

The methodology of TSP0 imaging with positron emission tomography

Federico E. Turkheimer^{*1}, Gaia Rizzo[†], Peter S. Bloomfield[‡], Oliver Howes^{*§}, Paolo Zanotti-Fregonara^{||},
Alessandra Bertoldo[†] and Mattia Veronese^{*}

^{*}Institute of Psychiatry, King's College London, London SE5 8AF, U.K.

[†]Information Engineering Dept., University of Padova, Padova 35131, Italy

[‡]Institute of Clinical Sciences, Imperial College London, London W12 0NN, U.K.

[§]MRC Institute of Clinical Sciences, Imperial College London, U.K.

^{||}University of Bordeaux, CNRS, INCIA, UMR 5287, France

Abstract

The 18-kDA translocator protein (TSP0) is consistently elevated in activated microglia of the central nervous system (CNS) in response to a variety of insults as well as neurodegenerative and psychiatric conditions. It is therefore a target of interest for molecular strategies aimed at imaging neuroinflammation *in vivo*. For more than 20 years, positron emission tomography (PET) has allowed the imaging of TSP0 density in brain using [¹¹C]-(*R*)-PK11195, a radiolabelled-specific antagonist of the TSP0 that has demonstrated microglial activation in a large number pathological cohorts. The significant clinical interest in brain immunity as a primary or comorbid factor in illness has sparked great interest in the TSP0 as a biomarker and a surprising number of second generation TSP0 radiotracers have been developed aimed at improving the quality of TSP0 imaging through novel radioligands with higher affinity. However, such major investment has not yet resulted in the expected improvement in image quality. We here review the main methodological aspects of TSP0 PET imaging with particular attention to TSP0 genetics, cellular heterogeneity of TSP0 in brain tissue and TSP0 distribution in blood and plasma that need to be considered in the quantification of PET data to avoid spurious results as well as ineffective development and use of these radiotracers.

Introduction

Positron emission tomography (PET) is an imaging technology that assays the distribution of ligands labelled with positron emitters (e.g., ¹⁸F, ¹¹C, ¹⁵O) *in vivo*, by measuring the emitting annihilation photons with a ring of detectors. It is a functional imaging technology that best suits the three principles of tracer measurements because PET instrumentation (a) allows absolute quantification, (b) PET labels do not modify tracer properties and (c) PET ligands are in trace concentrations. These properties stem from the unique ability of the technology to use high energy radiation emitted from the nucleus as opposed to lower energy modalities, such as computed tomography, where X-rays are emitted by the orbiting electrons. PET labels radiate positrons, the anti-matter counterpart of the electron that annihilate with slowly moving electrons emitting two γ -rays at an 180° angle that are detected by the ring of detectors.

Figure 1 illustrates a typical dynamic PET acquisition where, after injection, radioactivity in blood and in the organ

of interest is monitored throughout the experiment generally lasting an hour or more. Once the study is completed, the coincident detections of the γ -rays are reconstructed into a set of time frames that measure the distribution of the radioligand during the acquisition. At the same time, the blood data are corrected for radioactive impurities (radioactive metabolites, radioactivity in blood cells and platelets, plasma protein bound fractions) to obtain the concentration of the free tracer in plasma. The availability of absolute measures of the ligand in tissue and plasma, the latter being a very good approximation of the concentration in the capillaries, allows the calculation of functional measures such as blood flow, enzymatic rates and protein densities via a kinetic model.

It is important to underline that the use of a kinetic model that describes with some degree of fidelity the underlying biology of the tissue is key to obtain meaningful parameters. At the same time, both tissue and plasma concentrations need to be measured accurately; this is occasionally impeded by limitations of the instrumentation. For example, the protein free plasma fraction of certain PET tracers is very low (<5%) and standard laboratory instrumentation lacks the necessary sensitivity to measure this fraction accurately. This may not be an issue as long as the free fraction is constant through the experimental setting and the correction can be disregarded. However, it becomes a serious matter when the free fraction of the ligand in plasma is subject to change in pathological conditions.

