets, we conducted a follow up GLM analysis of the data extracted from the same regions, in order to evaluate the significance of the Site effect and the Group * Site interaction, using *TSPO* genotype and injected dose as regressors of no interest, and to compute the effect size, using Cohen's d , for each site independently. The primary aim of these analyses was to assess whether the group differences identified in the voxelwise analyses were driven solely by one site, or could rather be similarly observed at both sites. In order to avoid circularity, we do not report the p-values for the Group effects at each site independently, as these analyses were performed on data extracted from regions preselected because they already exhibited a Group effect in the voxelwise analyses.

For all demographic, correlation, and follow-up analyses, significance was set as $p < 0.05$, uncorrected.

Unpaired t-tests were used to assess group differences in [^{11}C]-deprenyl- $\text{D}_2\lambda_3$ for each region with elevated *TSPO* signal, which included: aMCC, dLPFC, dmPFC, frontoinsular cortex, S1/M1, PCC, precuneus, pmCC, SMA, and SPL (see Results). Unpaired t-tests were also

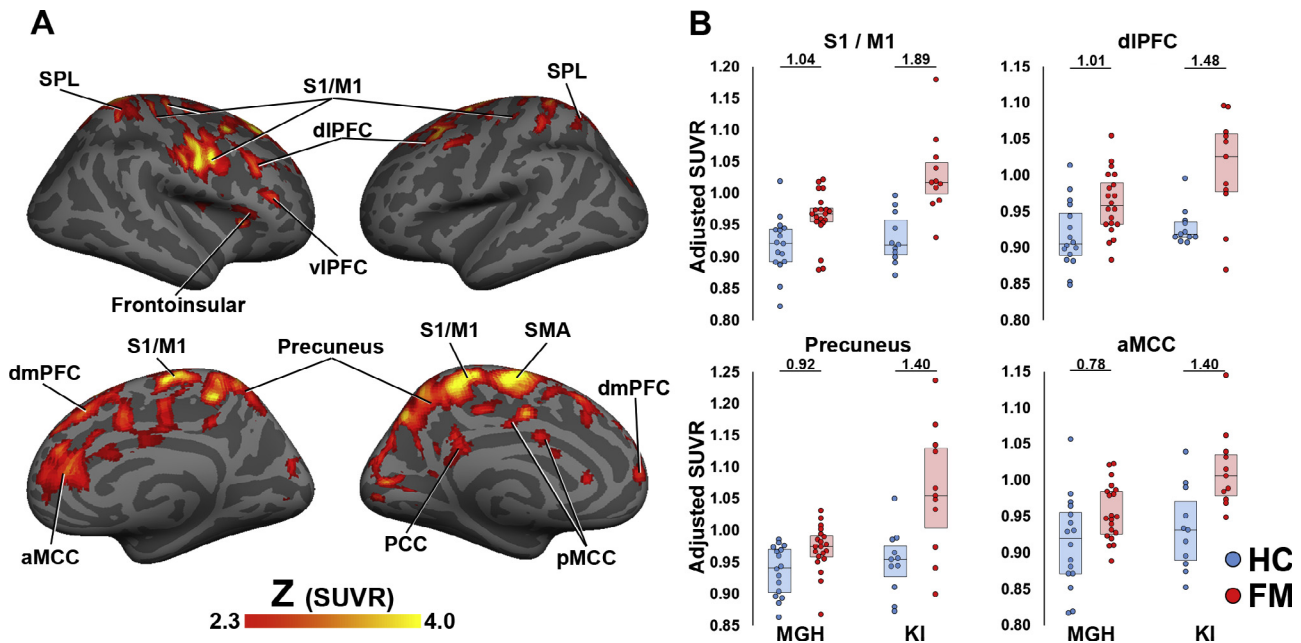


Fig. 2. Voxelwise group differences in [^{11}C]PBR28 SUVR. **A.** Surface projection maps displaying areas with significantly elevated [^{11}C]PBR28 SUVR in FM patients compared to controls (FM – $n = 31$; HC – $n = 27$), in voxelwise analyses (KI + MGH sample). **B:** average \pm standard deviation SUVR extracted from several of the clusters identified as statistically significant in the voxelwise SUVR analysis. Data from individual research sites (MGH or KI) are displayed separately, and the number above each ROI pairing corresponds to the effect size (Cohen's d) of PET signal differences between FM patients and controls for each site. These data show that overall SUVR group differences, while larger for the KI dataset, are elevated in FM patients compared to controls in both datasets when evaluated independently. pmCC – posterior middle cingulate cortex, aMCC – anterior middle cingulate cortex. All data have been adjusted for genotype and injected dose.

