PET imaging of neuroinflammation in neurological disorders

William C Kreisl, Min-Jeong Kim, Jennifer M Coughlin, Ioline D Henter, David R Owen, Robert B Innis

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Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, New York, NY, USA (W C Kreisl MD); **Molecular Imaging Branch, National Institute of Mental Health, Bethesda, MD, USA** (M-J Kim MD, I D Henter MA, Prof R B Innis MD); **Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD, USA** (J M Coughlin MD); **and Department of Brain Sciences, Imperial College London, London, UK** (D R Owen PhD)

Correspondence to:
Prof Robert B Innis, National Institute of Mental Health, Bethesda, MD 20892–1026, USA
robert.innis@mail.nih.gov

A growing need exists for reliable in-vivo measurement of neuroinflammation to better characterise the inflammatory processes underlying various diseases and to inform the development of novel therapeutics that target deleterious glial activity. PET is well suited to quantify neuroinflammation and has the potential to discriminate components of the neuroimmune response. However, there are several obstacles to the reliable quantification of neuroinflammation by PET imaging. Despite these challenges, PET studies have consistently identified associations between neuroimmune responses and pathophysiology in brain disorders such as Alzheimer's disease. Tissue studies have also begun to clarify the meaning of changes in PET signal in some diseases. Furthermore, although PET imaging of neuroinflammation does not have an established clinical application, novel targets are under investigation and a small but growing number of studies have suggested that this imaging modality could have a role in drug development. Future studies are needed to further improve our knowledge of the cellular mechanisms that underlie changes in PET signal, how immune response contributes to neurological disease, and how it might be therapeutically modified.

Introduction

The discovery of glial-expressed risk variants associated with neurodegenerative diseases,[1] coupled with increasing evidence supporting neuroimmune modulation as a strategy for drug development, underscore the crucial need for reliable in-vivo measurement of neuroinflammation, which would enable research into the neuroimmune mechanisms that contribute to neurological disease and inform clinical trial design. Because of its ability to measure selected proteins at low concentrations, PET is particularly well suited to quantify neuroinflammation and has the potential to discriminate components of the neuroimmune response. However, in our experience, obstacles to the reliable measurement of neuroinflammation by PET imaging include misperceptions and limitations regarding 18 kDa TSPO (ie, the most common neuroinflammatory target), such as high non-specific binding and sensitivity to a genetic polymorphism that affects the binding affinity of early TSPO radioligands, as well as a paucity of non-TSPO targets with validated radioligands. The only validated non-TSPO target in use for research is the astrocyte-expressed MAOB, which has its own limitations (eg, expression by neurons).

Fortunately, there are several reasons for optimism. First, combining TSPO PET with other biomarkers has provided insights into the temporal and spatial relationships between neuroinflammation and the canonical pathologies underlying brain disorders such as Alzheimer's disease. Second, tissue studies have begun to clarify the meaning of increased TSPO-PET signal in some diseases. Third, improved second-generation and third-generation TSPO radioligands, particularly ^{11}C-ER176, have overcome some of the major disadvantages of earlier tracers. Finally, novel non-TSPO targets are under investigation, and several radioligands are in various stages of early development.

This Review assesses the role of PET imaging of neuroinflammation in patients with neurological disorders. Rather than provide an exhaustive, historical list of all published PET studies, this Review discusses only disorders for which at least one study that used a second-generation TSPO radioligand discriminated patients from healthy controls or showed an association with another disease-related outcome measure. These disorders include multiple sclerosis, HIV-associated cognitive impairment, Alzheimer's disease, frontotemporal dementia, chronic traumatic encephalopathy, Huntington's disease, amyotrophic lateral sclerosis, epilepsy, and stroke. Other disorders, such as corticobasal degeneration, progressive supranuclear palsy, and dementia with Lewy bodies, were excluded from the Review because they have not yet been studied with second-generation TSPO radioligands. Although second-generation radioligand studies have not observed increased TSPO in patients with Parkinson's disease, the possible role of TSPO PET in drug discovery for this disorder is discussed in the context of a clinical drug trial, in which a novel anti-inflammatory drug reduced TSPO signal. Disorders are discussed on the basis of similarities in terms of proposed relatedness to neuroinflammatory response. Classic immune-related disorders are discussed first, followed by neurodegenerative disorders, epilepsy, and stroke.

Overcoming obstacles to identifying neuroinflammation by use of PET imaging

This section discusses the major obstacles to reliable PET measurement of neuroinflammation, including misperceptions regarding TSPO as a biomarker, the limitations associated with early TSPO radioligands, and the paucity of non-TSPO targets with validated radioligands. Fortunately, multimodal imaging and tissue studies have increased understanding of the meaning of the TSPO-PET signal, and radioligand development has improved the ability to measure TSPO in vivo.

Although widely used as an inflammatory biomarker, TSPO has important caveats that require consideration to avoid misinterpreting PET-imaging results. First, although TSPO is predominantly expressed in the brain by microglia,[2] expression by other cell types should be considered. TSPO was originally found in peripheral