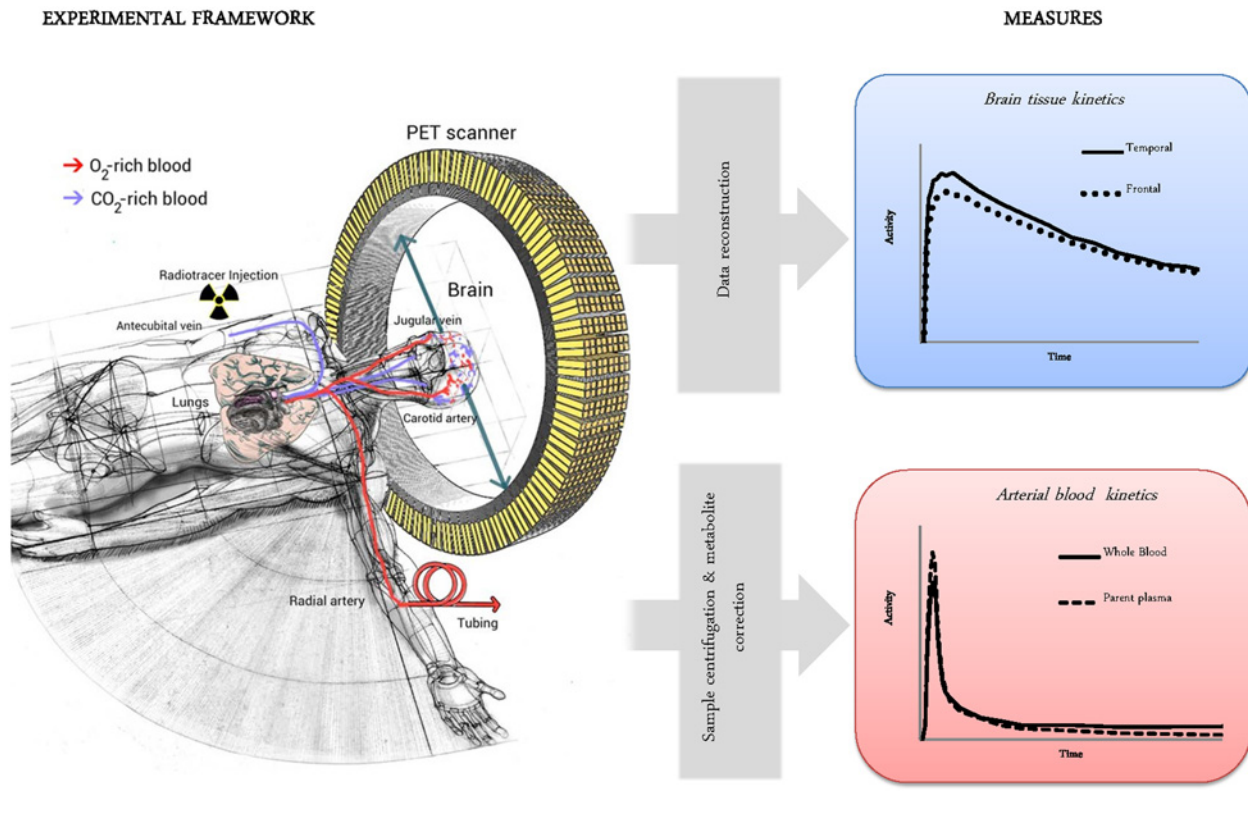
Key words: endothelium, genetics, heterogeneity, kinetic modelling, methodology, microglia, neuroinflammation, peripheral benzodiazepine receptor (PBR), PK11195, peripheral benzodiazepine receptor (PBR)28, plasma free fraction, positron emission tomography, schizophrenia, translocator protein (TSP0).

Abbreviations: BBB, blood-brain barrier; CNS, central nervous system; HAB, high-affinity binder; LAB, low-affinity binder; MAB, mixed-affinity binder; PBR, peripheral benzodiazepine receptor; PET, positron emission tomography; TAC, time-activity curve; TSP0, translocator protein; UHR, ultra-high risk.

¹ To whom correspondence should be addressed (email federico.turkheimer@kcl.ac.uk).

Figure 1 | Schematic representation of a standard dynamic PET study

The PET instrumentation allows the quantification of the concentration of the radiotracer in the organ of interest whereas additional instrumentation is used to quantify radioactive concentrations in blood. The latter is then corrected for those tracer fractions bound to red cells, platelets, plasma proteins etc., to recover the free tracer concentration in plasma. The plasma used as input function for the mathematical model that is applied to the data to obtain the parameters of interest. Kinetics curves refer to a representative [^{11}C]-PBR28 PET study in healthy HAB.



The use of PET for the quantification of translocator protein (TSPO) density in brain tissue poses a number of the challenges described above. Here, we briefly review the background of TSPO imaging and the past and present problems in quantification that are specific to this protein in brain imaging. We focus on three major determinants: (1) polymorphisms of the TSPO gene, (2) complex tracer kinetics due to heterogeneity of TSPO distribution in brain tissue and (3) variability of plasma free fractions across human clinical cohorts. We look at the impact of these factors in practice by referring to an exemplary dataset acquired in patients with schizophrenia and matched normal controls.

PET imaging of the TSPO with [^{11}C]-PK11195

In the past 20 years or so, PET imaging of the TSPO [at the time still called the peripheral benzodiazepine receptor (PBR)] has utilized the antagonist ligand PK11195, an isoquinoline-carboxamide derivative with nanomolar affinity

(9.3 nmol). After an initial application to the imaging of the TSPO in the heart [1], PK11195, labelled with ^{11}C , was used to target the TSPO in activated microglia and infiltrating macrophages in the central nervous system (CNS) [2]. Since then [^{11}C]-(*R*)-PK11195 has been the radiotracer of choice to image neuroinflammation *in vivo* which has been demonstrated in stroke [3], neurodegeneration [4–12], traumatic brain injury [13] and neoplasia [14,15]. Please see [16] for a review. The good sensitivity of [^{11}C]-(*R*)-PK11195 allows its use in clinical trials for pharmacological agents acting on microglia such as minocycline. This broad-spectrum tetracycline antibiotic is commonly used in the treatment of acne whereas it also demonstrates an interesting profile for use as neuroprotective agent given its lipophilicity, penetration into the brain and action over microglia [17,18].