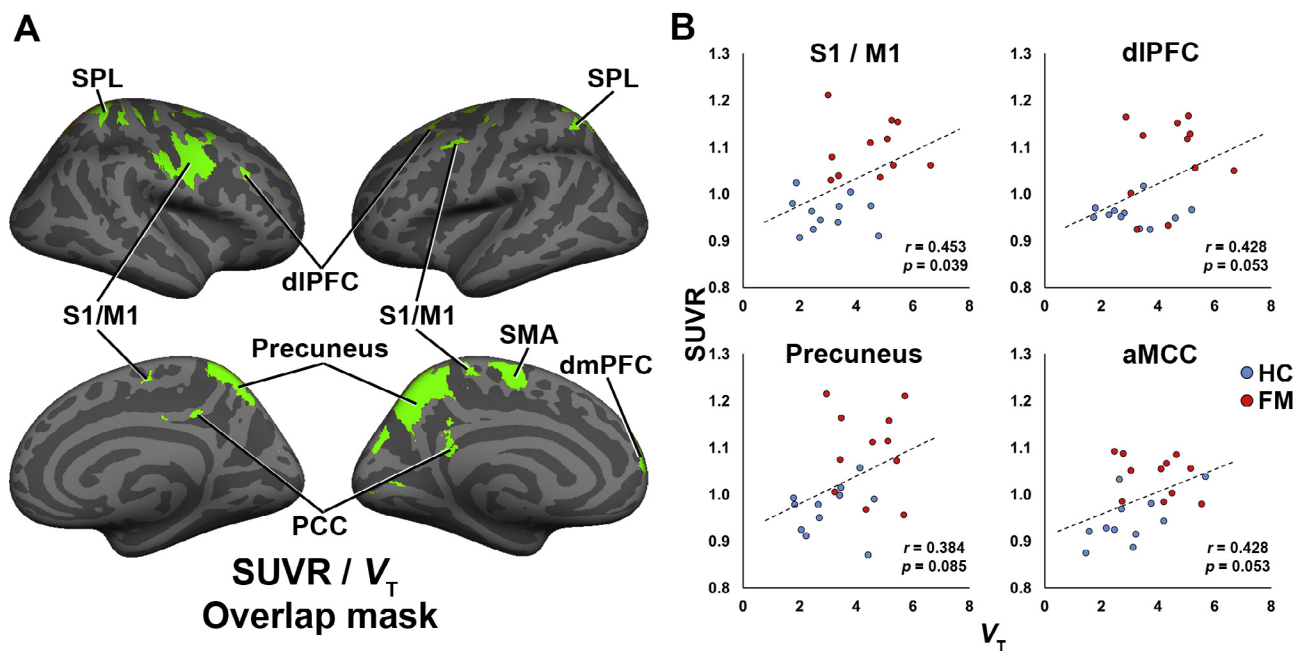


Fig. 3. Agreement between SUVR and V_T analyses. **A.** Surface projection displaying the regions showing elevated PET signal across both SUVR (KI + MGH combined dataset) and V_T (KI-only) analyses. **B:** Cross-correlations between SUVR and V_T , extracted from regions identified in the overlap mask or, for the aMCC, from the SUVR analyses, in FM patients and HC from the KI site.

performed for the exploratory analysis of 23 anatomically defined ROIs, and whole brain and gray matter. However, because no group differences were significant in any ROI, all analyses were followed up by exploratory uncorrected analyses to provide more convincing support to the claim that the lack of group effects reported in the deprenyl analyses are likely to reflect the lack of astrocytic activation in FM.

Please refer to [Supplementary Table 2](#) for a summary of the statistical analyses.

3. Results

3.1. Subject characteristics

Demographic and other key characteristics for all subjects are shown in [Table 1](#). FM patients had significantly higher BMI than controls in the MGH dataset ($p < 0.001$), but there were no significant patient-control differences for any other variable (p 's ≥ 0.071) for any dataset. Clinical characteristics of all FM patients are presented in [Table 2](#). For the [^{11}C]PBR28 analysis, FM patients from the KI site had significantly higher scores than FM patients from the MGH site on several of the ACR 2011 items ($p < 0.047$; [Table 2](#)). KI patients included in both the [^{11}C]PBR28 and the [^{11}C]-L-deprenyl- D_2 analyses

were also less medicated ([Table 3](#)). There were no significant differences between FM patients at the KI site in the [^{11}C]PBR28 and [^{11}C]-L-deprenyl- D_2 datasets for any demographic characteristic or clinical measure evaluated (p 's ≥ 0.29).

