PTSD is associated with neuroimmune suppression: evidence from PET imaging and postmortem transcriptomic studies

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Despite well-known peripheral immune activation in posttraumatic stress disorder (PTSD), there are no studies of brain immunologic regulation in individuals with PTSD. [11C]PBR28 Positron Emission Tomography brain imaging of the 18-kDa translocator protein (TSPO), a microglial biomarker, was conducted in 23 individuals with PTSD and 26 healthy individuals—with or without trauma exposure. Prefrontal-limbic TSPO availability in the PTSD group was negatively associated with PTSD symptom severity and was significantly lower than in controls. Higher C-reactive protein levels were also associated with lower prefrontal-limbic TSPO availability and PTSD severity. An independent postmortem study found no differential gene expression in 22 PTSD vs. 22 controls, but showed lower relative expression of TSPO and microglia-associated genes TNFRSF14 and TSPAP1 in a female PTSD subgroup. These findings suggest that peripheral immune activation in PTSD is associated with deficient brain microglial activation, challenging prevailing hypotheses positing neuroimmune activation as central to stress-related pathophysiology.
Posttraumatic stress disorder (PTSD) develops in 6–10% of persons experiencing a traumatic event. Existing interventions for PTSD have relatively low efficacy; this has generated interest in identifying biomarkers, particularly immune markers, that may underlie the development and persistence of PTSD symptoms. C-reactive protein (CRP) is an acute phase reactant protein secreted by the liver in response to pro-inflammatory cytokine signaling. Higher levels of CRP have been linked with exposure to a traumatic event, greater risk for developing PTSD following a traumatic event, and greater severity of PTSD symptoms. These findings, as well as its translational potential due to widespread use in clinical settings, lend support for CRP as a critical target of translational potential due to widespread use in clinical settings. CRP, interferon-α, and tumor necrosis factor-α (TNF-α), and have found PTSD to be associated with higher levels of IL-6, IL-1β, and interferon-γ (IFN-γ), but not TNF-α. In contrast to these findings, subsequent studies have observed lower levels of TNF-α and IFN-γ immediately following trauma in individuals who later developed PTSD, and higher levels of TNF-α in a combat-exposed PTSD group. Investigations of cytokine levels in cerebrospinal fluid (CSF) have also been mixed. Some studies showed higher levels in PTSD of IL-6 and IL-1β compared to trauma-exposed and non-exposed individuals, respectively, while others reported no differences in multiple pro-inflammatory cytokines between PTSD and non-trauma-exposed individuals. Despite these inconsistencies, more recent reviews have corroborated previous meta-analyses, providing additional evidence for elevation of pro-inflammatory IL-6, CRP, IFN-γ, and TNF-α in PTSD, using this as a basis to advocate for testing anti-inflammatory treatments.

Peripheral immune modulators, including cytokines, are thought to affect PTSD pathophysiology by traversing the blood–brain barrier and acting on microglia, the resident immune cells of the central nervous system. These actions have been thought to trigger immunologic activation of microglia, which subsequently release pro- and anti-inflammatory cytokines that modify the brain response to stress, as observed in rodent models of stress and PTSD. Many such preclinical models have observed “classical activation” or pro-inflammatory responses of microglia in stress-responsive regions of the brain, with increased stimulated cytokine levels or expression of IL-1β, IL-6, and TNF-α or increased microglia-specific proteins and cell-surface markers. However, other rodent studies have demonstrated no changes or variable changes in pro-inflammatory cytokines, or evidence of “alternately activated” microglial response with decreased pro- but increased anti-inflammatory cytokines. The variability of neuroimmune responses to severe stress in these models may depend on differences in species, as well as duration, type, and intensity of stress paradigms, and behaviors assessed. The differing findings might also reflect true heterogeneity in stress-induced neuroimmune pathology, but these animal models are likely limited in their ability to recapitulate the complex pathophysiology and symptomatology that characterizes PTSD in humans.

The inconsistency of peripheral and neural immune alterations in association with PTSD pathophysiology underscores the importance of studying the neuroimmune system in the brains of humans living with PTSD. The goal of our study was to use the radiotracer [11C]PBR28 and positron emission tomography (PET) brain imaging to measure a marker of the neuroimmune system in PTSD. [11C]PBR28 targets the 18-kDa translocator protein (TSPO), which is a mitochondrial outer membrane protein expressed in microglia, and a molecule that has been extensively studied as a biomarker of microglia in humans with PET brain imaging. [11C]PBR28 is a second-generation PET radioligand with high specific binding to TSPO, favorable kinetics, and good reproducibility in quantifying TSPO availability. Careful characterization by our group has demonstrated that [11C]PBR28 PET is sensitive to a decrease in microglial number after pharmacological depletion, as well as LPS-induced increases in microglial activation in non-human primates and humans.