tissue[3] but is also expressed in the brain by astrocytes[4] and in the vascular endothelium.[5] Migration of peripheral myeloid cells into the brain can also contribute to the TSPO signal.[6] Therefore, the relative contribution of TSPO radioligand binding by microglia compared with other cells depends on the disease studied. For example, some,[7,8] though not all,[9] autopsy studies have shown that microglia are the predominant TSPO-positive cells in the brains of individuals with Alzheimer's disease. Similarly, TSPO in human multiple sclerosis lesions is mostly expressed in microglia, although it is also expressed in astrocytes to a lesser extent.[5] However, disease stage can, in some cases, also influence cellular expression of TSPO. For instance, in an experimental stroke model in rats, TSPO-expressing microglia were first found in the ischaemic lesion; days later, TSPO-expressing astrocytes were found in the surrounding area.[10] Additionally, the meaning of increased TSPO binding is controversial, even within the proportion of signal due to microglia. Rodent studies noted that increased TSPO expression signals a shift from resting to activated morphology in microglia, resulting in the widely held view that increased TSPO binding equates to microglial activation.[11] However, studies of human tissue do not necessarily support this view. Proinflammatory conditions did not increase TSPO expression in a study of human microglia, suggesting that radioligand binding might reflect microglial density rather than microglial phenotype.[12] Furthermore, although autopsy studies in humans identified TSPO-expressing microglia proximal to neuritic plaques in the brains of patients with Alzheimer's disease,[7,8] one study of patients with Alzheimer's disease showed that the area fraction of TSPO immunoreactivity was not associated with that of microglial activation (defined as CD68 immunoreactivity).[9] Additionally, although increased TSPO immunoreactivity was detected in brain and spinal cord tissue from individuals with multiple sclerosis, TSPO was identified in both proinflammatory and anti-inflammatory microglia (defined by coexpression of CD40 in proinflammatory microglia or MMR in anti-inflammatory microglia).[5] Nevertheless, immunohistochemistry results do not always agree with PET results. TSPO antibodies attach to the C terminus of the target protein, whereas radioligands bind to its active site. Furthermore, autoradiography and PET represent the available number of binding sites, not just the total amount of protein, and both techniques are inherently more quantifiable than immunostaining. Therefore, although TSPO binding should not be broadly assumed to reflect the extent of microglial activation, disease-specific and species-specific tissue studies should guide the interpretation of increased TSPO signal.

Another challenge intrinsic to TSPO PET is establishing which radioligand to use, given the tracers available and their varying limitations. The prototypical radioligand ^{11}C-(R)-PK11195 has a low signal-to-noise ratio, which reduces its ability to detect subtle changes in TSPO density.[13] Although second-generation radioligands have improved the ratios of specific to non-specific binding, they are sensitive to a common polymorphism (rs6971) in the *TSPO* gene.[14] Individuals with two copies of the rare allele (ie, low-affinity binders) bind these radioligands with a lower affinity than people with two copies of the major allele (ie, high-affinity binders), and people who are heterozygous for this allele (ie, mixed-affinity binders) express both high-affinity and low-affinity binding sites in similar proportions. Thus, individuals with the same TSPO density but different genotypes will produce different PET signals. This obstacle has been addressed by doing *TSPO* genotyping before imaging (to exclude low-affinity binders) and by including binding affinity as a statistical covariate.[15,16] One study that compared four ^{11}C-labelled TSPO radioligands (^{11}C-(R)-PK11195, ^{11}C-PBR28, ^{11}C-DPA-713, and ^{11}C-ER176) reported that ^{11}C-DPA-713 had the greatest signal-to-noise ratio in the human brain.[17] However, ^{11}C-ER176 also had a high signal-to-noise ratio, was least likely to generate metabolites that could penetrate the blood–brain barrier, and was sufficiently insensitive to the rs6971 polymorphism to allow reliable TSPO measurement in low-affinity binders.[17] Thus, evidence suggests that ^{11}C-ER176 is the best available TSPO radioligand. Although ^{18}F-GE180 has been described as a third-generation TSPO radioligand, this tracer has unfavourable kinetics for imaging the human brain because of low penetration into the brain from the vascular compartment;[18] thus, we do not advise use of this tracer.

Another potential obstacle for TSPO PET is the absence of a true reference region. TSPO is diffusely expressed throughout the brain, and accurate measurement of its density relies on kinetic modelling through use of the metabolite-corrected arterial input function. Different methods have attempted to circumvent the need for arterial sampling, including cluster sampling techniques[19] and use of so-called pseudoreference regions.[20,21] Methods that do not use the arterial input function inherently underestimate TSPO binding because the reference voxels are not completely devoid of specific TSPO signal. However, several such studies have noted increased TSPO density in various neurological disorders, with colocalisation to abnormalities in other biomarkers (eg, with neurofibrillary tau and loss of grey matter volume in Alzheimer's disease).[22,23] Additionally, methods that do not use arterial input function often reduce variance in TSPO-PET data by accounting for differences in physiological TSPO expression between participants, thus improving power to detect pathological increases in TSPO density.[21] Because binding behaviour can differ among different radioligands and diseases, we recommend validating pseudoreference methods against arterial sampling before their application for each radioligand. The diffuse nature of TSPO is less of an issue in focal disorders, such as stroke, where contralateral tissue of the same volume can be used for comparison. However,