3.2. [^{11}C]PBR28 analyses: Group differences

3.2.1. [^{11}C]PBR28 V_T comparison (KI sample only)

In FM patients, [^{11}C]PBR28 V_T was elevated in several brain regions compared to healthy control subjects ([Fig. 1](#)), including dorsolateral prefrontal cortex (dlPFC), dorsomedial PFC (dmPFC), primary somatosensory and motor cortices (S1/M1), precuneus, posterior cingulate cortex (PCC), supplementary motor area (SMA), supramarginal gyrus (SMG), and superior parietal lobule (SPL). Additionally, an ROI analysis of the aMCC revealed elevated V_T in the FM patients that approached statistical significance ($p = 0.071$). There were no regions where control V_T was significantly higher than FM V_T .

3.2.2. [^{11}C]PBR28 SUVR comparison (KI + MGH)

A whole brain voxelwise analysis identified several brain regions where SUVR in FM patients was significantly greater than in HC ([Fig. 2](#)). These regions largely overlapped those identified in the V_T

Table 1
Participant characteristics.

	Massachusetts General Hospital [^{11}C]PBR28		Karolinska Institutet [^{11}C]PBR28		Karolinska Institutet [^{11}C]-L-deprenyl- D_2	
	FM ($n = 20$)	HC ($n = 16$)	FM ($n = 11$) ¹	HC ($n = 11$)	FM ($n = 11$) ²	HC ($n = 11$)
Age (years)	48.0 \pm 12	50.2 \pm 13	51.8 \pm 8.6	51.5 \pm 9.0	51.5 \pm 8.2	51.0 \pm 7.0
Sex	18F; 2M	14F; 2M	11F	11F	11F	11F
TSPO polymorphism	14 HAB; 6 MAB	10 HAB; 6 MAB	8 HAB; 3 MAB	8 HAB; 3 MAB	N/A	
BMI	30.0 \pm 5.7*	23.6 \pm 3.6	24.5 \pm 3.7	23.1 \pm 2.0	24.5 \pm 3.0	22.3 \pm 1.8
Injected dose (MBq)	505 \pm 40	457 \pm 57	385 \pm 71	416 \pm 40	366 \pm 45	364 \pm 46
Specific activity (GBq/ μmol)	71.8 \pm 26	77.4 \pm 30	239 \pm 74	306 \pm 195	213 \pm 82	193 \pm 105
Injected mass (μg)	2.78 \pm 1.1	2.32 \pm 0.8	0.61 \pm 0.21	0.60 \pm 0.34	0.36 \pm 0.13	0.48 \pm 0.30

* Significantly different from controls within-cohort, $p < 0.05$.

¹ Six of the FM subjects receiving a [^{11}C]PBR28 scan also received a [^{11}C]-L-deprenyl- D_2 scan.

² [^{11}C]-L-deprenyl- D_2 data from one FM subject was not used due to a technical issue during arterial blood collection.

Table 2
FM patient clinical characteristics: [¹¹C]PBR28 analysis.

	Massachusetts General Hospital [¹¹ C]PBR28	Karolinska Institutet [¹¹ C] PBR28	Karolinska Institutet [¹¹ C]- deprenyl-D ₂
N	20	11	11
Current VAS pain	37.8 ± 23	47.2 ± 15	42.9 ± 16
FIQ pain	5.15 ± 2.0	5.82 ± 1.2	5.80 ± 1.1
FIQ stiffness	6.35 ± 2.1	6.18 ± 2.4	6.13 ± 2.3
FIQ depression	2.75 ± 2.6	3.36 ± 3.3	3.35 ± 3.0
FIQ anxiety	3.55 ± 2.9	4.55 ± 3.4	4.62 ± 3.4
ACR total	18.6 ± 4.3	24.7 ± 3.3**	25.5 ± 3.2
ACR symptom severity	7.61 ± 1.9	9.09 ± 1.8*	9.00 ± 1.8
ACR widespread pain index	10.9 ± 3.3	16.1 ± 2.0**	16.5 ± 1.7
ACR fatigue	2.06 ± 0.7	2.55 ± 0.5#	2.64 ± 0.5
ACR trouble thinking	1.61 ± 0.9	2.64 ± 0.5**	2.18 ± 0.8
ACR waking tired	2.11 ± 0.6	2.27 ± 0.5	2.64 ± 0.5
BDI	12.5 ± 9.2	14.9 ± 6.1	12.2 ± 5.7
PCS	17.5 ± 9.1	17.2 ± 11	17.5 ± 11

FIQ – Fibromyalgia Impact Questionnaire; ACR – 2011 modifications of the American College of Rheumatology diagnostic criteria for fibromyalgia; BDI – Beck Depression Inventory; PCS – Pain Catastrophizing Scale.

* Significant difference between KI and MGH FM patients from the [¹¹C]PBR28 datasets, $p < 0.05$.