The quantification of [^{11}C]-(*R*)-PK11195 data have however presented a number of challenges. The first challenge stems from the affinity of PK11195 to a number of binding sites in blood, e.g. platelets, monocytes and plasma proteins. Platelets and monocytes have significant TSPO density and this is altered in disease with consistent reductions observed

in anxiety disorders and aggression [19–23]. The fraction of a radioligand bound to blood cells is usually stable at equilibrium but not necessarily so at capillaries where exchanges with tissue take place. The correction of the arterial radioactivity by separation of blood cells from plasma by spinning the samples can be a good approximation only if the bound fraction is small compared with the free fraction in plasma or if the binding does not change across subjects. However, this is problematic for PK11195 because of its poor free fraction in plasma (~1%–5% that, incidentally, does not allow accurate measurements either); to complicate matters further PK11195 also binds to plasma proteins that may be up-regulated in the course of peripheral inflammatory events that sometimes are a cofactor in brain disorders [24].

If the quantification of TSPO binding cannot be pursued using plasma concentrations, the alternative option is to use as input function the time-activity curve (TAC) of a reference region in the brain, a tissue with perfusion characteristics similar to the ones of the target tissue but lacking the specific target of the ligand. In the case of the TSPO, this is non-trivial for two reasons.

The first reason is that the localization of microglial activation and ensuing abnormal TSPO density is generally unknown so one cannot select an anatomical region of reference *a-priori*. As an alternative a pseudo reference region can be identified by modelling the TAC of the tracer in each voxel of the brain as a linear combination of kinetics of pre-defined classes; usually four classes are used, normal grey and white matter, vascular and inflamed tissue. The estimated coefficients of the normal grey matter component are then used to calculate a reference region TAC as the weighted average of all voxels' contributions [25,26].

The second challenge stems from the ubiquity of TSPO in the normal brain and in the blood-brain barrier (BBB) in particular [25]. In the case of [¹¹C]-(*R*)-PK11195 the BBB binding generates a homogeneous background signal of low intensity that does not hamper accurate quantification using reference region approaches [25,27]. However the density of TSPO at the BBB is disturbed in neurodegenerative conditions because of vascular fibrosis and needs to be corrected for. In this instance, the vascular TAC must be first identified in the image using the first minutes of the PET acquisition when most of the radioactivity injected is still in circulation. The vascular TAC, that contains both radiotracer in blood and bound to the BBB, is then incorporated into the reference TAC (usually fixed at a standard value, 5%–7%) and ultimately estimated as a variable in the target tissue [28]. This step brings further robustness to the quantification procedure and generates stable and reproducible values for the TSPO-specific signal [26].

PET imaging of the TSPO with second generation radioligands

The increasing amount of evidence demonstrating neuroinflammation as an important factor in disease and the ensuing

interest in biomarkers of CNS immune system has spurred unprecedented activity in the development of novel TSPO ligands; novel series of phenoxyphenyl acetamide derivatives (i.e., PBR06, DAA1106) or bicyclic linker derivatives (i.e., PBR111, DPA713) with high affinity for the TSPO have been radiolabelled for use in PET with the aim of improving the signal-to-noise properties of [¹¹C]-(*R*)-PK11195 [29]. Unfortunately, the promise of improved image quality has not been delivered. The reasons may be ascribed to the three main factors depicted in Figure 2.

