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Molecular imaging of neuroinflammation in preclinical rodent models using positron emission tomography

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Abstract

INTRODUCTION: Neuroinflammation (NI) is an adaptive response to different noxious stimuli, involving microglia, astrocytes and peripheral immune cells. NI is a hallmark of several acute and chronic diseases of central nervous system (CNS) and contributes to both damage and repair of CNS tissue.

EVIDENCE ACQUISITION: Interventional or genetically modified rodent models mimicking human neuropathologies may provide valuable insights on basic mechanisms of NI, but also for improving the development of new diagnostic and therapeutic strategies.

EVIDENCE SYNTHESIS: Preclinical positron emission tomography (PET) allows to investigate noninvasively the inflammatory response in CNS of rodent models at a molecular level, validating innovative probes for early diagnosis, and characterizing the time course of neuroinflammatory changes and their relationship with disease progression, as well as the effects of experimental treatments with high translational potential. In particular, recent efforts of preclinical PET field are intended to develop specific and selective radiotracers that target the activation of innate immune system in CNS.

CONCLUSIONS: Here, we have reviewed the state of art for PET in relevant rodent models of acute and chronic neuropathologies associated with NI, with particular regard on imaging of activated microglia and astrocytes.

KEY WORDS: Neuroinflammation- Microglial and astrocytes activation- Rodent models- Positron emission tomography.

INTRODUCTION

Neuroinflammation (NI)

Neuroinflammation (NI) is a dynamic and complex defensive response to pathological insults of various nature, involving microglia, astrocytes and infiltrating peripheral immune cells (neutrophils, macrophages and lymphocytes).[1] NI is typically associated with infections of the central nervous system (CNS), when blood–brain barrier (BBB) fails to stop biological insults, but it may occur also in acute non infectious neuropathologies such as stroke or chronic diseases such as multiple sclerosis (MS), neurodegenerative and psychiatric disorders. [2, 3] Acute lesions usually induce a strong and complex neuroinflammatory response leading to local changes of blood flow and vascular permeability, alteration of the BBB, infiltration of peripheral macrophages and activation of resident microglia. [4] Moreover, a sustained inflammatory response, mainly mediated by microglia and astrocytes, might be also involved in the pathogenesis and progression of chronic neurodegenerative diseases.[5] In this respect, it is noteworthy that microglia and astrocytes can be activated in response to aberrant endogenous proteins such as amyloid-β, mutant superoxide dismutase 1-SOD1 and others. In particular, microglial cells, the innate macrophages of CNS, seem to play a pivotal role in NI.[5] In physiological conditions, microglia have the function of maintaining cerebral tissue homeostasis, neuronal integrity and network functioning in the brain.[5] Following various noxious stimuli, however, microglia switches from a resting, ramified form to an activated, ameboid state.[1] Depending on the involved signaling pathways and tissue microenvironment, microglia may promote tissue remodeling/repair (anti-inflammatory M2-like phenotype) or may induce tissue damage (pro-inflammatory M1-like activation).[1, 6] Overall, the contribution of NI to pathophysiology of the CNS diseases is multifarious and not yet fully understood. Several factors may influence the balance of NI towards detrimental or beneficial outcomes, depending on the timing of expression after CNS injury, localization, extent, chronicity, and immune cell types involved.[6] Improving knowledge of these crucial aspects may provide new insight on the role of NI in acute and chronic CNS disorders as well as on their therapy.

EVIDENCE ACQUISITION

Preclinical models and imaging of NI

An increasing number of rodent models mimicking different acute or chronic human neuropathologies associated with NI have become available and are extensively characterized.[7-9] Genetically modified mice, carrying specific gene mutations present in human familial forms of diseases, are of particular relevance for further understanding of molecular pathways involved in