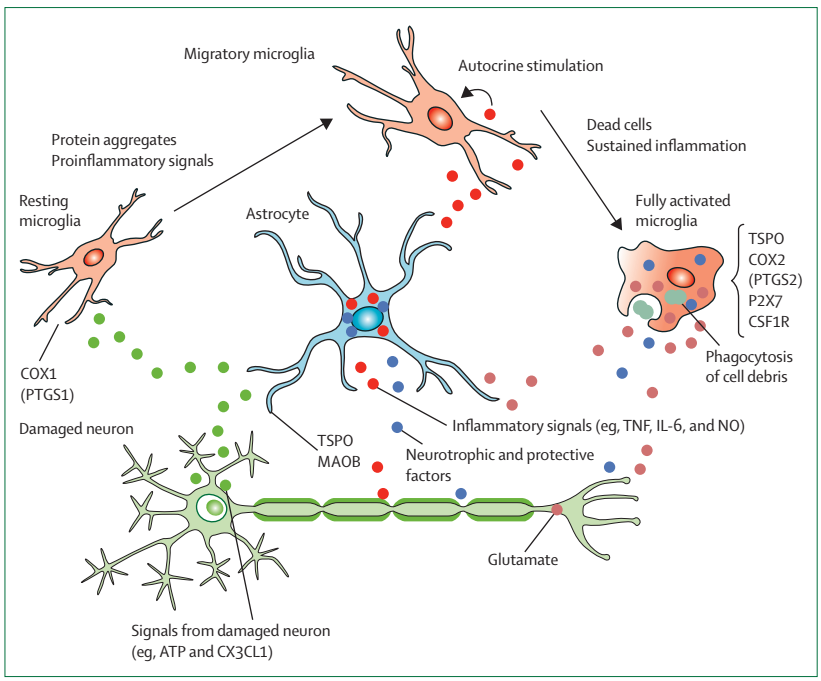

Figure 1: **Relationship between neurons, microglia, and astrocytes in neuroinflammation**
Microglia become activated in response to several immunological signals, including from cytokines and aggregated proteins. This activation might be either protective or toxic for the surrounding glial cells and neurons. Proteins expressed by glial cells are established and proposed targets for PET imaging to quantify neuroinflammation. Reproduced from Monk and Shaw,[27] by permission of Springer Nature. IL-6=interleukin-6. NO=nitric oxide.

Wallerian degeneration or diaschisis might affect regions that are distant to focal injury, potentially influencing PET-imaging results.[24]

Finally, although ^{11}C-ER176 has emerged as the preferred TSPO radioligand, TSPO binding appears to reflect a broad spectrum of immune responses; thus, more precise targeting of inflammatory mechanisms will require novel radioligands for novel biomarkers. The only non-TSPO radioligand in use for research is ^{11}C-deuterium-L-deprenyl, a radioligand for MAOB. However, although MAOB is expressed by astrocytes, it is also expressed by pyramidal neurons in the brains of patients with Alzheimer's disease,[25] and physiological signals in the basal ganglia—in which there is a high turnover of dopamine—might reduce the sensitivity of ^{11}C-deuterium-L-deprenyl to detect changes in this region.[26] Novel inflammatory targets include COX1 (PTGS1), COX2 (PTGS2), CSF1R, and P2X7 (figure 1). To date, no radioligand for any of these targets has been fully validated in human disease, although each shows promise for more precisely measuring neuroimmune response than does TSPO. Discussion of these emerging targets can be found in our companion Review in *The Lancet Psychiatry*.[28] The table summarises the neuroinflammatory radioligand studies done in humans in the past five years, noting the studies that used non-TSPO radioligands, arterial sampling, or autopsy tissue, as well as studies in which neuroinflammation PET has been incorporated into clinical trials.

PET imaging of neuroinflammation in patients with neurological disorders

Multiple sclerosis

Most PET studies aiming to quantify neuroinflammation in patients with multiple sclerosis have used TSPO as the PET target. Many studies have noted increased TSPO expression in white matter lesions identified with MRI in individuals with relapsing-remitting multiple sclerosis or secondary progressive multiple sclerosis.[36,73–75] The fact that white matter lesions, which are indistinguishable on MRI, have different patterns of binding on TSPO-PET imaging suggests that TSPO PET can detect pathophysiological heterogeneity to which MRI is insensitive.[36,73] As might be expected, the molecular changes detected by TSPO PET precede the structural changes detected by MRI.[75] In addition, non-lesional white matter in patients with multiple sclerosis also shows greater TSPO signal than in age-matched healthy controls.[36,76,77] Increased signal in non-lesional white matter is associated with greater brain atrophy and worse disability.[20,73,78] Higher amounts of signal in non-lesional white matter were also associated with the appearance of new lesions, worsening brain atrophy, and a more severe trajectory of disability worsening over the subsequent 12 months.[20,36] Increased signal in cortical grey matter has also been detected in individuals with multiple sclerosis and is associated with disability and cognitive impairment.[76,78] The effect of standard medications (including glatiramer acetate, fingolimod, and natalizumab) on TSPO signal has been assessed in a handful of studies, all of which have shown modest reductions in the signal in either lesional or non-lesional white matter.[33–35,79] Although previous neuropathology studies identified activated microglia as the source of TSPO signal in multiple sclerosis lesions,[8] a comprehensive post-mortem assessment of the brains of patients with multiple sclerosis showed that TSPO is not preferentially expressed on activated microglia but, rather, is expressed equally across all microglial phenotypes.[5] Furthermore, although microglia are the main contributors to TSPO signal in white matter lesions, a substantial contribution (approximately 25%) is made by astrocytes and, to a lesser extent, endothelial cells.[5] Notably, TSPO-PET studies in participants with multiple sclerosis relative to healthy controls identified much smaller differences than might be expected from pathological investigations. This finding could be due to the relatively small size of the lesions or could reflect differences in the configuration of TSPO between in-vitro and in-vivo states.

HIV-associated cognitive impairment

Cognitive impairment is prevalent among individuals with HIV, including people who use effective antiretroviral therapy.[39,80] Microglial activation and reactive astrocytosis are among the posited contributing factors to cognitive impairment in patients with HIV. One ^{11}C-(R)-PK11195-PET study noted higher binding in the frontal, temporal,