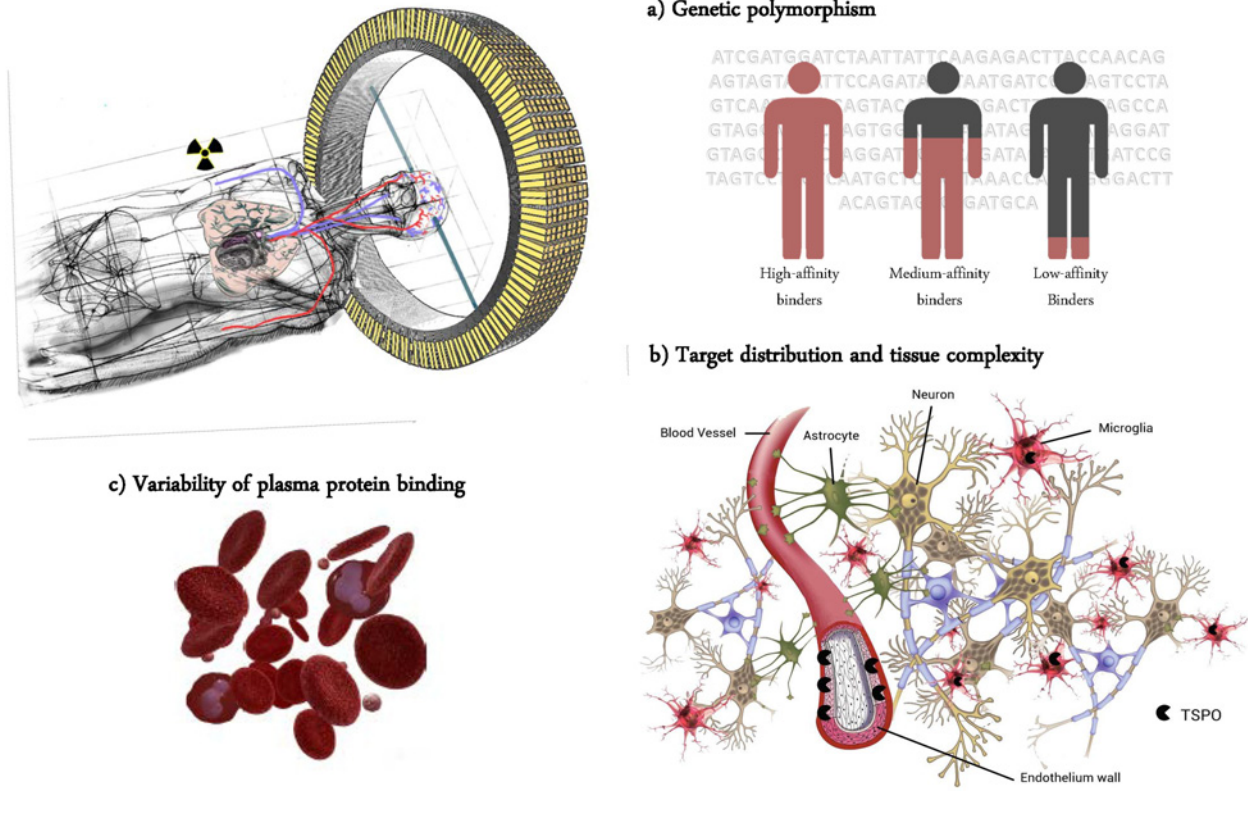
The first factor is genetics. A nucleotide polymorphism in the TSPO gene (rs6971) leads to an amino-acid substitution (A¹⁴⁷T) inducing a variation in binding affinity for second generation TSPO tracers at a population level [30]. This co-dominant monogenic trait defines a class of individuals characterized by high tracer-affinity binding for a single TSPO binding site (2 nmol for PBR28) and this class comprises ~66% of the Caucasian population [e.g., high-affinity binders (HABs)]. Five percent of the same population is characterized by a single low-affinity binding site (180 nmol for PBR28) and they are defined as low-affinity binders (LABs). The remaining 29% or so are characterized by a combination of one high-affinity and one low-affinity site; hence, they are labelled as MABs (mixed-affinity binders). All new classes of TSPO ligands display a dependency on the rs6871 genotype; however, in PET studies, the differences in binding across HAB/MAB/LAB classes vary depending on ligand affinity with the difference being the largest for PBR28, the tracer with highest affinity. For PBR28 the difference in affinity translates in a 2:1 TSPO signal in PET images in HABs compared with the MABs so that genetic stratification of the subjects to be included in the studies is required.

The second factor is that the very high affinity of some of the novel ligands disproportionately increases the signal from the TSPO on the BBB compared with that of the tissue [31]. This paradoxical result stems from the far greater concentration of the free ligand near the BBB compared with the remaining ligand that, after entering the brain and exchanging with a number of non-specific sites (e.g. cellular membranes in particular that attract lipophilic ligands) manages to reach microglia and enter the cell to bind to the TSPO in the mitochondria. Hence, the apparent affinity, that is the affinity of the ligand for the target multiplied by the concentration of the ligand at the site [32], is dramatically higher at the BBB compared with the tissue and the signal from the latter is obscured and needs an appropriate kinetic model to be correctly identified [31].

The third factor is obviously the difficulty in obtaining accurate estimates of free plasma concentrations that were described in the previous section. Accurate free plasma concentrations are critical because the use of tissue reference approaches is no longer possible or at least quite difficult, because the now large and ubiquitous TSPO component bound at the BBB masks the various brain tissues (e.g., grey matter, white matter and inflamed tissue as well) hampering the identification of a suitable reference region.

Figure 2 | Illustration of the three main factors affecting quantification of PET TSPO ligands in brain

(a) the A^{147T} genotype, (b) the presence of abundant TSPO on the endothelium besides activated microglia and macrophages in brain tissue (Reproduced with permission from [31]: Rizzo, G., Veronese, M., Tonietto, M., Zanotti-Fregonara, P., Turkheimer, F.E. and Bertoldo, A. (2014) Kinetic modeling without accounting for the vascular component impairs the quantification of [(11)C]PBR28 brain PET data. *J. Cereb. Blood Flow Metab.* **34**, 1060–1069 and (c) variability across human cohorts of plasma bound fractions for the tracers.



In the next section, we demonstrate these issues with an exemplary application of a second generation TSPO tracer to a clinical study in a psychiatric cohort.

Methodological issues of [¹¹C]-PBR28 in schizophrenia

Several studies provide evidence that impaired regulation of microglia has significant impact on already existing neurobehavioural deficits or that dysregulation of microglia induces motivational and neuropsychiatric disorders [33]. In the case of schizophrenia, it is remarkable to note that one of the most consistent genetic associations reported in this disorder is with the major histocompatibility complex, hence the innate immune system [34]. The pathology of schizophrenia has been also associated with neuroinflammation whereas, in patients, increased serum concentration of several pro-inflammatory cytokines have been reported [35]. The relevance of microglial activation in the disorder has also been demonstrated by the inhibitory