of particular relevance for further understanding of molecular pathways involved in the diseases.[8,9] Although none of these animal models show all aspects of human diseases, they might provide valuable information on basic molecular processes of NI that are of crucial importance for the development of novel therapeutic strategies. Molecular imaging (MI) using Positron Emission Tomography (PET) may be a unique and useful tool for in vivo study of NI in these models. PET neuroimaging is a quantitative noninvasive reliable method well established in human subjects and increasingly applied in small laboratory animals thanks to the availability of high resolution dedicated PET scanners (microPET). Different radioligands, targeting diverse functional aspects or molecular events involved in NI might be used.[1, 10] Thus, the combined use of rodent models of NI and microPET is an emerging approach that will more and more contribute to the characterization and monitoring of the spatio-temporal dynamic of the inflammatory changes occurring in neurological disorders since the earliest/asymptomatic stages, to the evaluation of their relationship with disease progression and to the investigation on the effects of potential treatments. Moreover, it represents a unique tool for the validation of new PET radiotracers for key targets of NI. Finally, the complementary use of immunohistochemistry would provide the characterization of histopathological changes underlying in vivo imaging. Despite these advantages, there are some methodological limitations to be addressed. Among these, the accuracy of in vivo absolute quantification of the PET CNS data represents a crucial issue in rodent, especially in mice, due to the difficulty of obtaining plasma arterial input function and to partial volume effects (PVE).[11] Simplified methods using standardized uptake value (SUV)[12] or SUV ratios calculating using an unaffected reference region[13] might be easier alternative approaches but they require validation.[14] Moreover, the development of appropriate methods for PVE correction should be encouraged. Another issue is the effect of tracer binding on the receptor sites. Due the small concentration of receptors sites in rodent brain the radiosynthesis should achieve very high specific radioactivity in order to inject small amounts of radioligand thus avoiding possible partial saturation of receptor sites. Finally a biological issue to be considered is the choice of the NI molecular targets, not always expressed in a similar manner in different species. Overall, despite these limitations, findings obtained in small laboratory animals with PET may be important for translational research from bench-to-bedside and back to the bench.

Molecular PET probes for NI

Different PET approaches have been developed and used for in vivo study of NI, including imaging of cerebral blood flow and brain glucose or arachidonic acid metabolism.[1, 10] However, these methods do not provide evaluation of specific molecular targets involved in NI. Here we will shortly review the main molecular targets and PET radiotracers developed/available for

shortly review the main molecular targets and PET radiotracers developed/available for imaging of activated microglia and astrocytes, a topic more extensively treated in recent reviews. [2, 15]

Translocator protein (TSPO) is the main target of PET radiotracers developed during the last years for in vivo imaging of glial activation both in preclinical and clinical studies. TSPO**,** previously known as peripheral benzodiazepine receptor (PBR), is a 18 kDa protein located on the outer mitochondrial membrane of organs such as the adrenal glands, testis and ovaries, and the pituitary glands, which is involved in steroid synthesis.[16] TSPO levels in the CNS are low in healthy condition but are dramatically increased in activated microglia and astrocytes during NI.[16] Interestingly, modulation of TSPO expression might be also implicated in neuronal survival and regeneration.[16] Thus, TSPO is an attractive imaging target for diagnostic, prognostic and therapeutic purposes. A huge number of TSPO radioligands have been synthesized for PET imaging in preclinical and clinical fields, including [^{11}C]PK11195, [^{11}C]DAA1106, [^{11}C]PBR28, [^{18}F]PBR06, [^{11}C]SSR180575, [^{18}F]GE-180, [^{18}F]DPA-713 and [^{18}F]DPA-714.[1, 2, 15] [^{11}C]PK11195 was the first TSPO radioligand to be developed, but its use, especially when the expected TSPO changes are small, is limited by the high non-specific binding and low bioavailability. A particular effort has been done during the last years for the development and validation of second generation TSPO radiotracers with higher affinity, selectivity and specific binding.[2, 15] Validated fluorinated radiotracers are actually available and have the advantage to be more easily implemented and distributed across imaging centers for larger studies. Although improved signal-to-noise ratios, these TSPO radioligands showed in human studies inter-subject variability in their binding potentials due to polymorphism in the gene that encodes TSPO, leading to three distinct binding affinity classes: high-affinity binder, low-affinity binder, and the mixed-affinity binder expressing both binding sites. [17] It is noteworthy that, to date, the polymorphism of TSPO in rodents has not been demonstrated. Thus, further validation in human studies is required when new promising radiotracers have been evaluated in preclinical research. In this respect new TSPO radioligands with different molecular structure not influenced by human polymorphism are under validation and development. Among these, [^{18}F]FEBMPA seems to be promising candidate since the specific binding is not significantly affected by TSPO polymorphism in post-mortem in human brain.[18] Another critical point is the selectivity of TSPO for activated microglia. Although microglial activation seems mainly underlying the TSPO radiotracer uptake, activated astrocytes might also contribute to the signal as suggested by results obtained in studies on different experimental models of NI [16] and in a rat model of selective astrocyte activation with minimal microglial response. [19] Finally, it should be pointed out that all current TSPO radiotracers are not able to discriminate between the different M1-like and M2-like activation states of microglia, so the development of new probes specific for microglial phenotype is desirable. Moreover there are