The present study is the first to undertake an investigation of TSPO in vivo in individuals with PTSD, and to relate this neuroimmune system marker to PTSD symptom severity, peripheral immune markers, and transcriptomic alterations in postmortem brain tissue in PTSD. Based on the large literature demonstrating higher pro-inflammatory peripheral immune markers in PTSD, we hypothesized that higher TSPO availability, measured by [11C]PBR28 PET, would be associated with greater PTSD symptom severity, and also with a diagnosis of PTSD. The primary examination of symptom severity reflects a deliberate dimensional approach to studying a disorder as heterogeneous in symptom presentation as PTSD. Our investigation was focused on a prefrontal-limbic circuit comprised of the amygdala, anterior cingulate cortex (ACC), hippocampus, insula, and ventromedial prefrontal cortex (vmPFC), which together are heavily implicated as part of neuroimmune, neuroendocrine, and transcriptomic networks in PTSD. Additionally, we sought to examine the relationship between peripheral immune markers and TSPO availability. Finally, in an independent dataset of postmortem brain tissue from individuals with and without PTSD, we examined transcript expression of TSPO and other microglia-associated genes that may be differentially expressed in relation to PTSD, to better understand what is likely a complex interaction of TSPO with other molecules to produce PTSD-related neuroimmune system alterations.

This study presents the first known evidence that, contrary to our hypothesis, lower prefrontal-limbic TSPO availability is significantly associated with greater PTSD symptom severity, and is significantly lower in individuals with a diagnosis of PTSD compared to controls. We confirm the association of peripheral inflammation, as measured by plasma CRP levels, with PTSD severity, and demonstrate that TSPO availability is negatively associated with CRP. In an independent sample of postmortem brain, we also report evidence of lower expression levels of TSPO and microglia-associated genes, TNFRSF14 and TSPOAP1, in prefrontal cortical tissue from females with PTSD relative to non-PTSD controls. Collectively, these findings suggest that peripheral immune activation in PTSD is associated with suppression of microglia. Thus, these data question the presence of neuroimmunologic activation in PTSD, one of the prevailing hypotheses on the pathophysiology of PTSD in the literature, and suggest that medications to restore neuroimmune function may be effective for the treatment of PTSD.

Results

Participant characteristics and injection parameters (Table 1) did not differ in the PTSD vs. control group with the exception of injected mass per body weight, which was significantly higher in the control compared to PTSD group. There was no significant effect in a linear regression analysis of injected mass per body weight on prefrontal-limbic TSPO availability across all participants (p = 0.22), so this was not adjusted for in subsequent modeling analyses. TSPO availability was higher in HABs vs. MABs, and data from the two excluded outliers were greater than 3 SD above the mean TSPO availability within HABs.
(Supplementary Fig. 1). Interpersonal trauma (sexual, physical, childhood) was more prevalent in the PTSD compared to control group.

Lower TSPO is associated with greater PTSD symptom severity. In the PTSD group, lower composite prefrontal-limbic [11C]PBR28 distribution volume (VT) was significantly associated with greater severity of PTSD symptoms (Fig. 1: \( \beta = -0.43, p = 0.046 \)) measured by total scores on the Clinician Administered PTSD Scale (CAPS). To investigate whether specific symptom clusters may be driving the association with overall symptom severity, we conducted post hoc analyses with PTSD symptom clusters, but observed no significant associations with CAPS re-experiencing, avoidance, numbing, and hyperarousal subscores. Sensitivity analyses revealed that the overall association of composite prefrontal-limbic TSPO availability with PTSD symptoms remained significant after adjusting for tobacco, cannabis, and alcohol use (all \( \beta > -0.43, \) all \( p < 0.05 \))

![Fig. 1 Prefrontal-limbic TSPO availability was associated with PTSD severity. Lower [11C]PBR28 VT was significantly associated with greater PTSD severity (\( R^2 = 0.51, p = 0.003, \beta = -0.43, p = 0.046 \)), quantified by normalized total severity scores on CAPS in the PTSD group (\( n = 23 \)). Coefficient of determination and standardized coefficients were assessed using linear regression. Displayed composite prefrontal-limbic [11C]PBR28 VT values are genotype- and sex-adjusted, with gray shading indicating 95% confidence intervals.](image)

![Fig. 2 Prefrontal-limbic circuit TSPO availability was lower in the PTSD vs. control group. [11C]PBR28 VT in the prefrontal-limbic circuit composed of amygdala, anterior cingulate cortex, hippocampus, insula, and vmPFC, was lower (\( F_{5,41} = 2.46, p = 0.049 \)) in the PTSD (pink triangles, \( n = 23 \)) compared to control (green circles, \( n = 26 \)) group. A 14% difference in [11C]PBR28 VT between groups was observed ininsula (\( p = 0.036 \)) and vmPFC (\( p = 0.023 \)). Group differences were assessed using MANOVA and within-ROI comparisons assessed using ANOVA. Displayed [11C]PBR28 VT values are adjusted for genotype and sex, with group-wise mean indicated by black point. AMYG: amygdala, ACC: anterior cingulate cortex, HIPP: hippocampus, INS: insula, VMPC: ventromedial prefrontal cortex. *p < 0.05, not corrected for multiple comparisons.](image)