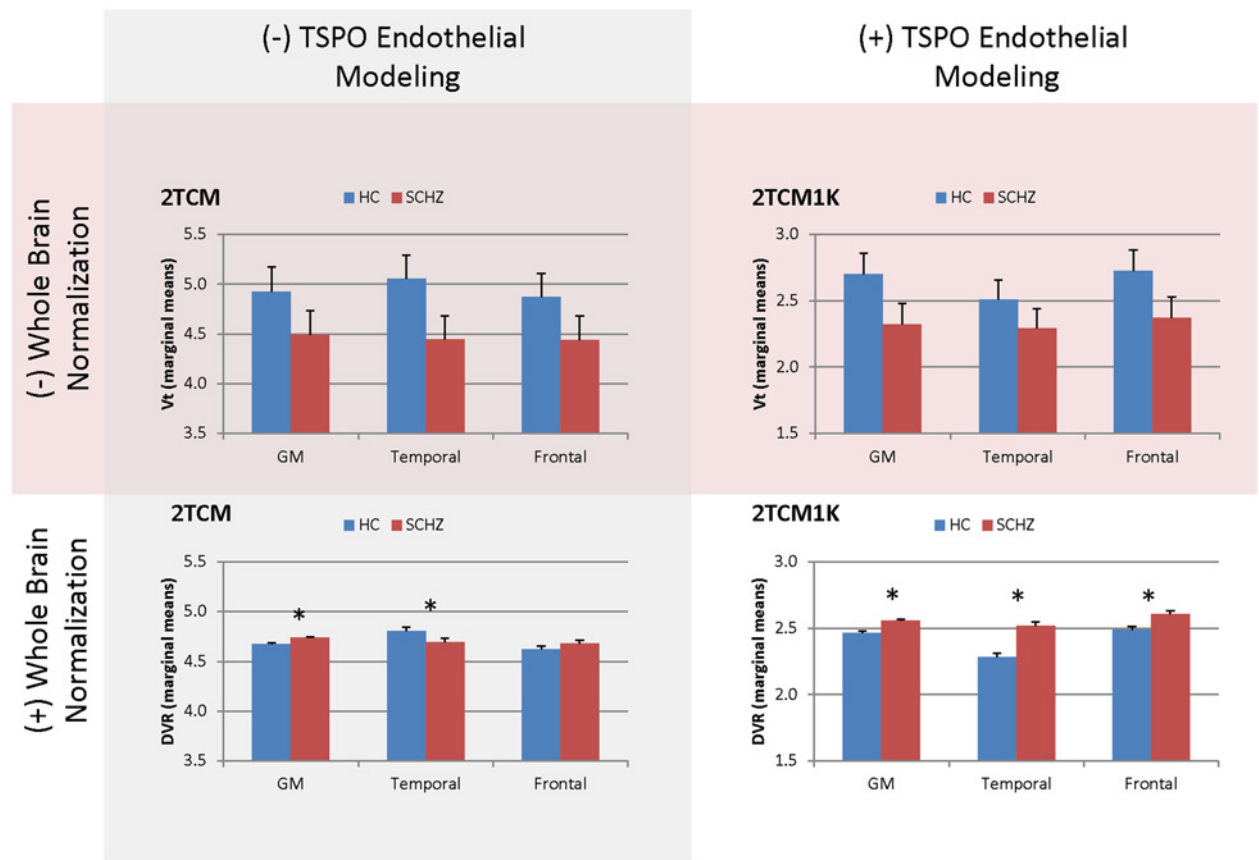
effects of some typical/atypical antipsychotics on activated microglia and by the remarkable clinical improvements achieved using minocycline as an adjuvant for therapy [35,36]. The imaging of microglia in schizophrenia has been reported by two studies using [¹¹C]-(*R*)-PK11195 demonstrating TSPO signal in either grey matter, temporal cortex or hippocampus [37,38].

Only one report has been published to date on the use of second generation TSPO tracer in schizophrenia and, although the sample size was adequate, (27 controls and 18 patients), no neuroinflammation was detected [39].

We have recently conducted a study on schizophrenia using the second generation TSPO tracer [¹¹C]-PBR28 using 22 healthy subjects (18 HABs, four MABs), 14 subjects at ultra-high risk (UHR; seven HABs, seven MABs) and 15 patients with schizophrenia (12 HABs, three MABs) [40]. The clinical and methodological details of the study are published elsewhere [40]. Plasma free fractions of the tracer could not be measured reliably because of the above reported technical difficulties and plasma was only separated from blood cells and corrected for the presence of

Figure 3 | The effect of correcting for endothelial binding and plasma variability, two of the main factors affecting quantification of TSPO ligand, on the final end-point for the PBR28 cross-sectional study comparing schizophrenics (SCHZ, red) and matched controls (HC, blue)

The figure shows the marginal averages of the volume of distribution corrected for the A¹⁴⁷T genotype and age for three regions (whole grey matter-GM, temporal and frontal cortex). The first row demonstrates the large variability of the results (with and without endothelial binding correction, right and left panel respectively) due to plasma variability; the reductions in the schizophrenic cohort are driven by the incorrect estimates of the free plasma concentration. Plasma variability is then taken into account by normalizing the data with whole brain uptake and results shown in the lower row. Here the incorporation of the endothelial binding in the model leads to the expected TSPO increases (right panel) whereas, without correction, end-points fluctuate erratically (left panel).



radiometabolites. Nevertheless we found large variability in the plasma radioactive concentrations that were abnormally associated with the groups as well as age and genotype with a significant increase in plasma concentration in the pathological cohorts. As a result, when plasma concentrations were used for quantification of TSPO tissue uptake, the end-points indicated implausible reductions in TSPO binding irrespective of the quantification model used (Figure 3, upper row). Given the impossibility of finding a suitable reference region, data were then normalized to total brain uptake and are illustrated in Figure 3 (bottom row) for kinetic models incorporating the TSPO-binding component in the endothelium (right panel) or not (left panel). The increases in whole grey matter, frontal and temporal cortex, regions generally involved in the pathology of schizophrenia, were demonstrated here only when BBB TSPO uptake was taken

into consideration. No other regional differences were found. Similar results were obtained in the UHR cohort (result not shown).