** Significant difference between KI and MGH FM patients from the [¹¹C]PBR28 datasets, $p < 0.01$.

Trend-level difference between KI and MGH FM patients from the [¹¹C]PBR28 datasets, $p < 0.10$.

Table 3
FM patient medications.

Medication	Massachusetts General Hospital	Karolinska Institutet [¹¹ C] PBR28	Karolinska Institutet [¹¹ C]- deprenyl-D ₂
N	20	11	11
NSAIDs	6	5	2
Acetaminophen (paracetamol)	2	6	4
Muscle relaxant	8	2	1
Sleep medication	2	2	3
SNRI	6	0	0
Anticonvulsant	2	0	0
Opioid	1	0	0
Benzodiazepines	9	0	0
Tricyclic antidepressant	6	0	0

Table 4
Site-specific comparison of SUVR.

Region	Effect of Site	Group * Site interaction	MGH – FM vs. HC effect size	KI – FM vs. HC effect size
aMCC	$F_{1,52} = 13.2, p = 0.001$	$F_{1,52} = 4.16, p = 0.047$	0.78	1.46
dIPFC	$F_{1,52} = 9.01, p = 0.004$	<i>$F_{1,52} = 3.38, p = 0.072$</i>	1.01	1.48
dmPFC	<i>$F_{1,52} = 0.01, p = 0.910$</i>	<i>$F_{1,52} = 1.43, p = 0.237$</i>	0.74	1.70
Frontoinsular cortex	$F_{1,52} = 5.22, p = 0.026$	<i>$F_{1,52} = 0.85, p = 0.362$</i>	0.74	1.27
S1/M1	$F_{1,52} = 18.1, p < 0.001$	$F_{1,52} = 8.94, p = 0.004$	1.04	1.89
pMCC	$F_{1,52} = 71.2, p < 0.001$	<i>$F_{1,52} = 2.88, p = 0.096$</i>	0.83	0.79
PCC	$F_{1,52} = 115, p < 0.001$	$F_{1,52} = 14.7, p < 0.001$	0.58	1.12
Precuneus	$F_{1,52} = 25.7, p < 0.001$	$F_{1,52} = 12.7, p < 0.001$	0.92	1.40
SMA	<i>$F_{1,52} = 3.22, p = 0.078$</i>	<i>$F_{1,52} = 0.24, p = 0.624$</i>	1.02	1.19
SPL	$F_{1,52} = 10.8, p = 0.002$	$F_{1,52} = 13.5, p < 0.001$	0.82	1.71

Statistically significant results are displayed in **bold**, statistical tests exhibiting trend-level results are displayed in *italics*. aMCC – anterior middle cingulate cortex, dmPFC – dorsomedial prefrontal cortex, dIPFC – dorsolateral prefrontal cortex, S1 – primary somatosensory cortex, M1 – primary motor cortex, pMCC – posterior middle cingulate cortex, PCC – posterior cingulate cortex, SMA – supplementary motor area, SPL – superior parietal lobule.

analysis (dlPFC, dmPFC, S1/M1, precuneus, PCC, SMA, SPL) but also revealed effects in additional regions (aMCC, posterior MCC [pMCC], and frontoinsular cortex). There were no regions where SUVR was significantly higher in healthy controls relative to FM patients. Supplementary voxelwise analyses, including specific activity and injected mass as regressors of no interest, showed similar outcomes to the primary analysis, which included injected dose as a regressor of no interest (Supplementary Fig. 1).

Following these voxelwise analyses, we performed a post-hoc regional analysis of the areas demonstrating SUVR group differences, in order to evaluate whether the effects emerging in the combined dataset could be similarly observed at each site independently (Table 4). This analysis revealed the presence of a statistically significant Site effect for all regions (p 's < 0.026), except for dmPFC ($p = 0.910$) and SMA ($p = 0.078$). It also revealed the presence of significant Site * Group interactions in SUVR for all regions examined (p 's < 0.047) except for dlPFC ($p = 0.072$), dmPFC ($p = 0.237$), frontoinsular cortex ($p = 0.362$), pMCC ($p = 0.096$), and SMA ($p = 0.624$). The Cohen's d for the difference between FM patients and HC ranged between 0.58 and 1.04 at MGH and between 0.79 and 1.87 at KI (Table 4; Fig. 2), indicating medium or large effect size for all examined regions at both sites (Cohen, 1988), although the magnitude of the difference was generally larger for the KI study.