	Radioligands used for each disorder	Studies that used arterial sampling	Autopsy studies	Use in clinical trials*	Key findings
Multiple sclerosis	^{11}C-(R)-PK11195; ^{11}C-PBR28; ^{18}F-PBR-111; ^{18}F-GE-180; ^{18}F-DPA-714	^{18}F-GE-180;[29,30] ^{18}F-DPA-714;[31] ^{11}C-PBR28[32]	NA	^{11}C-(R)-PK11195[33-35]	TSPO binding decreased after disease-modifying treatment;[33-35] TSPO predicted new lesions[20,36]
HIV-associated cognitive impairment	^{11}C-(R)-PK11195; ^{11}C-PBR28; ^{11}C-DPA-713	^{11}C-PBR28;[37] ^{11}C-DPA-713[38]	NA	NA	Regional TSPO binding was associated with domain-specific cognitive impairment[37,39]
Alzheimer's disease	^{11}C-(R)-PK11195; ^{11}C-PBR28; ^{18}F-FEPPA; ^{11}C-DAA1106; ^{18}F-DPA-714; ^{11}C-DPA-713; ^{11}C-deuterium-L-deprenyl†	^{11}C-PBR28;[21,40-42] ^{18}F-FEPPA;[43-45] ^{18}F-DPA-714‡[46]	^{11}C-(R)-PK11195[47]	NA	MAOB binding was increased in presymptomatic Alzheimer's disease;[48] TSPO binding was increased in amyloid-positive controls;[23,49] TSPO binding correlated with tau pathology[22,23,50]
Frontotemporal dementia	^{11}C-(R)-PK11195; ^{11}C-PBR28	^{11}C-PBR28[51]	^{11}C-(R)-PK11195[52]	NA	Patterns of TSPO binding discriminated frontotemporal dementia subtypes[52]
Chronic traumatic encephalopathy or traumatic brain injury	^{11}C-PBR28; ^{11}C-DPA-713	^{11}C-PBR28;[53] ^{11}C-DPA-713[54,55]	NA	^{11}C-PBR28[53]	TSPO binding was increased in medial temporal cortex and supramarginal gyrus in active and former National Football League players[54,55]
Huntington's disease	^{11}C-(R)-PK11195; ^{11}C-PBR28	NA	NA	NA	TSPO binding was increased in presymptomatic mutation carriers[56]
Amyotrophic lateral sclerosis	^{11}C-PBR28; ^{18}F-DPA-714; ^{11}C-deuterium-L-deprenyl;* ^{11}C-JNJ717†	^{18}F-DPA-714;[57] ^{11}C-JNJ717;†[57] ^{11}C-PBR28[58]	^{18}F-DPA-714;[57] ^{11}C-JNJ717†[57]	^{11}C-PBR28[59]	TSPO binding was correlated with changes in white matter integrity and cortical atrophy[60-62]
Epilepsy	^{11}C-(R)-PK11195; ^{11}C-PBR28; ^{11}C-DPA-713	^{11}C-PBR28;[63,64] ^{11}C-DPA-713[63,64]	NA	NA	TSPO binding was increased ipsilateral to seizure foci in temporal lobe epilepsy[63,64]
Stroke§	^{11}C-(R)-PK11195	NA	NA	NA	TSPO binding increased around haematoma in two-fifths of patients with acute intracerebral haemorrhage[65]

NA=not applicable. *Jucaite and colleagues[66] noted reduced ^{11}C-PBR28 binding in individuals with Parkinson's disease after treatment with a novel MPO inhibitor; however, subsequent PET studies published after 2015 that used either ^{11}C-PBR28 or ^{18}F-FEPPA[67,68] did not show increased TSPO binding in individuals with Parkinson's disease.[69] †Non-TSPO radioligand; binds to MAOB, a proposed marker of astrocytosis. ‡Second-generation TSPO radioligand study, in which *TSPO* genotype correction was not done. §Studies published before 2015 that used second-generation TSPO radioligands showed positive results in participants with stroke.[70-72]

Table: **Studies that used neuroinflammatory radioligands in humans between 2015 and 2020 by disorder**

and occipital cortices, thalamus, and putmen in participants with HIV (including participants with cognitive impairment and participants without cognitive impairment) compared with uninfected controls, with the highest binding observed in participants with HIV-associated dementia.[81] A second ^{11}C-(R)-PK11195-PET study noted higher binding in participants with HIV who did not have cognitive impairment compared with uninfected controls.[82] A third study found no difference between individuals with HIV, whether they had cognitive impairment or not, and healthy controls.[83]

For studies that used the second-generation radiotracers ^{11}C-DPA-713[84] or ^{11}C-PBR28,[37] greater binding was noted in individuals with HIV who were virally suppressed compared with uninfected controls in parietal and occipital cortices, globus pallidus, and white matter. Participants with HIV-associated dementia had elevated ^{11}C-DPA-713 binding in the frontal cortex,[84] and subsequent analyses that used data from individuals with HIV showed inverse correlations between regional ^{11}C-DPA-713 binding and performance in particular cognitive domains.[38] In individuals with HIV, ^{11}C-PBR28 PET also showed region-specific (ie, hippocampus and thalamus) associations between higher binding and lower scores on memory and verbal learning tests.[37] Inconsistencies between these studies could stem from differences between radioligands, clinical characteristics within patient and control groups, and analytical methods. Nevertheless, taken together, the data suggest that TSPO might be regionally elevated and linked to domain-specific cognitive impairment in patients with treated HIV.

Alzheimer's disease

Both human and preclinical PET studies have linked neuroinflammation with Alzheimer's disease pathology. Most TSPO-PET studies have shown increased binding in the brains of participants with Alzheimer's disease compared with healthy controls, particularly in frontotemporal regions, with more modest increases observed in neocortical regions in the brains of individuals with mild cognitive impairment (see Bradburn and colleagues[85] for a meta-analysis). Presymptomatic carriers of autosomal dominant Alzheimer's disease mutations had increased binding, as assessed with ^{11}C-deuterium-L-deprenyl, a radioligand for MAOB.[48] Although these studies suggest astrocytosis as a pathological entity in early stage Alzheimer's disease, MAOB is also expressed by pyramidal neurons in the brains of people with Alzheimer's disease.[25] Studies of transgenic mouse models of Alzheimer's disease identified increased TSPO and MAOB binding on PET imaging, often confirmed with autoradiography.[86,87]