Conclusion

The TSPO is a sensitive and robust biomarker of microglial activity in the CNS. Because of the heterogeneous distribution of TSPO in the blood and brain, the kinetics of PET-TSPO radioligands will produce spurious or null results unless biological variability is properly accounted for. Importantly, molecular strategies for the *in vivo* imaging of the TSPO require moderate affinity for the target as high-affinity ligands tend to accumulate on to the vascular-binding sites masking the TSPO in tissue. Hence, we recommend the analysis approaches outlined in this review to normalize for this large effect.

Funding

This work was supported by the PET Methodology Program [grant number Ref G1100809/1 (to F.E.T. and M.V.)]; and the Wellcome Trust Strategic Award.

References

- Charbonneau, P., Syrota, A., Crouzel, C., Valois, J.M., Prenant, C. and Crouzel, M. (1986) Peripheral-type benzodiazepine receptors in the living heart characterized by positron emission tomography. *Circulation* **73**, 476–483 [CrossRef PubMed](#)
- Myers, R., Manjil, L.G., Cullen, B.M., Price, G.W., Frackowiak, R.S. and Cremer, J.E. (1991) Macrophage and astrocyte populations in relation to [3H]PK 11195 binding in rat cerebral cortex following a local ischaemic lesion. *J. Cereb. Blood Flow Metab.* **11**, 314–322 [CrossRef PubMed](#)
- Ramsay, S.C., Weiller, C., Myers, R., Cremer, J.E., Luthra, S.K., Lammertsma, A.A. and Frackowiak, R.S. (1992) Monitoring by PET of macrophage accumulation in brain after ischaemic stroke. *Lancet* **339**, 1054–1055 [CrossRef PubMed](#)
- Banati, R.B., Newcombe, J., Gunn, R.N., Cagnin, A., Turkheimer, F., Heppner, F., Price, G., Wegner, F., Giovannoni, G., Miller, D.H. et al. (2000) The peripheral benzodiazepine binding site in the brain in multiple sclerosis: quantitative *in vivo* imaging of microglia as a measure of disease activity. *Brain* **123** (Pt 11), 2321–2337 [CrossRef PubMed](#)
- Cagnin, A., Brooks, D.J., Kennedy, A.M., Gunn, R.N., Myers, R., Turkheimer, F.E., Jones, T. and Banati, R.B. (2001) *In-vivo* measurement of activated microglia in dementia. *Lancet* **358**, 461–467 [CrossRef PubMed](#)
- Edison, P., Archer, H.A., Gerhard, A., Hinz, R., Pavese, N., Turkheimer, F.E., Hammers, A., Tai, Y.F., Fox, N., Kennedy, A. et al. (2008) Microglia, amyloid, and cognition in Alzheimer's disease: an [11C](R)PK11195-PET and [11C]PIB-PET study. *Neurobiol. Dis.* **32**, 412–419 [CrossRef PubMed](#)
- Gerhard, A., Banati, R.B., Goerres, G.B., Cagnin, A., Myers, R., Gunn, R.N., Turkheimer, F., Good, C.D., Mathias, C.J., Quinn, N. et al. (2003) [11C](R)-PK11195 PET imaging of microglial activation in multiple system atrophy. *Neurology* **61**, 686–689 [CrossRef PubMed](#)
- Gerhard, A., Pavese, N., Hotton, G., Turkheimer, F., Es, M., Hammers, A., Eggert, K., Oertel, W., Banati, R.B. and Brooks, D.J. (2006) *In vivo* imaging of microglial activation with [11C](R)-PK11195 PET in idiopathic Parkinson's disease. *Neurobiol. Dis.* **21**, 404–412 [CrossRef PubMed](#)
- Gerhard, A., Watts, J., Trender-Gerhard, I., Turkheimer, F., Banati, R.B., Bhatia, K. and Brooks, D.J. (2004) *In vivo* imaging of microglial activation with [11C](R)-PK11195 PET in corticobasal degeneration. *Mov. Disord.* **19**, 1221–1226 [CrossRef PubMed](#)
- Okello, A., Edison, P., Archer, H.A., Turkheimer, F.E., Kennedy, J., Bullock, R., Walker, Z., Kennedy, A., Fox, N., Rossor, M. and Brooks, D.J. (2009) Microglial activation and amyloid deposition in mild cognitive impairment: a PET study. *Neurology* **72**, 56–62 [CrossRef PubMed](#)
- Turner, M.R., Cagnin, A., Turkheimer, F.E., Miller, C.C., Shaw, C.E., Brooks, D.J., Leigh, P.N. and Banati, R.B. (2004) Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: an [11C](R)-PK11195 positron emission tomography study. *Neurobiol. Dis.* **15**, 601–609 [CrossRef PubMed](#)
- Pavese, N., Gerhard, A., Tai, Y.F., Ho, A.K., Turkheimer, F., Barker, R.A., Brooks, D.J. and Piccini, P. (2006) Microglial activation correlates with severity in Huntington disease: a clinical and PET study. *Neurology* **66**, 1638–1643 [CrossRef PubMed](#)
- Ramlackhansingh, A.F., Brooks, D.J., Greenwood, R.J., Bose, S.K., Turkheimer, F.E., Kinnunen, K.M., Gentleman, S., Heckemann, R.A., Gunanayagam, K., Gelosa, G. and Sharp, D.J. (2011) Inflammation after trauma: microglial activation and traumatic brain injury. *Ann. Neurol.* **70**, 374–383 [CrossRef PubMed](#)
- Pappata, S., Cornu, P., Samson, Y., Prenant, C., Benavides, J., Scatton, B., Crouzel, C., Hauw, J.J. and Syrota, A. (1991) PET study of carbon-11-PK 11195 binding to peripheral type benzodiazepine sites in glioblastoma: a case report. *J. Nucl. Med.* **32**, 1608–1610 [PubMed](#)
- Takaya, S., Hashikawa, K., Turkheimer, F.E., Mottram, N., Deprez, M., Ishizu, K., Kawashima, H., Akiyama, H., Fukuyama, H., Banati, R.B. and Roncaroli, F. (2007) The lack of expression of the peripheral benzodiazepine receptor characterises microglial response in anaplastic astrocytomas. *J. Neurooncol.* **85**, 95–103 [CrossRef PubMed](#)