3.2.3. [¹¹C]PBR28 analysis: Agreement between SUVR and V_T (KI sample only)

The regions identified in the voxelwise SUVR analysis displayed a large degree of spatial overlap with results from the V_T analysis. To visualize this, we created an image showing common regions identified in both analyses as significantly higher in FM patients. These regions included dlPFC, dmPFC, PCC, precuneus, S1/M1, SMA, and SPL (Fig. 3, left). In addition to this spatial agreement across analyses, we were able to evaluate the association across metrics in the KI participants, for whom both SUVR and V_T were available. Statistically significant positive correlations were observed for S1/M1 ($r = 0.453, p = 0.039$; Fig. 3, right), dmPFC ($r = 0.497, p = 0.022$), and PCC ($r = 0.491, p = 0.024$), whereas dlPFC ($r = 0.428, p = 0.053$), precuneus ($r = 0.384, p = 0.085$), and SPL ($r = 0.375, p = 0.094$) demonstrated only trend-level correlations.

3.3. [¹¹C]PBR28 analysis: Association with clinical variables (KI + MGH)

Within FM patients, an exploratory GLM analysis revealed that the scores of the Fatigue item of the ACR diagnostic criteria were significantly associated with SUVR in the aMCC ($F_{2,23} = 5.04, p = 0.015$, uncorrected) and pMCC ($F_{2,23} = 3.80, p = 0.03$, uncorrected), with higher SUVR in patients reporting severe fatigue compared to those reporting slight/mild fatigue in both regions (p 's ≤ 0.01 , uncorrected; Fig. 4). There were no significant associations between SUVR and any

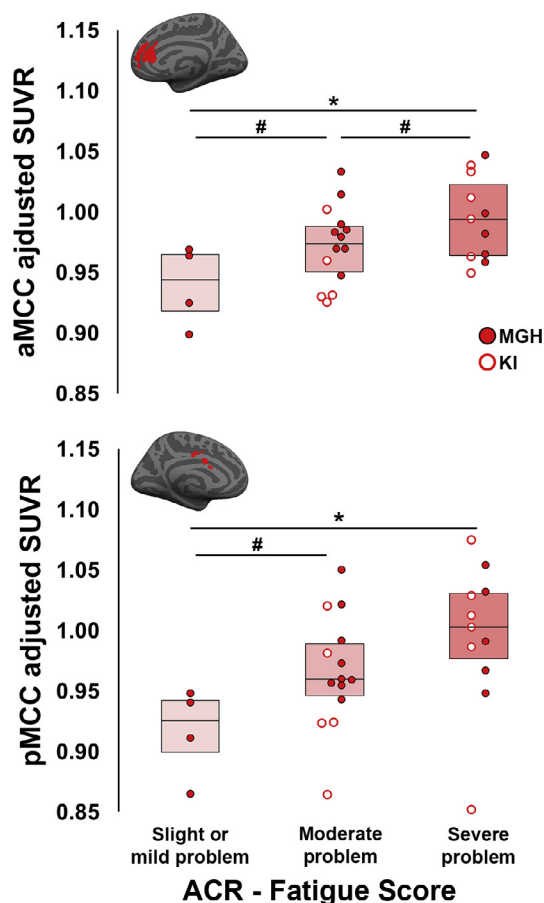


Fig. 4. Associations between $[^{11}\text{C}]\text{PBR28}$ SUVR and clinical variables. Significant differences in $[^{11}\text{C}]\text{PBR28}$ SUVR in the aMCC (top) and pMCC (bottom) between FM patients reporting different severity of fatigue on the ACR 2011 questionnaire. Surface projections of individual ROIs are displayed in red above the plots. All data have been adjusted for *TSPO* genotype. * – Significant difference in post-hoc tests between FM patients reporting different levels of fatigue severity ($p < 0.01$) # - Trend-level difference in post-hoc tests between FM patients reporting different levels of fatigue severity ($p < 0.10$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

other clinical variable.

3.4. $[^{11}\text{C}]\text{-L-deprenyl-D}_2$ analysis: Group differences (KI sample only)

Group comparisons of λk_3 values revealed no significant differences between FM patients and HC subjects, even when evaluated without correction for multiple comparisons, both within the regions demonstrating elevated $[^{11}\text{C}]\text{PBR28}$ SUVR (Fig. 5; p 's ≥ 0.53 , uncorrected), as well as in any of the additional 23 anatomically defined ROIs, or in the whole brain or whole gray matter regions (p 's ≥ 0.53 uncorrected; Supplementary Table 3).

Of note, even in the small subset of 6 FM patients who received both $[^{11}\text{C}]\text{PBR28}$ and $[^{11}\text{C}]\text{-L-deprenyl-D}_2$ scans, we were able to confirm elevated $[^{11}\text{C}]\text{PBR28}$ signal (p 's ≤ 0.007), but statistically indistinguishable $[^{11}\text{C}]\text{-L-deprenyl-D}_2$ signal (p 's ≥ 0.63), compared to controls (Supplementary Fig. 2).