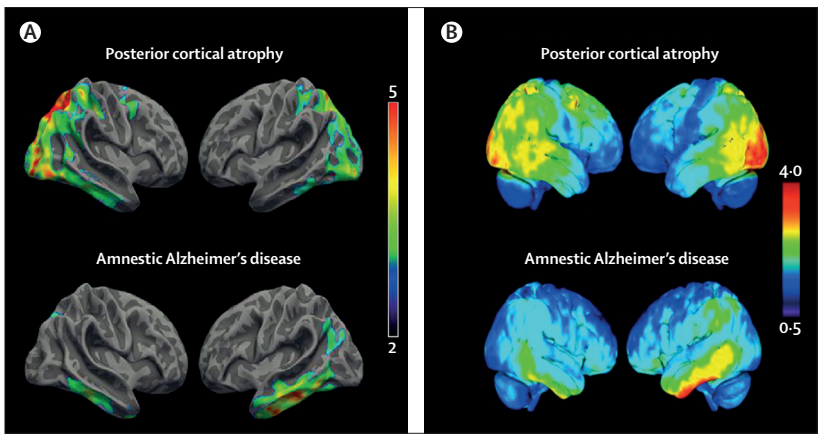

Figure 2: **TSPO and tau imaging: Alzheimer's disease versus posterior cortical atrophy**
Topographical distribution of TSPO binding resembles that of tau pathology in different clinical subtypes of Alzheimer's disease. Colour scale represents standardised uptake value ratio values. (A) Surface-based projection maps showing differences in ^{11}C-PBR28 binding (measured as standardised uptake value ratio, cerebellar reference) between individuals with Alzheimer's disease and age-matched controls, for posterior cortical atrophy (a visual variant of Alzheimer's disease) and typical amnestic presentation of Alzheimer's disease. Contrast threshold is p<0·05 after family-wise correction for multiple comparisons and with *TSPO* genotype, age, and education as covariates. Reproduced from Kreisl et al.[40] (B) Single-participant PET standardised uptake value ratio images from a separate study, in which ^{18}F-AV-1451 was used to label neurofibrillary tau deposits. Representative participants with posterior cortical atrophy or amnestic Alzheimer's disease are shown. Reproduced from Ossenkoppele et al,[94] by permission of Oxford University Press.

The exact pathological stimulus for increased TSPO binding in the brains of patients with Alzheimer's disease is unclear. Studies have observed increased TSPO binding in asymptomatic individuals with incidental amyloid positivity[23,49] and in participants meeting clinical criteria for amnestic mild cognitive impairment or mild Alzheimer's disease with absence of amyloid binding on PET.[23,88] These increases suggest that TSPO binding might increase in response to amyloid deposition and amyloid-independent neurodegeneration. Multimodal PET studies have looked for spatial correlations between TSPO binding and both amyloid plaque and neurofibrillary tau burden. Studies comparing TSPO and amyloid binding have been inconsistent, showing no correlation,[15,89] positive correlations,[49,50,90,91] and negative correlations.[92] Nevertheless, three of four studies reported positive correlations between TSPO and tau binding.[22,23,50,93] One study identified distinct patterns of TSPO binding in patients with different clinical variants of Alzheimer's disease,[40] similar to previously reported patterns of tau pathology (figure 2).[94] Notably, both TSPO and tau binding were greater in participants with early onset Alzheimer's disease (ie, symptoms began before age 65 years) compared with participants with late onset Alzheimer's disease (ie, symptoms began after age 65 years).[95,96] Therefore, TSPO might have a stronger relationship with tau than with amyloid, at least during the clinical stages of Alzheimer's disease.

Whether increased TSPO binding in the early stages of Alzheimer's disease represents a beneficial or maladaptive glial response is controversial. Cross-sectional studies in people with Alzheimer's disease reported that TSPO binding is associated with worse cognitive impairment.[15,43] Additionally, the first longitudinal TSPO-PET studies in patients with Alzheimer's disease showed overall increased binding as the disease advanced.[95,97] However, amyloid-positive individuals with mild cognitive impairment had greater ^{18}F-DPA-714 binding than individuals with Alzheimer's disease, and greater ^{18}F-DPA-714 binding was associated with a higher Mini-Mental State Exam score.[49] Another study noted that six of eight individuals with mild cognitive impairment (four of whom were amyloid-negative) showed a mean decrease in ^{11}C-(R)-PK11195 binding at follow-up.[98] The authors interpreted these results as evidence for a bimodal pattern of neuroimmune activation in patients with Alzheimer's disease, with a beneficial phase of glial behaviour occurring before dementia onset, followed by a detrimental phase of proinflammatory glial activity that worsened throughout the dementia stage.[99] In another study, ^{11}C-deuterium-L-deprenyl binding decreased over time in individuals with autosomal dominant Alzheimer's disease, although no change was seen in individuals with sporadic mild cognitive impairment.[100] That greater ^{11}C-PBR28 binding was associated with less cortical atrophy on MRI among participants with mild cognitive impairment supports the notion that TSPO-expressing microglia might have a beneficial effect early in Alzheimer's disease.[101] However, the results from this study could be interpreted another way, given that cortical volume is a marker of so-called brain reserve—the ability to retain cognitive function despite increasing pathology.[102] In that context, participants with mild cognitive impairment who have less atrophy could be more resilient to the damaging effects of microglial or astrocyte activation, or both, allowing a similar degree of cognitive impairment as people with more atrophy despite greater amounts of TSPO binding. Alternatively, inflammation-induced neuronal and glial swelling could result in increased cell volume, as posited by one ^{11}C-deuterium-L-deprenyl study showing that MAOB binding was associated with greater cortical thickness.[103] The bimodal hypothesis of TSPO binding in Alzheimer's disease progression has not been consistently supported in the literature. In particular, ^{18}F-DPA-714 binding was shown to increase in participants with mild cognitive impairment and in participants with Alzheimer's disease over time,[90] and several studies have observed a more or less linear increase in ^{11}C-PBR28 binding across the clinical continuum of Alzheimer's disease.[15,21,23]