- Jacobs, A.H., Tavitian, B. and consortium, I.N. (2012) Noninvasive molecular imaging of neuroinflammation. *J. Cereb. Blood Flow Metab.* **32**, 1393–1415 [CrossRef PubMed](#)
- Converse, A.K., Larsen, E.C., Engle, J.W., Barnhart, T.E., Nickles, R.J. and Duncan, I.D. (2011) 11C-(R)-PK11195 PET imaging of microglial activation and response to minocycline in zymosan- treated rats. *J. Nucl. Med.* **52**, 257–262 [CrossRef PubMed](#)
- Dodel, R., Spottke, A., Gerhard, A., Reuss, A., Reinecker, S., Schimke, N., Trenkwalder, C., Sixel-Doring, F., Herting, B., Kamm, C. et al. (2010) Minocycline 1-year therapy in multiple-system-atrophy: effect on clinical symptoms and [(11)C] (R)-PK11195 PET (MEMSA-trial). *Mov. Disord.* **25**, 97–107 [CrossRef PubMed](#)
- Martini, C., Chelli, B., Betti, L., Montali, M., Mancuso, M., Giannaccini, G., Rocchi, A., Murri, L. and Siciliano, G. (2002) Peripheral benzodiazepine binding sites in platelets of patients affected by mitochondrial diseases and large scale mitochondrial DNA rearrangements. *Mol. Med.* **8**, 841–846 [PubMed](#)
- Soreni, N., Apter, A., Weizman, A., Don-Tufeled, O., Leschiner, S., Karp, L. and Gavish, M. (1999) Decreased platelet peripheral-type benzodiazepine receptors in adolescent inpatients with repeated suicide attempts. *Biol. Psychiatry* **46**, 484–488 [CrossRef PubMed](#)
- Pini, S., Martini, C., Abelli, M., Muti, M., Gesi, C., Montali, M., Chelli, B., Lucacchini, A. and Cassano, G.B. (2005) Peripheral-type benzodiazepine receptor binding sites in platelets of patients with panic disorder associated to separation anxiety symptoms. *Psychopharmacology* **181**, 407–411 [CrossRef PubMed](#)
- Begni, B., Tremolizzo, L., Andreoni, S., Contri, M., Uccellini, O., Neri, F. and Ferrarese, C. (2009) Neuroligand binding endophenotypes in blood cells distinguish two subsets of borderline personality disorder patients. *Neurosci. Lett.* **462**, 144–146 [CrossRef PubMed](#)
- Ritsner, M., Modai, I., Gibel, A., Leschiner, S., Silver, H., Tsinovoy, G., Weizman, A. and Gavish, M. (2003) Decreased platelet peripheral-type benzodiazepine receptors in persistently violent schizophrenia patients. *J. Psychiatr. Res.* **37**, 549–556 [CrossRef PubMed](#)
- Lockhart, A., Davis, B., Matthews, J.C., Rahmoune, H., Hong, G., Gee, A., Earnshaw, D. and Brown, J. (2003) The peripheral benzodiazepine receptor ligand PK11195 binds with high affinity to the acute phase reactant alpha1-acid glycoprotein: implications for the use of the ligand as a CNS inflammatory marker. *Nucl. Med. Biol.* **30**, 199–206 [CrossRef PubMed](#)
- Turkheimer, F.E., Edison, P., Pavese, N., Roncaroli, F., Anderson, A.N., Hammers, A., Gerhard, A., Hinz, R., Tai, Y.F. and Brooks, D.J. (2007) Reference and target region modeling of [11C]-(R)-PK11195 brain studies. *J. Nucl. Med.* **48**, 158–167 [PubMed](#)
- Yaqub, M., van Berckel, B.N., Schuitemaker, A., Hinz, R., Turkheimer, F.E., Tomasi, G., Lammertsma, A.A. and Boellaard, R. (2012) Optimization of supervised cluster analysis for extracting reference tissue input curves in (R)-[11C]PK11195 brain PET studies. *J. Cereb. Blood Flow Metab.* **32**, 1600–1608 [CrossRef PubMed](#)
- Anderson, A.N., Pavese, N., Edison, P., Tai, Y.F., Hammers, A., Gerhard, A., Brooks, D.J. and Turkheimer, F.E. (2007) A systematic comparison of kinetic modelling methods generating parametric maps for [(11)C]-(R)-PK11195. *NeuroImage* **36**, 28–37 [CrossRef PubMed](#)
- Tomasi, G., Edison, P., Bertoldo, A., Roncaroli, F., Singh, P., Gerhard, A., Cobelli, C., Brooks, D.J. and Turkheimer, F.E. (2008) Novel reference region model reveals increased microglial and reduced vascular binding of 11C-(R)-PK11195 in patients with Alzheimer's disease. *J. Nucl. Med.* **49**, 1249–1256 [CrossRef PubMed](#)
- Chauveau, F., Boutin, H., Van Camp, N., Dolle, F. and Tavitian, B. (2008) Nuclear imaging of neuroinflammation: a comprehensive review of [11C]PK11195 challengers. *Eur. J. Nucl. Med. Mol. Imaging* **35**, 2304–2319 [CrossRef PubMed](#)
- Owen, D.R., Yeo, A.J., Gunn, R.N., Song, K., Wadsworth, G., Lewis, A., Rhodes, C., Pulford, D.J., Bennacef, I., Parker, C.A. et al. (2012) An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. *J. Cereb. Blood Flow Metab.* **32**, 1–5 [CrossRef PubMed](#)
- Rizzo, G., Veronese, M., Tonietto, M., Zanotti-Fregonara, P., Turkheimer, F.E. and Bertoldo, A. (2014) Kinetic modeling without accounting for the vascular component impairs the quantification of [(11)C]PBR28 brain PET data. *J. Cereb. Blood Flow Metab.* **34**, 1060–1069 [CrossRef PubMed](#)
- Delforge, J., Syrota, A. and Bendriem, B. (1996) Concept of reaction volume in the *in vivo* ligand-receptor model. *J. Nucl. Med.* **37**, 118–125 [PubMed](#)