specific for microglial phenotype is desirable. Moreover there are no radiotracers that are selective for different transmembrane domains of TSPO. Despite this complexity, the use of TSPO radiotracers allowed to characterize in vivo with PET microglial activation in human disorders and preclinical models of NI.

Cyclooxygenase (COX) is another potential interesting target implicated in the inflammatory cascade. COX is an enzyme converting arachidonic acid into prostaglandins (PGs) and is induced by acute and chronic inflammatory stimulations even in the brain. There are two isoforms: COX-1, constitutively expressed in all cell types, and COX-2, a form inducible in response to cytokines, growth factors and pro-inflammatory molecules. The potential role of COX isoforms in brain pathological conditions has been extensively reviewed.[2, 20] In particular, COX-2 contributes to different physiological functions of CNS, and can be expressed in the brain not only by neurons, but also from activated microglia and astrocytes, and from infiltrating blood cells (neutrophils, monocytes/macrophages) in course of NI associated to acute or chronic diseases.[21] COX-1 appears predominantly localized in microglia and could play an important role in NI by secreting PGs in response to microglia activation.[18] Thus both isoforms might be interesting targets for development of PET imaging probes. Different PET radiotracers have been developed during the last years by radiolabeling different COX inhibitors, most part of them however displayed poor selectivity for the different isoforms and unfavourable characteristics for in vivo imaging.[2, 15, 22] Interestingly, the results of a recent study performed in a rat model of unilateral intrastriatal injection of lipopolysaccharides (LPS) and in (APP-Tg) mouse model of AD suggested that the S-enantiomer of [^{11}C]-KTP-Me might be a potential selective and sensitive radiotracer for brain imaging of COX-1 expression and microglial activation.[23] Furthermore, in vitro studies have reported promising candidates for the development of [^{18}F]-radiolabeled COX-2 radiotracers.[13]

The **cannabinoid receptor type-2 (CB_2R)** is predominantly involved in anti-inflammatory and immunosuppressive actions. In healthy brain tissue, resting microglia expresses low levels of CB_2R, whereas its expression is increased by activated microglia in diseased brain.[24] Post-mortem studies reported increased CB_2R expression in brain areas showing increased microglial activation in patients with AD,[25] in lesioned striatum of a rat model of HD and in the spinal cord of a murine model of MS.[24] Moreover pharmacological experiments suggested that the protective effects of cannabinoid treatment might be related to the prevention of microglial activation in a rodent model of AD.[25] A series of radiotracers were developed for imaging CB_2R expression in the brain.[2, 15] Among these, [^{11}C]-NE40 and [^{11}C]-A-836339 revealed favorable characteristics for in vivo studies.[2, 15] [^{11}C]-NE40 showed small amount of brain radiometabolites in vivo in normal rodents and increased uptake in a rat model with human CB_2R overexpression as expected.[2, 15] However, a

PET study with [^{11}C]-NE40 demonstrated lower CB_2R expression in the brain of AD patients than in controls, an unexpected result possibly reflecting [^{11}C]-NE40 binding also to neuronal CB_2R, that might be reduced in AD.[26] [^{11}C]-A-836339, an agonist with subnanomolar affinity for CB_2R provided interesting results in AD murine models. [27]