Frontotemporal dementia

PET studies have consistently observed increased TSPO binding in individuals with clinically diagnosed frontotemporal dementia. The first study, which used a reference region method, reported that five participants with frontotemporal dementia showed a mean average increase in ^{11}C-(R)-PK11195 binding in the left dorsolateral prefrontal cortex, right hippocampus, right parahippocampus, and

bilateral putamen compared with eight healthy controls.[104] An ^{11}C-PBR28 study that used arterial sampling noted increased binding in four individuals with frontotemporal dementia and further reported the absence of comorbid Alzheimer's disease pathology with amyloid PET (figure 3).[51] Although participants did not have a genetic or neuropathological determination of underlying histopathology, the pattern of ^{11}C-PBR28 binding mirrored that of fluorodeoxyglucose hypometabolism. In both studies, the patients had varied clinical presentations and patterns of atrophy on brain MRI.

In a larger study, the topographic pattern of ^{11}C-(R)-PK11195 binding discriminated frontotemporal dementia subtypes from each other and from healthy controls.[52] This study also reported that, in autopsy tissue, the density of microglia, particularly in patients with activated morphology, correlated with the extent of abnormal protein aggregation (ie, phosphorylated tau or TDP43).

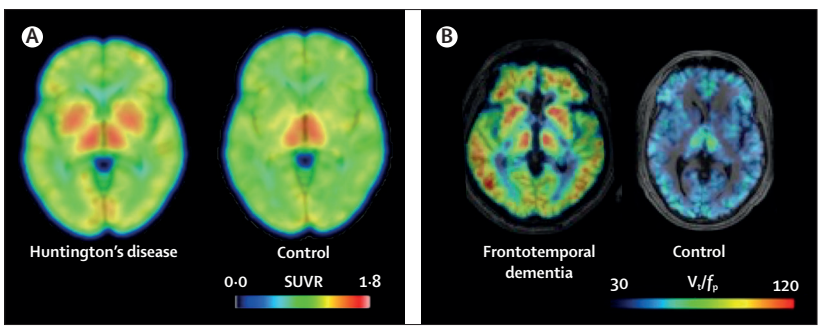

Figure 3: **TSPO imaging in Huntington's disease and frontotemporal dementia**
(A) Averaged ^{11}C-PBR28-PET images from seven individuals with Huntington's disease and three healthy controls. PET images represent SUVR (normalised to whole brain activity) by use of images acquired 60–90 min post-injection. Increased binding in bilateral basal ganglia was noted in the participants with Huntington's disease. (B) Representative ^{11}C-PBR28-PET images from an individual with frontotemporal dementia and an age-matched healthy control, both high-affinity binders. Images represent total distribution volume, corrected for V_T/f_P. Increased binding was most notable in frontal and temporal lobes. Reproduced from Kim et al,[51] and Lois et al,[105] by permission of American Chemical Society. SUVR=standardised uptake value ratio. V_t/f_p=free fraction of radioligand in the plasma.

Chronic traumatic encephalopathy

Chronic traumatic encephalopathy is pathologically defined by deposits of phosphorylated tau in a perivascular distribution, particularly in the depths of cortical sulci.[106] Chronic traumatic encephalopathy has been noted in the human brain following traumatic brain injury, including sports-related, repetitive concussion incurred through playing American football.[106,107] Prolonged microglial activation after repeated traumatic brain injury has been hypothesised to contribute to chronic traumatic encephalopathy.[108] Indeed, increased ^{11}C-(R)-PK11195 or ^{11}C-PBR28 binding has been reported in the brains of individuals with traumatic brain injury,[109,53] even years after injury.[110] Additionally, higher ^{11}C-DPA-713 binding was reported in young National Football League players in the medial temporal cortex and supramarginal gyrus (figure 4).[54] High levels of ^{11}C-DPA-713 binding were also observed in the supermarginal gyrus in a study of older players (ie, aged 57–74 years) decades after the last time that they played in the National Football League.[55] Whole brain images included in each ^{11}C-DPA-713-PET study suggested widespread distribution of high TSPO expression throughout the brain.[54,55]

Notably, however, the clinical implications of high TSPO signals in former National Football League players are elusive. No cognitive deficits were reported in the cross-sectional population of active and former National Football League players with high ^{11}C-DPA-713 binding.[54] Longitudinal investigation is needed to elucidate the relationship between neuroimmune activation marked by high TSPO expression and behavioural decline associated with traumatic brain injury.

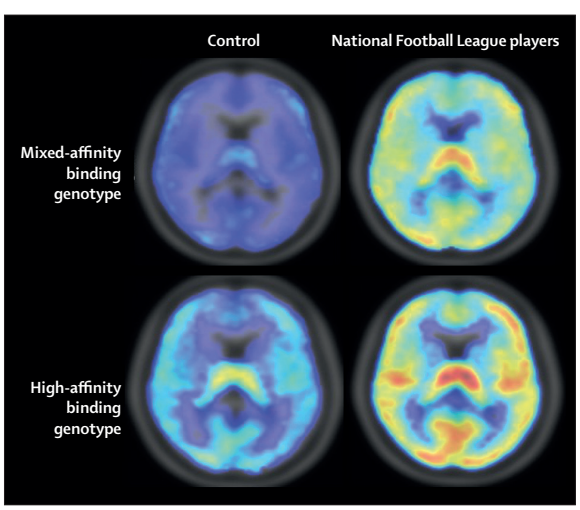

Figure 4: **TSPO imaging of patients with chronic traumatic encephalopathy**
Comparative mean ^{11}C-DPA-713 binding (total distribution volume) is displayed for individuals with the mixed-affinity binding genotype (six healthy controls, five National Football League players) and those with the high-affinity binding genotype (five healthy controls, seven National Football League players). Compared with healthy controls, matched for age, sex, race, body-mass index, years of education, and *TSPO* genotype, binding of ^{11}C-DPA-713 in grey matter was 53% higher in the brains of former National Football League players with the mixed-affinity binding genotype and 34% higher in players with the high-affinity binding genotype. Reproduced from Coughlin et al, by permission of JAMA Neurology.[54]