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**Table 1 Participant characteristics and injection parameters.**

| Characteristics | Control \((n = 26)\) | PTSD \((n = 23)\) | \(p\)-value |
|-----------------|---------------------|-----------------|------------|
| Age: years      | 32 ± 12             | 38 ± 10         | 0.10       |
| Sex             | 8 F, 18 M           | 10 F, 13 M      | 0.36       |
| Body mass index | 27.3 ± 4.3          | 27.8 ± 5.4      | 0.68       |
| rs6971 Genotype | 17 HAB, 9 MAB       | 18 HAB, 5 MAB   | 0.32       |
| Ethnicity (%)   |                     |                 | 0.32       |
| African American| 6 (23)              | 9 (39)          |            |
| Asian/Pacific   | 1 (4)               | 1 (4)           |            |
| Caucasian       | 12 (46)             | 9 (39)          |            |
| Hispanic        | 7 (27)              | 4 (17)          |            |
| **Clinical Characteristics** | | | |
| PTSD severity   |                     |                 |            |
| CAPS-IV \((n)\) | —                   | 71 ± 24 (10)    |            |
| CAPS-5 \((n)\)  | —                   | 32 ± 10 (13)    |            |
| Trauma exposure(s) \((n = 16)\) | — |                 |            |
| Sexual trauma   | 3                   | 12              |            |
| Physical trauma | 10                  | 21              |            |
| Childhood trauma| 11                  | 23              |            |
| Combat          | 3                   | 9               |            |
| Accident/natural| 13                  | 17              |            |
| Disaster        |                     |                 |            |
| Years since primary trauma \((n)\) | 16 ± 10 (12) | 16 ± 11 (22)    | 0.91       |
| Current comorbid MDD | —       | 18              |            |
| Currently on psychotropic medication | — | 5               |            |
| Current tobacco users | 12 | 8              | 0.41       |
| Current cannabis users | 4 | 7              | 0.21       |
| Current alcohol users | 17 | 13             | 0.53       |
| **Injection parameters** | | | |
| Injected dose (MBq) | 502.6 ± 189.3       | 573.9 ± 165.7   | 0.17       |
| Injected mass (µg/kg) | 0.03 ± 0.03 | 0.01 ± 0.01    | 0.022      |
| \(f_p\) | 0.024 ± 0.008       | 0.020 ± 0.007   | 0.14       |

*Significant at \( p < 0.05 \); assessed from two-sided independent samples t-test. Values are Mean ± SD, unless otherwise specified.

Group differences in TSPO availability. Analysis in the primary regions of interest comprising a prefrontal-limbic circuit (i.e., amygdala, ACC, hippocampus, insula, vmPFC) revealed that [11C]PBR28 VT was significantly lower in the PTSD than the control group (Fig. 2: MANOVA: \( F_{5,41} = 2.46, p = 0.049 \)), reaching regional significance in the insula (14% lower, \( p = 0.036 \)) and vmPFC (14% lower, \( p = 0.023 \)). Sensitivity analyses showed that the overall PTSD vs. control group difference in prefrontal-limbic TSPO availability remained significant after adjusting for tobacco, cannabis, and alcohol use (all \( F > 2.47, \) all \( p < 0.05 \)). There were no differences in prefrontal-limbic TSPO availability between individuals in the PTSD group with or without comorbid MDD (\( p = 0.60 \)) or current psychotropic medication use (\( p = 0.59 \)), and between individuals in the control group with or without trauma exposure (\( p = 0.85 \)).
A global pattern of lower $[^{11}C]PBR28$ $V_T$ in the PTSD group was visually apparent (Supplementary Fig. 2) with $[^{11}C]PBR28$ $V_T$ across all regions displayed in Supplementary Table 1, but a multivariate analysis showed no main effect of PTSD vs. control group across non-primary ROIs (MANOVA: $F_{10,36} = 1.07, p = 0.41$). Based on our finding of a significant relationship between TSPO availability and symptom severity, post hoc analyses were conducted among PTSD subgroups stratified by median of total symptom severity on the CAPS. In the high-severity PTSD compared to control group (but not in low-severity vs. control), $[^{11}C]PBR28$ $V_T$ in the prefrontal-limbic circuit was even lower (MANOVA: $F_{5,20} = 3.34, p = 0.021$) than in the overall PTSD vs. control group comparison, reaching regional significance in ACC (19% lower, $p = 0.022$), insula (21% lower, $p = 0.009$), and vmPFC (22% lower, $p = 0.005$) (Supplementary Fig. 3). There were no significant differences between high- vs. low-severity PTSD groups.

**C-reactive protein levels.** Driven by the evidence for CRP as a robust and differentially modulated peripheral immune marker in PTSD, blood CRP was assayed in a majority of participants (41 total—21 PTSD, 20 control). In the PTSD group, CRP levels were significantly negatively associated with composite prefrontal-limbic $[^{11}C]PBR28$ $V_T$ (Fig. 3a: $\beta = -0.92, p = 0.029$), and significantly positively associated with greater PTSD symptom severity measured as a normalized total CAPS score (Fig. 3b: $R^2 = 0.21, p = 0.037$), confirming previous observations of an association between elevated CRP and PTSD symptomatology.\(^6\)\(^7\)\(^8\)\(^9\) In the group as a whole, CRP levels were significantly negatively associated with composite prefrontal-limbic $[^{11}C]PBR28$ $V_T$ ($\beta = -0.82, p = 0.003$). This finding also held within the control group ($n = 20$, $\beta = -0.76, p = 0.044$). Both findings remained significant after removal of the single CRP sample analyzed by a different assay.