The **$P2X_7$ receptor ($P2X_7R$)** is a complex target of particular interest, because its overexpression seems to be mainly associated with the anti-inflammatory microglial phenotype.[15] $P2X_7$ is a purinergic ionotropic receptor activated by ATP (ATP-gated ion channel) expressed in a variety of cells including macrophages and microglia, and appears to be involved in NI (Ory 2014, Monif 2010). [2, 28] Increased $P2X_7R$ expression in CNS and enhanced microglial activation have been reported post-mortem in different animal models of NI and neurodegeneration.[28] Thus $P2X_7$ might be a promising target for imaging NI by PET. A variety of antagonists for $P2X_7R$ have been developed showing however different interspecies affinity [2] thus limiting the preclinical validation of potential new PET radioligands. Interestingly, using a transfected rat model expressing the human $P2X_7R$ ($hP2X_7R$) in the striatum, Ory *et al.*[29] recently demonstrated that in vivo [^{11}C]-JNJ-54173717 was able to selectively and specifically bind to $hP2X_7R$. Moreover, preliminary results in non-human primates also showed that [^{11}C]-JNJ-54173717 specifically bound to brain $P2X_7R$, suggesting that this would be a promising radiotracer for further studies of neuroinflammatory pathologies. Other radiotracers for $P2X_7R$, such as [^{11}C]-A-740003 and [^{11}C]-GSK1482160 are under evaluation [15]

Matrix Metalloproteinases (MMPs) are endopeptidases secreted as inactive zymogens and activated in the extracellular space, and were also proposed as NI targets. MMPs not only degrade the extracellular matrix and contribute to tissue repair, but also act on pro-inflammatory cytokines, chemokines and other inflammation related proteins. In vitro, activated human microglial cells showed increased mRNA levels of MMP-1, MMP-3, MMP-8, MMP-10 and MMP-12. Moreover, overexpression of MMP-12 was found post-mortem in patients with MS and in a mouse model of MS.[2] Recently, [^{18}F]-BR-351 has demonstrated to be a suitable radiotracer for imaging of activated MMP-2 and MMP-9 in early phases of experimental stroke associated to BBB damage and potentially later to glial activation.[30]

α4β2 nicotinic acetylcholine receptor (α4β2-nAChR) is not only expressed in neurons of but also in microglia and macrophages and might be involved in inflammatory responses.[31] Recently, Martin *et al.*[31] suggested that 2[^{18}F]-fluoro-A85380, a specific radiotracer for α4β2-nAChR, might be used in vivo with PET for imaging increased microglial activation in a stroke rat model. Further studies are required to confirm the potential usefulness of radiotracers targeting α4β2-nAChR for studying NI.

Monoamine oxidase type B (MAO-B) is an interesting target proposed for in vivo imaging of activated astrocytes. This enzyme, that catalyzes the deamination of monoamines in neurotransmitters synthesis, is located at the outer mitochondrial membrane in serotonergic neurons but also in astrocytes where it is upregulated in activated states during NI.[32] [^{11}C]-L-deprenyl, an irreversible MAO-B inhibitors, and its deuterated form [^{11}C]-deuterium-L-deprenyl ([^{11}C]-DED), a more suitable PET radioligand showing lower blood flow dependence and slower metabolism, were the first radiotracers successfully developed for imaging MAO-B activity. They allowed to image astrocytes activation in post-mortem [30] as well as in vivo studies in AD patients [33] and in APPswe mouse model of AD.[34] Recently a bis-deuterium substituted L-deprenyl analog has been labeled with [^{18}F] ([^{18}F]-fluorodeprenyl-D2). Preliminary results suggest that it is a suitable PET radioligand for in vivo study of MAO-B activity. [35] Other radiotracers have been developed but did not show favorable characteristics for in vivo PET studies or are under development and evaluation as extensively reported by Janssen *et al.* [15]

Preclinical PET imaging in rodent models of NI

We have reviewed here the main preclinical PET imaging studies in the most relevant rodent models of acute and chronic neuropathologies associated with NI, in particular stroke, MS, AD, ALS, PD and HD.

Ischemic stroke

Stroke is a common disease worldwide leading to long-term disability and death, and acute ischemic stroke is the most common type. Cerebral ischemia results from occlusion of major brain arteries and reperfusion within the first hours remains the treatment of choice for improving clinical outcome [1, 36] A large body of evidence suggests that inflammation triggered by the focal cerebral ischemia plays an important role on tissue outcome at different stages of cerebral ischemic injury. [36] Moreover, early reperfusion may also worse cerebral injury promoting a stronger neuroinflammatory response.[37] Thus NI in the ischemic stroke appears an attractive therapeutic target with a wide therapeutic window. [36] Different innate and peripheral immune cell populations infiltrate the ischemic brain tissue over time after stroke and might contribute to tissue damage and/or repair.[36] The temporo-spatial dynamic of these events has been in part reported ex-vivo in experimental stroke models, [38] but it is poorly known in vivo. The combined use of PET and experimental animal models might provide further insight to this issue and thus might contribute to the development and evaluation of new therapeutic strategies. Currently the most common experimental model of focal brain ischemia, also used in the most part of in vivo microPET studies, is the occlusion of the middle cerebral artery (MCAO) in rodents. MCAO may be either permanent (pMCAO) or transient (tMCAO) followed by reperfusion after a variable