Huntington's disease

The neurodegenerative disorder Huntington's disease is associated with increased gliosis and expression of GFAP and complement proteins in the brain, particularly in the striatum.[111] Increased proinflammatory cytokines are found in carriers of the mutated *HTT* gene, which causes Huntington's disease.[112]

Early PET studies showed that asymptomatic gene carriers and individuals with Huntington's disease had increased ^{11}C-(R)-PK11195 binding.[113,114] In another study, premanifest carriers of the mutation for Huntington's disease had greater ^{11}C-(R)-PK11195 binding in cortical, basal ganglia, and thalamic brain regions;[56] radioligand binding in the somatosensory cortex also correlated with plasma concentrations of interleukin-1β (IL-1β), IL-6, IL-8, and TNF. Second-generation TSPO radioligands have

shown similar results. For instance, one study reported that participants with Huntington's disease had greater ^{11}C-PBR28 binding in the putamen and pallidum than healthy controls (figure 3);[105] however, arterial sampling was not done, and only ^{11}C-PBR28 binding relative to the whole brain was reported for each region. Although a similar approach has been used in ^{11}C-PBR28 studies of chronic pain[115] and amyotrophic lateral sclerosis,[116] this method has not been validated against a gold-standard kinetic modelling approach.

Amyotrophic lateral sclerosis

An ^{11}C-(R)-PK11195 study first identified increased TSPO binding in the motor cortex and associated brain regions of individuals with amyotrophic lateral sclerosis,[117] and the finding has since been reproduced with second-generation TSPO radioligands. Interestingly, increased TSPO binding in the motor cortex was associated with the severity of upper motor neuron symptoms with both ^{11}C-(R)-PK11195 and ^{11}C-PBR28.[116,117] Furthermore, large cohort studies of individuals with either amyotrophic lateral sclerosis or primary lateral sclerosis reported that increased ^{11}C-PBR28 binding in the motor cortex was also associated with other MRI parameters, such as diffusion tensor imaging and cortical thickness measured both cross-sectionally and longitudinally.[60–62] ^{18}F-DPA714 studies also noted increased TSPO binding in the motor cortex, although these studies did not evaluate correlation with clinical severity.[118]

As an extension of these findings, a therapeutic trial with ^{11}C-PBR28 PET as a biomarker of neuroinflammation was done in individuals with amyotrophic lateral sclerosis, but the results identified no difference between TSPO uptake before and after treatment, possibly because of low statistical power.[59] With regard to radioligands targeting other neuroinflammatory mediators than TSPO, a preliminary result with ^{11}C-JNJ717, a P2X7 radioligand, showed no such increase in individuals with amyotrophic lateral sclerosis.[57]

Epilepsy

Several PET studies targeting TSPO support the role of neuroinflammation in epileptogenesis (ie, the mechanism that causes brain changes that lead to epilepsy) or ictogenesis (ie, the mechanism that causes an individual seizure), although most of these studies were based on small sample sizes. Initial case reports with ^{11}C-(R)-PK11195-PET imaging showed a focal increase of TSPO uptake colocalised with the seizure focus;[119,120] a subsequent study showed that this uptake had even greater intensity and spatial extent in postseizure status (approximately 36 h after seizure) compared with a seizure-free period for 1 month, perhaps because of transient seizure-induced inflammation.[121]

In larger studies done with second-generation TSPO radioligands, ^{11}C-PBR28 uptake was noted to be higher ipsilaterally to the seizure focus in 16 participants with unilateral temporal lobe epilepsy.[122] When full quantification of TSPO binding was used, results in the same group showed that ^{11}C-PBR28 binding was higher in participants with temporal lobe epilepsy than in healthy controls for all ipsilateral and some contralateral temporal regions.[63] When the same evaluation was done in participants with neocortical seizure foci, nine of 11 participants had asymmetric binding patterns, with signal ipsilateral to the seizure foci greater than that contralateral to the seizure foci, although the absolute binding levels in the patients did not differ from those in healthy controls.[64] PET imaging of neuroinflammation in epilepsy has thus far investigated pathophysiology without correlating it with clinical severity or prognosis, suggesting that additional studies with full quantitative methods are needed before clinical application.

Stroke

Studies that used ^{11}C-(R)-PK11195 in patients with acute ischaemic stroke observed widespread TSPO binding at the primary infarct site and in the peri-infarct lesions.[123] During the chronic phase, increased TSPO binding also involved sites distant from the primary stroke lesion, perhaps as a result of Wallerian degeneration of neuronal tracts.[123,124] One case report noted increased binding in a subacute lacunar infarction as assessed via ^{11}C-PBR28.[70] Another study that used ^{11}C-vinpocetine, a radioligand that binds with moderate affinity to TSPO but has favourable penetration of the blood–brain barrier, also found increased TSPO binding in the peristroke region for several weeks after ischaemic stroke.[71] An ^{18}F-DPA714 study in nine individuals 8–18 days after ischaemic stroke reported colocalised uptake within areas of ischaemic infarction and extension beyond the region corresponding to damage to the blood–brain barrier.[72]

In contrast to such well reproduced findings in acute or subacute ischaemic stroke, haemorrhagic stroke has rarely been investigated with PET imaging of neuroinflammation. One ^{11}C-(R)-PK11195 study reported low uptake in haematomas, with two of five participants showing widespread increases in TSPO binding in the perihaematomal region compared with the contralateral hemisphere.[65] However, it is unknown whether these peri-lesional or distant increases of TSPO binding are associated with any favourable or unfavourable clinical outcomes.