- 33 Blank, T. and Prinz, M. (2013) Microglia as modulators of cognition and neuropsychiatric disorders. *Glia* **61**, 62–70 [CrossRef PubMed](#)
- 34 Stefansson, H., Ophoff, R.A., Steinberg, S., Andreassen, O.A., Cichon, S., Rujescu, D., Werge, T., Pietilainen, O.P., Mors, O., Mortensen, P.B. et al. (2009) Common variants conferring risk of schizophrenia. *Nature* **460**, 744–747 [PubMed](#)
- 35 Monji, A., Kato, T. and Kanba, S. (2009) Cytokines and schizophrenia: microglia hypothesis of schizophrenia. *Psychiatry Clin. Neurosci.* **63**, 257–265 [CrossRef PubMed](#)
- 36 Miyaoka, T., Yasukawa, R., Yasuda, H., Hayashida, M., Inagaki, T. and Horiguchi, J. (2008) Minocycline as adjunctive therapy for schizophrenia: an open-label study. *Clin. Neuropharmacol.* **31**, 287–292 [CrossRef PubMed](#)
- 37 van Berckel, B.N., Bossong, M.G., Boellaard, R., Kloet, R., Schuitmaker, A., Caspers, E., Luurtsema, G., Windhorst, A.D., Cahn, W., Lammertsma, A.A. and Kahn, R.S. (2008) Microglia activation in recent-onset schizophrenia: a quantitative (R)-[11C]PK11195 positron emission tomography study. *Biol. Psychiatry* **64**, 820–822 [CrossRef PubMed](#)
- 38 Doorduyn, J., de Vries, E.F., Willemsen, A.T., de Groot, J.C., Dierckx, R.A. and Klein, H.C. (2009) Neuroinflammation in schizophrenia-related psychosis: a PET study. *J. Nucl. Med.* **50**, 1801–1807 [CrossRef PubMed](#)
- 39 Kenk, M., Selvanathan, T., Rao, N., Suridjan, I., Rusjan, P., Remington, G., Meyer, J.H., Wilson, A.A., Houle, S. and Mizrahi, R. (2014) Imaging neuroinflammation in gray and white matter in schizophrenia: an in-vivo PET study with [18F]-FEPPA. *Schizophr. Bull.* **41**, 85–93 [CrossRef PubMed](#)
- 40 Bloomfield, P.S.S.S., Veronese, M., Rizzo, G., Bertoldo, A., Owen, D.R., Bloomfield, M.A.P., Bonoldi, I., Kalk, N., Turkheimer, F., McGuire, P. et al. Microglial activity in people at ultra high risk of psychosis and in schizophrenia; an [11C]PBR28 PET brain imaging study. Society of Biological Psychiatry, 70th Annual Meeting, 14–16 May 2015

Received 25 February 2015
doi:10.1042/BST20150058