**Scan-day cytokine levels.** Relationships between peripheral cytokine levels and TSPO availability were of interest, but these analyses were conducted in an exploratory manner, due to the lack of previously demonstrated associations between measured peripheral cytokines and TSPO availability, in either baseline or LPS-induced inflammatory states.\(^32\) IFN-γ was associated with prefrontal-limbic $[^{11}C]PBR28$ $V_T$ (Supplementary Fig. 4: $\beta = -0.64, n = 36, p = 0.046$) across all participants, with an effect for interaction of group by IFN-γ level ($p = 0.002$) reflected in a negative association within the controls ($\beta = -0.65, n = 20, p = 0.031$) and a positive association within the PTSD group ($\beta = 0.60, n = 16, p = 0.17$) of IFN-γ with prefrontal-limbic $[^{11}C]PBR28$ $V_T$. However, these effects were not significant after correction for multiple comparisons. No other cytokine levels were significantly associated with prefrontal-limbic TSPO availability (Supplementary Table 2) or found to be significantly different in the PTSD compared to control group (Supplementary Table 3).

**Immune gene alterations in postmortem brain transcriptome.** Given both the large magnitude difference in TSPO availability that was observed in vmPFC, and the limited extant literature implicating BA25 and BA11 as regions of differential gene expression in PTSD\(^38\)\(^39\), postmortem analyses targeted the prefrontal cortical areas BA25 (subgenual prefrontal cortex) and BA11 (orbitofrontal cortex). TSPO expression was non-significantly lower in prefrontal cortex BA11 ($-1.66$-fold lower ($\pm 0.16$), $p = 0.07$) and in BA25 ($1.02$-fold lower ($\pm 0.08$), $p = 0.33$) in tissue from individuals with PTSD ($n = 22$) relative to non-PTSD controls ($n = 22$) (Fig. 4a). The possibility that effects of group might be obscured by not accounting for effect of sex on TSPO expression was investigated, based on evidence of sex effects on TSPO availability in the present and previous PET analyses\(^40\) and of sex effects on TSPO gene expression in the brain in preclinical studies.\(^41\) There was a significant effect of sex ($p = 0.048$) on TSPO expression in BA11 in PTSD relative to controls, and a non-significant interaction effect ($p = 0.32$). Contrasting findings for TSPO expression in within-group, female vs. male comparisons also suggested an interaction. Gene expression was significantly higher in female relative to male controls (BA11: $2.60$-fold higher ($\pm 0.10$), $p = 0.002$; BA25: $2.30$-fold higher ($\pm 0.08$), $p = 0.04$) but lower, albeit non-significant, in females vs. males with PTSD (BA11: $1.4$-fold lower ($\pm 0.91$), $p = 0.50$; BA25: $1.2$-fold lower ($\pm 0.70$), $p = 0.85$). Thus, secondary analyses were conducted to investigate gene expression in a sex-specific manner in female and male subgroups. In females with PTSD ($n = 11$), TSPO expression was significantly lower relative to female controls ($n = 11$) in BA11 ($-2.30$-fold lower ($\pm 0.10$), $p = 0.01$) but did not reach significance in BA25 ($-1.40$-fold lower ($\pm 0.06$), $p = 0.08$) (Fig. 4a). There was no difference in relative TSPO expression in males with PTSD compared to male controls ($n = 10$).

\[\text{Fig. 3 CRP was negatively associated with prefrontal-limbic TSPO availability and positively associated with PTSD severity. a Lower }[^{11}C]PBR28 V_T \text{ was significantly associated with higher levels of CRP (}R^2 = 0.52, p = 0.005\text{); }\beta = -0.92, p = 0.029\text{) in the PTSD group (}n = 21\text{). Displayed }[^{11}C]PBR28 V_T \text{ values are adjusted for genotype and sex. b Higher CRP was significantly associated with greater PTSD severity (}R^2 = 0.21, p = 0.037\text{) in the PTSD group (}n = 21\text{). Coefficient of determination and standardized coefficients were assessed using linear regression. Gray shading indicates 95\% confidence intervals.}\]
expression in males with PTSD compared to male controls in either region. Exploratory analyses examined expression of microglia-associated genes, TNFRSF14 and TSPOAP1, to provide a broader characterization of microglial regulation. There was evidence of non-significant group × sex interaction effects on differential expression of TNFRSF14 (BA11: p = 0.063; BA25: p = 0.15) and TSPOAP1 (BA11: p = 0.055; BA25: p = 0.12), resulting in subsequent sex-specific investigations of differential expression of these genes in PTSD vs. controls. TNFRSF14 expression was significantly lower in prefrontal cortical areas in females with PTSD relative to female controls (BA11: −1.60-fold lower (±0.03), p = 0.01; BA25: −1.30-fold lower (±0.02), p = 0.04). In females with PTSD, there was also significantly lower relative expression of TSPOAP1 in BA11 (−1.60-fold lower (±0.06), p = 0.04) but not in BA25 (−1.40-fold (±0.06), p = 0.05) (Fig. 4b).

There was no difference in males with PTSD compared to male controls in relative expression of these microglia-associated genes.

Discussion

This study sheds new light on alterations in microglial regulation associated with PTSD based on data from in vivo PET imaging using [11C]PBR28 and analysis of microglia-associated gene expression changes in post-mortem brain tissue. The present results replicated the widely reported finding of higher blood CRP levels in relation with PTSD severity, suggestive of a pro-inflammatory state in PTSD. However, this study found significantly lower TSPO availability in prefrontal-limbic regions in PTSD. Both higher CRP levels and greater PTSD severity were associated with lower rather than higher TSPO availability. Finally, postmortem brain transcriptome analysis in an independent sample revealed lower expression of TSPO, TSPOAP1 and TNFRSF14 genes in prefrontal cortical tissue from females but not males with PTSD, providing convergent evidence of compromised microglial function potentially representing an overall neuroimmune suppression.