Neuroinflammation PET in drug development

Although TSPO PET does not have an established clinical application, evidence supports its potential use in drug development. First, TSPO density might predict treatment response in some instances. For example, greater TSPO binding in individuals with major depressive episodes was associated with greater reduction of symptoms after treatment with celecoxib,[125] suggesting that TSPO PET could play a potential role in participant stratification, similar to how amyloid PET is used to select participants for Alzheimer's disease trials. Second, TSPO has served as

a surrogate biomarker in proof of concept studies, although some results have been difficult to interpret. For instance, although minocycline treatment led to reduced ^{11}C-PBR28 binding in individuals with brain trauma, it also increased plasma concentrations of neurofilament light chain (NFL), raising the question of whether reduced TSPO signal is necessarily beneficial.[53] In another example, after treatment with a myeloperoxidase inhibitor, individuals with Parkinson's disease showed mean reductions in ^{11}C-PBR28 binding in nigrostriatal regions, with a 13·2–15·7% decrease in distribution volume from baseline.[66] However, similar decreases were identified in all other measured brain regions, indicating a global effect on TSPO binding, and raising the question of whether the treatment resulted in selective reduction of activated microglia (or astrocytes), diffuse reduction in TSPO expression, or depletion of glial cells in the brain. Notably, despite three well designed studies that used ^{11}C-PBR28 or ^{18}F-FEPPA, no second-generation TSPO study has shown increased binding in individuals with Parkinson's disease.[67–69] Conversely, the same myeloperoxidase inhibitor did not reduce ^{11}C-PBR28 binding in individuals with multiple system atrophy (NCT02388295). Phase 3 studies are needed to establish whether these changes in TSPO binding equate to clinical efficacy. Emerging non-TSPO biomarkers are also expected to be useful in evaluating novel treatments, particularly treatments that target the same protein as the radioligand. For example, CSF1R radioligands could be used for target engagement studies of CSF1R antagonists.

Conclusions and future directions

Although neuroinflammatory PET has largely been limited to targeting TSPO, this imaging modality has far-reaching potential. Emerging non-TSPO radioligands might soon allow more precise investigation of the mechanisms underlying specific immune response; however, for the time being, TSPO PET is the most extensively studied method for spatial measurement of neuroinflammation.

In neurology, the most consistent TSPO imaging results have arguably been in patients with Alzheimer's disease, in whom neuroinflammation might more closely reflect the distribution of tau than of amyloid and have a meaningful (although not necessarily linear) relationship to disease progression, and in patients with chronic traumatic encephalopathy, where five of five studies of traumatic brain injury noted elevated TSPO levels.[53–55,109,110] Yet, discrepancies exist in the TSPO literature, the most striking of which is in patients with multiple sclerosis, in whom the in-vivo TSPO signal is much lower than expected given the prominent in-vitro TSPO signal. This discrepancy and other in-vitro or in-vivo discrepancies for TSPO could reflect disruption of the in-vivo multimeric complex during tissue preparation, as occurs for other multimeric complexes. This phenomenon could be present in other disorders and could explain the subtle genotype effect on in-vivo binding to peripheral organs with ^{11}C-(R)-PK11195 and ^{11}C-ER176, despite these two radioligands having similar in-vitro affinity between high-affinity and low-affinity binders.[126,127]

As previously noted, TSPO PET has the potential for drug development in selected instances. For instance, TSPO binding changes over time in patients with Alzheimer's disease, is modifiable by minocycline and myeloperoxidase inhibition, predicts response to COX inhibition during major depressive episodes, and is increased in presymptomatic carriers of the gene mutation for Huntington's disease. Thus, this imaging modality might have important clinical applications in establishing which individuals are most likely to respond to novel drugs and which stage of disease is optimal for treatment.

Overall, our view is that this is a promising time for neuroinflammation PET, in that improved TSPO radioligands can be used as broad markers of immune response. Depending on their success, emerging biomarkers might allow more precise targeting of specific proteins involved in immune response; these could be used alone or in combination to delineate the mechanisms underlying neurological disease. Finally, neuroinflammation PET might be most useful in the context of clinical trials, in terms of both predicting and monitoring response to treatment in early drug discovery.

Search strategy and selection criteria

References for this Review were identified by searching PubMed between Jan 1, 2015, and March 15, 2020, and by further examining the reference lists from relevant articles. Combinations of the following search terms were used: "PET", "TSPO", "translocator protein", "peripheral benzodiazepine receptor", "inflammation PET", "microglia PET", "Alzheimer's disease", "progressive supranuclear palsy", "corticobasal degeneration", "dementia", "Huntington's disease", "multiple sclerosis", "TBI", "CTE", "HIV", "HAND", "epilepsy", "stroke", "Parkinson's disease", "dementia with Lewy bodies," and "amyotrophic lateral sclerosis". There were no language restrictions. We limited disorders to those for which at least one study that used a second-generation TSPO radioligand discriminated patients from healthy controls or showed an association with another disease-related outcome. The final reference list was generated on the basis of the relevance to the theme of this Review.

Contributors

All authors contributed equally to the literature search, generation of figures, writing, and revision of this manuscript. All authors approved the final version of the paper.

Declaration of interests

We declare no competing interests.

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