4. Discussion

The current study provides evidence of elevated *TSPO* binding, as measured with $[^{11}\text{C}]\text{PBR28}$ PET, in patients with fibromyalgia (FM) compared to healthy controls (HC). This marker of glial activation was

increased in several brain regions implicated in FM pathology from previous neuroimaging studies. We also report positive associations between *TSPO* PET signal in several of these regions and subjective ratings of fatigue, one of the most common symptoms reported by FM patients (Clauw, 2014; Wolfe et al., 2011). Our observations are supportive of a role for neuroimmune/glial activation in FM pathology.

These results conform to a body of clinical data suggesting a possible association between neuroinflammation and FM. Several studies of FM patients demonstrated elevated CSF levels of molecules implicated in neuroglial signaling, such as fractalkine and IL-8 (Backryd et al., 2017; Kadetoff et al., 2012; Kosek et al., 2015). Furthermore, previous studies showed increased endogenous opioidergic tone in FM (Schrepf et al., 2016), which could be of relevance as opioid-induced hyperalgesia is associated with glial activation (Roedel et al., 2016). In line with this evidence, some pharmacological treatments involving the opioid system and/or with putative inhibitory actions on glial cells are beneficial for FM. One example of this is low-dose naltrexone, an opioid antagonist, which is proposed to inhibit glial activation (Mattioli et al., 2010) and has been shown to have beneficial effects in FM (Younger and Mackey, 2009; Younger et al., 2009). Additionally, serotonin/noradrenaline reuptake inhibitors (SNRIs; e.g., duloxetine, milnacipran, etc.) are among the most commonly prescribed pharmacological treatments for FM, and show moderate effectiveness in reducing some FM symptoms (Welsch et al., 2018). While the primary mechanism of action of SNRIs is to normalize concentrations of endogenous monoamine neurotransmitters, which are thought to be imbalanced in FM (Albrecht et al., 2016; Kosek et al., 2016; Russell et al., 1992; Wood, 2008), one potential additional mechanism may be glial modulation, as both duloxetine (Yamashita et al., 2016) and milnacipran (Shadfar et al., 2018) attenuate microglial activation in animal models. Interestingly, among the regions demonstrating neuroimmune activation in our current study was the PCC/precuneus, a core region of the default mode network, where post-treatment changes in pain related activation were specifically related to the degree of positive clinical response to milnacipran treatment in fibromyalgia patients (Jensen et al., 2014). Further studies are needed to better understand the specific cellular and molecular mechanisms of FM pharmacotherapies, and the potential role

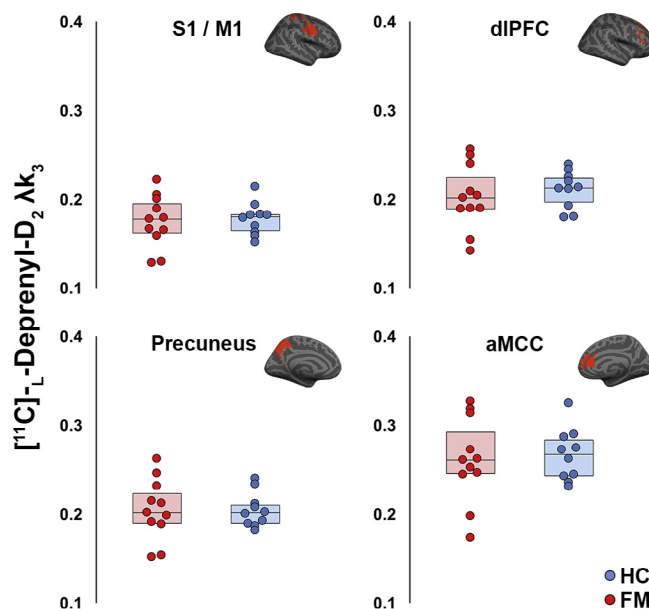


Fig. 5. Absence of group differences in $[^{11}\text{C}]\text{-L-deprenyl-D}_2$ λk_3 . Four representative regions showing significant group differences in the $[^{11}\text{C}]\text{PBR28}$ SUVR analysis (Fig. 2) show no differences in $[^{11}\text{C}]\text{-L-deprenyl-D}_2$ λk_3 ($p \geq 0.53$ uncorrected). Surface projections of individual regions are displayed in red above the plots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

glial cell inhibition plays in their effectiveness.