Compromised microglial function could contribute meaningfully to the pathophysiology of PTSD, particularly compromised cortico-limbic connectivity

42,43. Microglia have been implicated in the immunologic regulation of synaptic plasticity in part through production of neurotrophic factors such as IGF-1 and BDNF
44,45, and in a neuroprotective response in rodent models of spinal cord injury, with selective microglial depletion exacerbating neurodegeneration and motor impairment.

Adding to this is a growing consensus that higher TSPO levels do not simply represent neuroinflammatory M1-type microglial activation, but rather that TSPO likely represents a dynamically-regulated balance between microglial M1- and M2-type activation.
states. For instance, observations of M1-predominant activation did not result in any increase in TSPO in in vitro human microglia cell culture, while TSPO overexpression was associated with M2-predominant activation and reduced pro-inflammatory cytokine production in rodent microglial cells. Furthermore, direct viral overexpression of brain TSPO prior to a footshock stressor promoted a neuroprotective function and ameliorated the ensuing PTSD-like behaviors in rodents. Thus, we surmise that lower TSPO availability in association with PTSD severity may reflect lower microglial TSPO expression related to reduced activation of a neuroprotective microglial type, or progressive neuroimmune suppression marked by depletion of neuroprotective microglia themselves, or possibly, the presence of both processes. TSPO PET would be sensitive to such alterations based on our group’s previous demonstrations that TSPO PET can detect increases in TSPO expression as well as microglial depletion. While it is not clear whether low levels of TSPO are cause or consequence of PTSD, results of these studies indicate a suppressed ability to mount an adequate neuroprotective microglial response, which may in turn contribute to the chronicity and severity of PTSD. Longitudinal studies are required to disentangle possible causal and temporal relationships between trauma, neuroimmune responses, and PTSD symptoms.

Our investigation of peripheral immune markers in relation to the neuroimmune system revealed that lower TSPO was significantly associated with higher levels of the acute phase immune reactant, CRP, while confirming a significant association of higher CRP with greater PTSD severity in our PET study sample. Erady et al. found that higher CRP prior to combat-related trauma was a stronger predictor for developing PTSD and hyperarousal and numbing symptoms, and Michopoulos et al. found CRP levels to be positively associated with overall PTSD severity. Thus, our data in combination with previous findings suggest that higher CRP may be a risk factor, while its association with lower prefrontal-limbic TSPO suggests a possible circuit-specific mechanism linking higher CRP with the development or maintenance of clinical PTSD pathologies. No significant associations were found in exploratory cytokine analyses, due to limited post hoc power after correction for the 7 different cytokines assayed. Overall, in contrast to previous suppositions of higher levels of all peripheral immune markers driving greater “neuroinflammation”, our finding of higher CRP in association with lower TSPO availability lends further support for a peripheral-to-neural immune system relationship that is more complex than previously proposed.

Results of our PET study converged with postmortem brain analyses showing lower relative gene expression—statistically significant only in females with PTSD—of prefrontal cortical TSPO, TNFRSF14, which encodes TNF-α receptors expressed on microglia; and TSPOAPI1, encoding TSPO-associated protein 1, also known as peripheral benzodiazepine receptor-associated protein 1 (PRAX1). These data provide evidence of altered postmortem expression of TSPO and TSPO-related genes in humans with PTSD, adding to recent findings of reduced dorsolateral prefrontal cortex I11A gene expression in PTSD. The observation in PTSD of lower relative expression of TNFRSF14 concurrently with TSPO, may reflect a reduction of microglia that are responsive to pro-inflammatory TNF-α signaling and, in fact, parallels the association, though non-significant, of lower TSPO availability with lower peripheral TNF-α levels in our PET study. The additional finding of lower relative expression of TSPOAPI1 is of further interest, considering recent studies demonstrating upregulation of Tspoa1 mRNA in hippocampus and amygdala of non-stressed rats in response to antidepressant treatment. Lower expression of these three genes in PTSD may thus represent a downregulation of a network of genes associated with neuroprotective microglial function and, encouragingly, such gene network dysregulation may be modifiable.

It is important to note the sex-specific nature of the postmortem findings. We explored possible effects of sex in both PET and postmortem studies, based on compelling evidence of sex differences in both brain TSPO availability from a recent multi-site study with [11C]PBR28 PET, and of an apparent group by sex interaction effect on TSPO gene expression in our study, supported by evidence of differential effects of sex on TSPO expression in brain and other tissues in several preclinical studies. Though we were not powered to conduct sex-specific subgroup analyses within the PTSD group in the PET study, we covaried for sex based on the improved statistical fit of our regression models of the PET data. In our postmortem study, we found that TSPO expression was significantly lower in a female PTSD relative to control subgroup, even though it was non-significantly lower in PTSD relative to controls combined across sex. This highlights the importance of sex-specific analysis of postmortem TSPO gene expression, and is underscored by the demonstration of sex differences in baseline TSPO gene expression and sex-stereotyped modulation of TSPO expression and abundance of TSPO protein, which may extend to a role for sex in the differential regulation of TSPO gene expression and TSPO availability in our present study as well. The findings nevertheless represent the first known corroboration of neuroimmune suppression in individuals with PTSD between an independent in vivo assessment of TSPO availability and ex vivo assay of TSPO gene transcription.