(pMCAO) or transient (tMCAO) followed by reperfusion after a variable time. [39] These two models show different pathophysiological features that have to be considered in the interpretation of results.

Activated microglia have been the main target of PET studies in rodent stroke models, and the radioligands for monitoring changes in TSPO sites have been mostly developed and used (Jacobs 2012, Janssen 2016) [1, 15] [^{11}C](R)-PK11195 was the first and most widely applied in both human and experimental stroke studies. [1, 15] After tMCAO, increased [^{11}C](R)-PK11195 uptake was found in the infarct core and in the peri-infarct region at 4 and 7 days in outbred rats [40] and at 2 and 14 days in spontaneous hypertensive rats [41]. Increased radiotracer uptake was less marked at 2 and 4 than at 7 and 14 days and was mainly associated with increased expression of TSPO by activated microglia/macrophages. Only some reactive astrocytes at the infarct margin expressed TSPO sites at 7 days after stroke. [40] Similar findings were reported using second generation TSPO radioligands in tMCAO rodent stroke models. [30, 42-44] Using [^{18}F]DPA-714 and immunohistochemistry, Martin *et al.* [42] further characterized in rats the time-course of TSPO expression and glial activation in the ischemic hemisphere after tMCAO. In vivo PET imaging revealed a strong and significant increase of [^{18}F]DPA-714 uptake on the ischemic hemisphere involving the core and the peri-infarct area at 7, 11, 15 and 21 days after ischemia, peaking at 11 days and slowly decreasing thereafter until 30 days. Displacement with excess of unlabeled DPA714 or PK11195 demonstrated the specificity of the [^{18}F]DPA-714 binding in the ischemic tissue at 7 days after ischemia. Interestingly, the spatio-temporal profile of PET [^{18}F]DPA-714 binding matched with that of microglia/macrophages activation but not with that of activated astrocytes as evaluated by immunohistochemistry. A migration of GFAP/TSPO-positive astrocytes was showed progressively from the peri-infarct rim toward the infarct core reaching highest values at 30 days after stroke. A similar time-course of in vivo [^{18}F]DPA-714 binding and post-mortem microglial activation was also reported in the ischemic hemisphere of mice after induction of tMCAO [30, 44] (Figure 1.A). Interestingly, this response was also present, although to a lesser extent, as early as 1-2 days after tMCAO, in line with post-mortem studies in mice (Gelderblom) or with PET studies in living rats.[41] Recently, tMCAO model in rats was also used to validate [^{18}F]FEBMP, a new TSPO radioligand that might be potentially less affected by human polymorphisms. Preliminary results showed specific binding of [^{18}F]FEBMP in the infarcted striatum of rats 7 days after tMCAO, as confirmed by displacement studies.[18] The time course of neuroinflammatory processes has been also characterized in rat models of permanent focal cerebral ischemia. In contrast to tMCAO, increased [^{11}C]PK11195 uptake was not observed 7 days after pMCAO in the infarct core, presumably due to lack of reperfusion and reduced tissue radiotracer availability, but only in normoperfused peri-infarct zone.[45] Peri-infarct region also

perfused peri-infarct zone.[45] Peri-infarct region also showed increased glucose metabolism at PET and increased microglia/macrophages activation at immunohistochemistry, and might represent tissue at risk of secondary damage.[45] The time-course of [^{11}C]PK11195 uptake after pMCAO, studied in rats between 2-45 days and at 7 months after stroke, seems to be also different from that reported in tMCAO, showing mainly an early peri-infarct increase followed by a late shift towards secondary degenerative regions such as the thalamus and the pyramidal tracts, as confirmed by Iba1 staining at 28-56 days and 7 months.[46, 47] However, the long term neuroinflammatory effects in chronic stages after stroke were studied only in pMCAO. Interestingly, persistent microglial activation and increased [^{11}C]PK11195 binding might be observed in regions remote from the ischemic lesion even 7 months after pMCAO, a finding in line with previous observation in human stroke. [48] The role of this long lasting neuroinflammatory response in the final tissue and clinical outcomes deserve further investigation. Second-generation TSPO radioligands were also tested in pMCAO showing similar results. [49]