The utility of TSPO as a marker of glial activation is supported by numerous preclinical and post-mortem studies. While TSPO is ubiquitously expressed by many cell types, it can be used as a sensitive marker of glial activation *in vitro* because it is dramatically upregulated in glial cells in the context of a neuroinflammatory response. TSPO upregulation has been colocalized with activated microglia and/or astrocytes across a spectrum of CNS disorders, including animal models of neuropathic pain (Liu et al., 2016; Wei et al., 2013), both animal MS models and human MS lesions (Abourbeh et al., 2012; Chen and Guilarte, 2006; Cosenza-Nashat et al., 2009), and both animal models of Alzheimer's disease (AD) and human post-mortem tissue (Cosenza-Nashat et al., 2009; Gulyas et al., 2009; James et al., 2017), among many others. The utility of TSPO as a marker of glial activation is further supported by numerous *in vivo* human PET imaging studies. In many of these, elevated TSPO PET signal is observed in brain regions where glial activation is known to occur. TSPO elevations have been documented in the primary motor cortex in Amyotrophic and Primary Lateral Sclerosis (Alshikho et al., 2016; Alshikho et al., 2018; Paganoni et al., 2018; Zurcher et al., 2015), in white matter or gray matter lesions in Multiple Sclerosis (Datta et al., 2017; Herranz et al., 2016), in amyloid positive regions in Alzheimer's Disease (Kreisl et al., 2013b; Parbo et al., 2017), and in the basal ganglia in Huntington's Disease (Lois et al., 2018). However, while a plethora of human and preclinical studies support TSPO as a glial marker, it is important to note that not all studies have detected TSPO upregulation in neuropathologies with a hypothesized inflammatory component. For instance, previous work showed no differences in TSPO PET signal in cocaine dependence (Narendran et al., 2014), and decreased signal in alcohol dependence (Hillmer et al., 2017; Kalk et al., 2017). In patients with psychosis, initial studies with first generation TSPO tracers showed an increase, whereas recent studies using second-generation radioligands are supportive of a decrease in TSPO levels (Plaven-Sigra et al., 2018). Thus, further work is needed to better assess the potential usefulness of TSPO as a means to image neuroinflammation, and the meaning of the observed TSPO signal changes, particularly in certain pathologies.

In addition, even in conditions for which TSPO may be more established as a marker of glial activation, the specific functional significance of its upregulation remains unclear, and represents an active area of investigation. Numerous preclinical studies show analgesic and anti-inflammatory effects of TSPO agonism, such as increased expression of anti-inflammatory IL-10 and other M2-related microglial genes, indicating that changes in TSPO expression might be an adaptive response to a homeostatic challenge (Bae et al., 2014; Liu et al., 2016; Wei et al., 2013). Similarly, recent *in vitro* human studies suggest that immune challenges induce TSPO upregulation in anti-inflammatory M2-like macrophages, and TSPO reduction in inflammatory M1-like macrophages (Narayan et al., 2017; Owen et al., 2017). We previously documented significantly higher CSF concentrations of IL-10 and other anti-inflammatory cytokines in FM patients, as opposed to a more classical (M1-like) pro-inflammatory CSF cytokine profile in patients with rheumatoid arthritis (RA; Kosek et al., 2015; Lampa et al., 2012). Altogether, these observations suggest that the [¹¹C]PBR28 PET signal increases in FM patients might be reflective of a M2-like glial phenotype, although in the absence of PET tracers with a higher degree of phenotype specificity this remains speculative.

As mentioned above, elevated TSPO during a neuroinflammatory response may colocalize with both microglia and astrocytes, depending on the specific circumstances. As such, the exact cellular contributions of these glial subtypes to the TSPO PET signal are uncertain. In order to disambiguate the cellular specificity of the TSPO elevations observed in this study, a smaller sample of FM patients, partially overlapping with the sample scanned with [¹¹C]PBR28, was evaluated with [¹¹C]-L-deprenyl-D₂, to quantify brain levels of MAO-B. The expression of this protein in glial cells is thought to be predominantly, if not exclusively, within astrocytes, with little to no contribution from monocytes or

microglia (Ekblom et al., 1994). For instance, MAO-B upregulation was found to be colocalized with reactive astrocytes in post-mortem tissues from patients with AD (Nakamura et al., 1990) and ALS (Ekblom et al., 1993; Ekblom et al., 1994), conditions that also demonstrate elevations in [¹¹C]-L-deprenyl-D₂ PET signal (Johansson et al., 2007; Scholl et al., 2015). Because in the current study we observed no group differences in [¹¹C]-L-deprenyl-D₂ binding, our data suggest that elevated [¹¹C]PBR28 signal in FM patients might be driven by activated microglia rather than astrocytes. Furthermore, the lack of group differences in [¹¹C]-L-deprenyl-D₂ signal in any of the other anatomically defined brain regions, including whole brain and whole gray matter, suggests that astrocyte activation may not be relevant to the FM pathophysiology.