The divergence of our observation of lower TSPO availability in PTSD from higher TSPO availability in major depressive disorder (MDD) is noteworthy. Distinct neuroimmune phenotypes of PTSD and MDD are in line with the “hypocortisolemic” HPA-axis phenotype observed in PTSD that is notably the opposite of the “hypercortisolemic” phenotype of MDD. An association of neuroimmune suppression with HPA-axis suppression in PTSD is supported by evidence of reduced glucocorticoid signaling and associated reduced microglial activation in mice exposed to chronic unpredictable stress. Furthermore, we observed no independent association of comorbid MDD in the PTSD group with TSPO availability, implicating the observed association as PTSD-specific. Collectively, these findings implicate a neuroimmune-neuroendocrine basis for the distinct clinical presentations and prognoses observed between PTSD and MDD.

Some limitations of our study should be noted. First, our study samples are representative of the heterogeneity in the PTSD population for factors such as use of tobacco and psychotropic medications, and we recognize that these factors could contribute to greater heterogeneity in the relationship between TSPO availability and PTSD symptoms. However, we did not observe any relationship of these factors with TSPO availability, and we consider it a strength of the study that our sample is more clinically generalizable. Second, our analysis of relationships between TSPO availability and cytokine levels was limited by a few factors. Single time-point measurements of plasma cytokines do not provide the most reliable indicator of peripheral immune state in each individual. Additionally, though we imputed values below assay detection limits according to recommended methods, this approach could introduce bias in the measurements. These factors led to the exploratory nature of this analysis. Third, due to exclusion criteria for eligibility to participate in a PET scan, our sample happens to be relatively healthy in terms of BMI and lack of metabolic and cardiovascular disease other than hypertension. These disorders may be driving the elevation of pro-inflammatory cytokines that have been observed in other PTSD samples, but not in ours, and it is possible that PTSD with high medical
comorbidity may have a distinct neuroimmune phenotype. Fourth, our PET and postmortem samples inevitably differ on some clinical characteristics. For instance, the prevalence of cardiovascular and overdose-related deaths in the postmortem dataset is somewhat inherent to samples available in the post-mortem PTSD brain bank, but may also contribute to different degrees of lower TSPO observed between the postmortem and PET data. Additionally, the absence of comorbid MDD in the postmortem dataset may limit the cross-validity of the lower TSPO findings. Nevertheless, the complementary findings of lower prefrontal cortical TSPO expression in the postmortem PTSD-only dataset and the lack of association of comorbid MDD with lower TSPO availability in the PET study, if anything, provide further support for lower TSPO being more specifically associated with PTSD symptomatology and less likely to be driven by comorbid MDD. Fifth, our targeted TSPO gene qPCR was performed in subgenual prefrontal cortex (BA25) and orbitofrontal cortex (BA11) as an attempt to capture tissue comprising the greatest amount of the larger prefrontal cortex, but these regions are not precisely analogous to the AAL atlas-defined vmPFC region in which we found the greatest magnitude difference in TSPO availability in our PET study. Sixth, despite the extensive array of peripheral immune markers examined in association with TSPO availability, these analyses are not able to directly inform underlying mechanisms of microglial function in PTSD, such as delineation of the balance between M1 or “classical activation” representing neuroinflammation and M2 or “alternative activation” representing neuroprotection. Nevertheless, we assert that our associations of lower TSPO levels with greater symptom severity, peripheral immune markers, and alterations in other putatively neuroprotective microglia-associated genes, represent initial descriptive evidence of a functional microglial characterization which should, in the future, be incorporated into mechanistic models of microglial function specifically in the context of PTSD.

Taken together, with data from previous literature, our findings challenge previous conceptions of concurrent peripheral and neural inflammation in PTSD, instead providing support for a novel model of neuroimmune pathophysiology underlying PTSD: deficient neuroimmune neuroprotective function. We propose that this deficient neuroimmune neuroprotective function is characterized by lower TSPO and alterations in other microglia-associated molecular markers, TNFRSF14 and TSPOAPI. The association of lower TSPO with greater PTSD severity, highlights the specificity of the observed neuroimmune dysregulation to PTSD clinical pathology. Additional associations of both lower TSPO and greater symptom severity with higher levels of CRP, previously shown to be a risk factor for PTSD, implicate higher CRP as a possible risk factor making individuals more vulnerable to developing this particular neuroimmune phenotype of PTSD. Importantly, preclinical studies provide hope that a chronic neuroimmune suppression phenotype may not be permanent. Ongoing experiments include assessment of the dynamic functioning of the neuroimmune system in PTSD to see if microglia mount a comparable response to the previously validated LPS challenge, and to examine if LPS might even improve symptoms in a neuroimmune suppressed PTSD phenotype, similar to the LPS-induced reversal of numbing-like behaviors and accompanying microglial suppression observed in rodent PTSD models. Direct agonism of TSPO may provide an even more specific manipulation of microglial activity, with overexpression of TSPO previously shown to reduce anxiety-like behaviors analogous to PTSD re-experiencing and hyperarousal symptoms. Such avenues of investigation will help inform the development of pharmacological agents targeting microglia for potential therapeutic intervention to restore neuroimmune system function in a subset of individuals with a particularly severe and unremitting presentation of PTSD.