Treatment with minocycline, a neuroprotective agent, reduced [^{18}F]DPA-714 uptake in the ischemic brain region of tMCAO rats at 7 days after stroke, without a relevant effect on the final infarct size.[50] A similar decrease of [^{18}F]DPA-714 uptake was reported in the ischemic brain region of tMCAO mice at 3 and 7 days after stroke following treatment with AMD3100, an anti-inflammatory agent mainly acting on peripheral immune cells.[18, 51]

To date, potential NI targets other than TSPO have been only poorly explored in vivo with microPET in experimental stroke models. In a first original study, Martin *et al* [31] tested 2[^{18}F]-fluoro-A85380, a radioligand that specifically bound to α4β2-nAChR, and demonstrated that increased expression of this receptor might be detected in vivo in tMCAO rats, with a similar time-course of [^{11}C]PK11195 measured in vivo in the same animals and of microglial activation measured post-mortem using TSPO and CD11b immunostaining. Which could be the modulatory role of α4β2-nAChR in neuronal injury should be investigated. Recently, Zinnhartd *et al* [30] have characterized the spatio-temporal profile of MMPs activity and microglial activation using [^{18}F]BR-351, an inhibitor of MMPs, and [^{18}F]DPA-714, respectively. Compared to [^{18}F]DPA-714, [^{18}F]BR-351 uptake showed an earlier increase (between 24 hours and 7 days after stroke) followed by a rapid decrease, suggesting a different time-dependent MMPs expression and overall, the results of these studies showed that microPET imaging of TSPO and/or of other relevant molecular targets of NI may allow to characterize and monitor in vivo the spatio-temporal dynamic of microglial activation in rodent models of focal cerebral ischemia, as well as the effects of therapies.

Multiple sclerosis (MS)

Multiple sclerosis (MS) is considered a chronic neuroinflammatory and neurodegenerative disease of CNS. The pathological hallmarks of MS are focal demyelinated plaques within the CNS showing different degree of inflammation, gliosis and axonal damage depending the timing and clinical phenotype.[1] Although MS has been considered a disease primarily affecting the white matter, recent evidence suggest that demyelinated lesions might be found in the cortical gray matter of MS patients.[52] Acute active plaques are most frequent in acute and relapsing-remitting MS and are characterized by T and B cells infiltrates, activated microglia and macrophages, myelin debris and BBB damage, whereas chronic lesions in remitting phases display low levels of inflammatory infiltration and no evident demyelination.[1] The relationships between inflammatory aspects and neuronal/axonal loss are controversial. The balance between M1-like and M2-like phenotypes of microglia seems to play an important role in demyelination, neuronal damage, and remyelination in MS.[53] Experimental autoimmune encephalomyelitis (EAE) in rodents reproduce the key pathological features of MS, and is the most widely applied in the research field.[54] The use of EAE model contributed to a better understanding of pathophysiology, diagnosis and therapy of MS. EAE might be induced in a variety of rat and mice strains by parenteral immunization with different myelin proteins or peptides. Depending of the immunization used, different clinical phenotypes of MS and histological/neuroinflammatory changes can be obtained: proteolipid protein peptide (PLP139-151)-induced relapsing remitting in SJL mice; myelin basic protein (MBP)-induced non-demyelinating acute monophasic disease in PL/J mice; chronic-progressive models of myelin oligodendrocyte glycoprotein (MOG35-55) peptide-induced disease in C57/BL6 mice (Gold 2006). Different PET studies have been performed in animal models of MS using [^{18}F]FDG as a marker of increased metabolic rate of activated immune cells as well as specific radioligands for more selective targets of NI. MicroPET studies of EAE-MOG chronic-progressive model reported increased [^{18}F]FDG PET uptake in the spinal cord of C57BL/6 mice from day 8 to 21 after immunization[55] and of dark agouti rats between 10-14 days from immunization.[56] The increased [^{18}F]FDG uptake was associated with the presence of inflammatory infiltrates as determined at histology [55,56] and correlated especially with CD68-positive cells in rats.[56] Moreover, immunosuppressive therapy with dexamethasone, performed in mice, reduced glucose metabolism in the spinal cord.[56] However, the high background of [^{18}F]FDG in the normal CNS and the lack of specificity for glial cells limit the use of this radiotracer. Recently, PET studies using specific TSPO radioligands for imaging microglial activation were performed in experimental MS models. Increased [^{18}F]DPA-714 uptake was found in thoracic and lumbar spinal cord of a rat model of acute monophasic EAE at 10–12 days after immunization with MBP as compared to controls. The specificity of [^{18}F]DPA-714 binding was