In line with our current findings of elevated TSPO PET signal in FM patients, we have previously reported brain TSPO elevations in patients with another pain condition, chronic low back pain (Loggia et al., 2015b). In the cLBP study, we observed a different spatial pattern of glial activation that was localized to the thalamus and areas of the somatosensory and motor cortices consistent with the somatotopic representation of the back and leg, regions in which those participants experienced pain. In the FM patients, by contrast, we observed a pattern which was more spatially extended, and involved only cortical areas. The larger cortical spread of neuroinflammation in FM patients compared to cLBP patients might be reflective of the differences in clinical presentation of these two patient groups, as the former report more widespread pain, and a higher incidence of cognitive issues and affective comorbidities (Clauw, 2014). Of note, the majority of FM patients also report low back pain but, unlike in our previous [¹¹C]PBR28 cLBP study, we did not observe statistically significant elevations in thalamic TSPO PET signal in FM, suggesting that similar pain symptoms in the two disorders may be mediated by distinct mechanisms. On the other hand, TSPO PET signal in the cingulate cortex, which in our FM patients was associated with fatigue scores, was also found to be elevated in patients with chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME; Nakatomi et al., 2014), suggesting glial activation in this region as a potential mechanism underlying pathological fatigue across different conditions.

Importantly, our results showed that elevated [¹¹C]PBR28 signal in FM, which was initially identified in the main analysis combining datasets from two different sites (KI + MGH), could be observed within each site separately in follow-up analyses (Fig. 2). The reproducibility of the effects across sites strengthens confidence in the solidity of our observations. However, we also noticed that the effect sizes for the KI dataset were overall larger compared to those for the MGH dataset. This difference in magnitude of group differences between sites could be a result of several contributing factors, including differences in imaging procedures, e.g., different PET scanners, attenuation correction methodology, tracer injection parameters, tracer synthesis, etc. Additionally, patients from the KI site had significantly higher scores on several ACR items, including symptom severity and trouble thinking clearly (Table 2), and were less medicated (Table 3).

Finally, we observed an overlap in the spatial pattern of [¹¹C]PBR28 PET group differences between the SUVR and V_T analyses (Fig. 3), indicating that these analytical techniques may have similar abilities to detect regions exhibiting neuroinflammation in FM. This observation is in accordance with previous studies comparing [¹¹C]PBR28 metrics computed with (e.g., V_T , DVR) or without (e.g., SUVR) an arterial input function in different populations (Albrecht et al., 2017; Lyoo et al., 2015). While in the current study we also observed significant positive correlations between SUVR and V_T in several cortical regions, it should be noted that an association between these metrics has not been consistently observed in the literature (Matheson et al., 2017). A more thorough investigation of the relationship between SUVR and V_T is warranted to better understand this discrepancy across studies.

There are several caveats to take into consideration when interpreting results of the current study. In our voxelwise analyses, we

implemented a cluster-forming threshold of $z > 2.3$, which has been criticized by some for being susceptible to false positives (Eklund et al., 2016). However, we were able to show elevations in [^{11}C]PBR28 signal independently for each study site, indicating that the effect is likely to be the result of a true physiological effect. Additionally, in the analyses evaluating the association between [^{11}C]PBR28 signal and clinical variables, no correction for multiple comparisons was utilized. While we feel that this exploratory approach is warranted, given that this is the first study demonstrating TSPO signal elevations in FM, the clinical significance of [^{11}C]PBR28 signal elevation in FM awaits further investigation. Furthermore, the ACR data for all KI patients and 2 MGH patients were acquired during the screening visit, but not at the scan visit. Though scores on this questionnaire show temporal stability, it is possible that scores for some patients may have changed between screening and scanning. Finally, the results of the [^{11}C]-L-deprenyl- D_2 analysis should also be interpreted with caution. Importantly, the absence of a significant effect cannot be used as conclusive proof of no difference, especially with a small sample size as in the current study. Further analysis with larger samples will be thus needed to confirm that astrocytes do not have a key role in the pathophysiology of FM.

In conclusion, our work shows that brain levels of the glial marker, TSPO, as measured using [^{11}C]PBR28 PET imaging, are elevated in the cortex of FM patients relative to healthy controls. Furthermore, we found an association between the TSPO PET signal and fatigue, a predominant FM symptom. The lack of elevated [^{11}C]-L-deprenyl- D_2 binding in FM may be viewed as support of an involvement of microglial, rather than astrocytic, activation. Future studies will need to test whether glial modulation may be a viable therapeutic strategy for FM.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbi.2018.09.018>.

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