**Methods**

**Participant recruitment.** Forty-nine individuals participated in the current study and were recruited from 2011 to present via advertisements in public forums or through clinician referral. Written informed consent was obtained from all participants after a complete explanation of study procedures. A Structured Clinical Interview for the DSM (IV or 5) was administered to all participants to screen for history of trauma, PTSD among trauma-exposed individuals, presence of comorbid Major Depressive Disorder in the PTSD group, and exclusion of all other psychiatric diagnoses, including alcohol dependence (DSM-IV) and alcohol use disorder (DSM-5) in both PTSD and control groups. Clinical characterization of PTSD (n = 23) was performed using the Clinician Administered PTSD Scale (DSM-IV: n = 10; DSM-5: n = 13) administered by trained clinical research assistants under the supervision of a licensed clinical psychologist. Social drinking, and tobacco and cannabis use were permitted and quantified using the past month Timeline Followback. Current use of psychotropic medications, other than antidepressants and psychostimulants, was permitted in the PTSD group, and was determined at screening and confirmed on scan-day (Total: n = 5 of 23, serotonin reuptake inhibitors: n = 3, norepinephrine-dopamine reuptake inhibitors: n = 1, GABA<sub>A</sub> receptor agonists: n = 3, see Supplementary Table 4 for greater detail). Medical examination was performed and clinical laboratory tests were reviewed by the study physicians to confirm absence of significant medical illness- in particular individuals using non-steroidal and other anti-inflammatory medications on a daily or regular basis were excluded. All participants were screened for TSPO rs6971 genotype (NeoGenomics Laboratories, Houston, TX, USA) and individuals with homozygous T/T at the rs6971 locus or low-affinity binders were excluded, based on previous work establishing the effect of TSPO rs6971 genotype on affinity of [11C]PBR28 for TSPO. All screening and investigational procedures were in accordance with Code of Federal Regulations Title 45, Part 46 policy on protection of human subjects in research, and were approved and overseen by the Yale University Institutional Human Investigation Committee and the Yale New Haven Hospital Radiation Safety Committee.

**Trauma history assessment.** History of at least one traumatic event was assessed in all participants by screening with the SCID Post Traumatic Stress Disorder (DSM-IV) or SCID Trauma and Stress-related Disorders screen (DSM-5). History of particular types of trauma as listed in Table 1 was assessed by combination of participant report during clinician interview, self-report on the Life Events Checklist, a well-validated 17-item tool for assessing trauma history, and the Childhood Trauma Questionnaire Short Form (CTQ), a well-validated 28-item measure for assessing history of traumatic events before 18 years of age.

**Imaging acquisition.** Synthesis of [11C]PBR28 was achieved at high molar activity of 233 ± 173 MBq/nmol according to previously established methods and described in Supplemental Methods. Participants received a 1-min intravenous bolus of 536 ± 180 MBq [11C]PBR28 and were subsequently scanned for 120 min (apart from one scan terminated at 90 min due to participant discomfort) on the High-Resolution Research Tomograph (HRRT; Siemens, Medical Solutions, Knoxville, TN, USA). A T1-weighted structural MRI image acquired with a sagittal gradient-echo (MPRAGE) sequence (Siemens 3.0 T Prisma Fit; 176 sagittal slices, thickness = 1 mm, TR = 2530 ms, TE = 2.26 ms, flip angle = 7°, FoV = 256 mm, matrix size = 256 × 256, 1 × 1 × 1 mm<sup>3</sup> voxels) was also obtained in all individuals for co-registration of PET images and for definition of anatomical regions of interest.

**C-reactive protein analysis.** Levels of C-reactive protein were measured in 42 individuals. CRP level was measured on scan day in 41 participants using hs-CRP Wide Range Reagent (Clniqa Corporation, San Marcos, CA, USA), and on initial screening day in 1 control participant using ultraseensitive CRP (Graham Massey Analytical Labs, Shelton, CT, USA). CRP levels greater than 10 were excluded from subsequent analyses.

**Cytokine analysis.** Levels of cytokines IL-1β, IL-6, IL-8, IL-10, TNF-α, IL-17, IL-1β, IFN-γ, and MCP-1 were measured in plasma prior to radiotracer injection in 37 individuals using the MILLIPLEX panel assay (MillporeSigma, Burlington, MA, USA). Measurements below the assay-specific detection limit were imputed as the assay-specific detection limit normalized by a factor of 2 or V<sub>2</sub>, previously shown to be appropriate for normal and log-normal distributions, respectively, of data with values below the detection limit.

**Arterial input function measurement.** An arterial catheter was placed in the radial artery contralateral to [11C]PBR28 injection site for arterial sampling throughout the duration of the scan. Blood samples were acquired to measure plasma radioactivity concentration either manually every 10 s (n = 8) or continuously for the first 3 min using an automated blood sampler (PBS-101, Venstra Instruments, Joue, Netherlands), and then at 3, 5, 8, 12, 15, 20, 25, 30, 40, 50, 60,
corrected arterial input function was used to estimate regional $^{[11C]}$PBR28 volume in prefrontal cortex (vmPFC), cerebellum, frontal cortex, hippocampus, insula, occipital cortex, orbitofrontal cortex, parietal cortex, putamen, temporal cortex, thalamus, and ventromedial prefrontal cortex (vmPFC).

The Multilinear Analysis method (MA1 with $r^2 = 30\%$) with metabolite-corrected arterial input function was used to estimate regional $^{[11C]}$PBR28 volume in prefrontal cortex: the subgenual prefrontal cortex (sgPFC; BA25) and the orbitofrontal cortex.