ificity of [^{18}F]DPA-714 binding was confirmed with displacement studies using both unlabeled PK11195 and DPA-714. Immunohistochemistry showed that TSPO expression colocalized with activated microglia and macrophages, but not with astrocytes.[57] In this study the binding of [^{18}F]DPA714 was not investigated in the brain because inflammatory changes in this model are prevalent in the spinal cord.[57] Interestingly, increased binding of [^{18}F]DPA714 could be detected between 12-16 days after immunization in the cerebellum and in the brainstem of PLP-EAE SJL/J mice, a model of RR-MS known to involve also brain areas (Figure 1.B).[58] Increased TSPO expression colocalized with increase microglial activation as evaluated by Iba1 immunolabeling.[58] These findings were similar to those reported in symptomatic EAE-PLP mice using microPET and [^{18}F]PBR11, another high affinity second generation TSPO radioligand.[59] Interestingly these authors also showed that a mild but significant increase of tracer uptake was found in the pre-symptomatic stage in several brain areas, including the cerebellum and midbrain.[59] Using a striatal focal chronic EAE-like model of MS, Airas *et al*.[60] demonstrated that increased [^{18}F]GE180 uptake could be detected in lesional and perilesional areas of ipsilateral hemisphere at 127 days after EAE induction, corresponding to activated microglia. Importantly, they showed that microPET with [^{18}F]GE180 was able to demonstrate in vivo reduction of microglial activation (both in lesion volume and in binding potential) induced by 28 days treatment with fingolimod, a finding confirmed by immunohistochemistry. Interestingly Liu *et al*.[61] have used the new radiotracer [^{11}C]TZ3321 that specifically binds to sphingosine-1-phosphate receptor 1 (S1PR1), the target of fingolimod therapy to evaluate its expression in the inflammatory lesions of MBP induce EAE rat model. The results showed that in vivo increased [^{11}C]TZ3321 uptake could be detected in the lumbar spinal cord in symptomatic mice, corresponding to increased S1PR1 expression by activated microglia and astrocytes in the white matter as demonstrated by immunohistochemistry. These preliminary data suggest that microPET and new radioligands for therapeutic targets, such as S1PR1, might provide a new and useful tool for evaluation of inflammatory processes and therapy. Finally, using a longitudinal multitracer PET study approach, Martin *et al*.[62] have investigated the relationship between glutamate excitotoxicity, microglial activation and oligodendrocytes/neuronal death using [^{18}F]FSPG, [^{11}C]PK11195, and [^{18}F]FDG respectively. Both [^{11}C]PK11195 and [^{18}F]FSPG uptake values were significantly increased in the lumbar spinal cord at the peak of clinical symptoms, indicating that microglial activation in this region could contribute to glutamate release, a finding also supported by Iba1 immunostaining and reduced [^{18}F]FSPG uptake following depletion of microglial population. In contrast, [^{18}F]FDG seems to have low sensitivity in monitoring disease progression, possibly due to mixed and opposite signal related to neuronal death and inflammatory response.[62]

Overall, the results of these studies suggest that the use of microPET with radiotracers targeting microglial activation or other interesting NI molecular targets is feasible in rodents model of MS and might provide new insight on diagnosis, pathogenesis and therapy.