For the postmortem gene expression analysis, quantitative real-time PCR (qRT-PCR) was performed on dissected tissue from 22 donors with PTSD (mean age: 46 ± 11 years, 11 females) and 22 matched non-PTSD control donors (mean age: 48 ± 12 years, 11 females) shown in Supplementary Table 5, in two areas within the prefrontal cortex: the subgenual prefrontal cortex (sgPFC; BA25) and the orbitofrontal cortex (OF; BA11).

Clinical diagnosis was based on psychiatric history and demographic data obtained through psychological autopsy performed postmortem. Trained clinicians with informants (usually next of kin) acquainted with the deceased subject provided all of the necessary information for diagnosis. To avoid systematic biases, PTSD and control cases were characterized by the same psychological methods, and were evaluated for presence of PTSD as well as lifetime alcohol and substance abuse and dependence, and for exclusion of other psychiatric and neurological illness, as determined by interview screening, medical examiner report, and by consensus diagnosis between two clinicians using DSM-IV Axis I lifetime diagnoses criteria.

Demographic and clinical characteristics, $n=971$ genotype, injection parameters, and peripheral inflammatory markers were compared across groups using Student’s $t$-tests for continuous variables and chi-squared tests for categorical variables. Distributions of all dependent variables were examined for normality using Shapiro–Wilk tests and inspection of histograms and Q-Q plots, and $^{[11C]}$PBR28 $V_T$ values were normally distributed. PTSD was defined as having a CAPS severity score of 30 or above, and control donors were defined as having a CAPS severity score of 0. Mann Whitney U tests were used to assess differences in cytokine levels. The exploratory nature of these analyses was due to the lack of detection of reliable associations between baseline and LPS-stimulated cytokine levels with TSPO availability in a previous study as well as the need to impute values below the assay-specific detection limits. A Benjamini-Hochberg procedure with $q = 0.05$ was used for correction of multiple comparisons in post hoc and exploratory analyses. Uncorrected $p$ values were reported for data not surviving false detection rate or FDR correction, and adjusted $p$ values were reported for data surviving FDR correction. Analyses of imaging, symptom severity, and peripheral immune marker data were conducted in RStudio version 1.0.133 with R programming language version 3.4.1.

The postmortem gene expression analysis, relative differences in transcript expression between PTSD and non-PTSD control donors were evaluated using Graphpad Prism v7 (Graphpad Software, San Diego, CA) and reported as fold changes calculated using the 2-DDCt method. Secondary sex-specific analyses of postmortem transcript expression were conducted, based on preclinical literature supporting sex-specific regulation of TSPO gene expression throughout tissues including in cortical limbic tissue.

The relationship of log-transformed CRP and prefrontal-limbic TSPO availability was assessed using a linear modeling approach, adjusting for $rs6971$ genotype and sex, and a general linear model was also used to compare log-transformed CRP and PTSD severity as measured by normalized CAPS total score. The exploratory analyses were conducted using linear modeling to assess possible relationships of prefrontal-limbic TSPO availability with levels of pro- and anti-inflammatory cytokines, and using two-sided Student’s $t$-tests to assess group differences in cytokine levels. These analyses were conducted using the past month Timeline Follow-back, comorbid MDD and psychotropic medication in the PTSD group, and trauma exposure vs. no exposure in the control group.

Statistical analysis. Demographic and clinical characteristics, $n=971$ genotype, injection parameters, and peripheral inflammatory markers were compared across groups using Student’s $t$-tests for continuous variables and chi-squared tests for categorical variables. Distributions of all dependent variables were examined for normality using Shapiro–Wilk tests and inspection of histograms and Q-Q plots, and $^{[11C]}$PBR28 $V_T$ values were normally distributed. PTSD was defined as having a CAPS severity score of 30 or above, and control donors were defined as having a CAPS severity score of 0. Mann Whitney U tests were used to assess differences in cytokine levels. The exploratory nature of these analyses was due to the lack of detection of reliable associations between baseline and LPS-stimulated cytokine levels with TSPO availability in a previous study as well as the need to impute values below the assay-specific detection limits. A Benjamini-Hochberg procedure with $q = 0.05$ was used for correction of multiple comparisons in post hoc and exploratory analyses. Uncorrected $p$ values were reported for data not surviving false detection rate or FDR correction, and adjusted $p$ values were reported for data surviving FDR correction. Analyses of imaging, symptom severity, and peripheral immune marker data were conducted in RStudio version 1.0.133 with R programming language version 3.4.1.

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source data for Figs. 1–3a, b, andable Supplementary Figs. 1–3a, b, and 4a, b are available as a Source Data file.

**Code availability**

Software codes used for image pre-processing and analysis are custom and proprietary, but specific parts of code may be made available upon reasonable request.

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Competing interests Dr. Jean-Kristal acknowledged the following relevant financial interests. He has a patent for glutamate modulator, riluzole, in augmentational treatment of anxiety disorders that was licensed by Biohaven Medical Sciences. His annual income from consultation for any of several pharmaceutical companies is not expected to exceed $10,000 in 2019. He holds stock in Biohaven Pharmaceuticals, Sage Pharmaceuticals, and Spring Care. He also receives over $10,000 from Society of Biological Psychiatry for his services as editor of the journal, Biological Psychiatry. The remaining authors declare no competing interests.

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