Alzheimer's Disease (AD)

A body of evidence indicates that the innate and adaptive immune systems is implicated in AD and may play a role in the pathogenesis and progression of the disease.[5] It is hypothesized that in early stages of AD, microglia are protective by promoting amyloid clearance. At later stages, however, the microglia become less efficient resulting in increased production of neurotoxic pro-inflammatory molecules that might contribute to neurodegeneration and neuronal loss. [2, 63, 64] Interestingly, bone marrow-derived mononuclear phagocytes or ''peripheral macrophages'' might also contribute to amyloid plaque-associated microgliosis, [65] and chronic systemic inflammation such as obesity, smoking, diabetes and atherosclerosis are often associated with AD.[64] Overall, several aspects of NI in AD remain controversial or poorly known such as the dynamic, topography and severity of neuroinflammatory response during the course of disease since the earliest stages, as well as the differentiation of beneficial or detrimental role. Preclinical PET combined with immunohistochemistry and behavioural studies may contribute to improve our understanding of mechanisms underlying NI in AD and to the development of effective therapeutic strategies. The most common experimental models of AD are transgenic mice overexpressing presenilin (PS) 1 and 2 and amyloid precursor protein (APP), the three genes identified in autosomal dominant mutations in familial AD. [7] Imaging of microglia activation using TSPO radiotracers has been one of the main target for PET preclinical studies in rodent models of AD. At difference of acute NI models such as stroke that is characterized by a strong NI response, in chronic neurodegenerative diseases such as AD the degree of microglia activation is relatively low as well as the TSPO expression. This represents a challenge for in vivo imaging and requires the development of radiotracers with very high specific binding and affinity for TSPO. The first TSPO radioligand, [^{11}C](R)-PK11195, widely used in human studies, did not reveal in vivo significant brain binding increase in 13-14 month-old APP/PS1 mice despite evidence of increased CD11b immunostaining.[66] Higher [^{11}C](R)-PK11195 brain retention could be detected only at late stages (16-19month-old APP/PS1 mice),[63] when microglial activation was more prominent as shown by immunohistochemical studies. Recently, numerous second-generation highly specific TSPO radiotracers have been used to image in vivo microglia activation in preclinical AD mice models. In a pioneering study, increased [^{18}F]fluoroethyl-DAA1106 binding induced by anti-amyloid treatment could be detected in the hippocampus of in 20-month-old APP23 (Tg) mice, corresponding to increased microglial activation as confirmed ex-vivo by

croglial activation as confirmed ex-vivo by immunohistochemistry. [67] The same authors showed, however, that [^{11}C]-AC-5216 was more sensitive than [^{18}F]FEDAA1106 in measuring increased hippocampal and entorhinal cortex TSPO density at 11 months of age in a different Tg model of AD, the PS19 line over-expressing human Tau. [68] Very slow washout of [^{18}F]FEDAA1106 from the brain and high unspecific binding might in part account for these results, suggesting that [^{18}F]FEDAA1106 is not a suitable radioligand for PET studies of AD. Increased [^{18}F]-PBR06 brain uptake was detected in 15-16-month-old Thy1-hAPPLond/Swe (APPL/S) mice compared with WT controls, but not in younger mice (9-10 months of age) despite extensive increases in CD68 and TSPO immunoreactivity.[69] These results are in line with those reported using the [^{18}F]-GE180 in APP/PS1dE9 Tg mice showing increased uptake in the hippocampus of old Tg mice compared to age-matched WT but not in young Tg mice. To investigate in vivo and ex-vivo the relationships between microglial activation and amyloid load, microPET multitracer studies were performed using PET amyloid and TPSO radiotracers. Progressive increase in the [^{18}F]GE180 SUV forebrain to white matter ratio was observed with age in PS2APP (Tg) mice when compared with age-matched WT mice reaching 25% at 16 months-age.[70] At this latter age, a positive correlation was found between this TSPO in vivo labeling and the β-amyloid load assessed by [^{18}F]-florbetaben, as previously also shown in another Tg mouse model of amyloidosis (APPswe PS1-dE9) using [^{18}F]DPA-714 and [^{18}F]-florbetabir. [71] In both these studies in vivo data were well-correlated with in vitro immunostaining of activated microglia and β-amyloid plaques. These findings demonstrated the close relationships between microglial activation and β-amyloid load in different Tg models of AD. Using a very high affinity second-generation TSPO radiotracer, [^{11}C]PBR28, and 5XFAD mice, a very aggressive model with early amyloid deposition, Mirzaei *et al.*[72] showed that [^{11}C]PBR28 brain uptake was increased in Tg mice at early stages (6 month of age) as compared with age-matched WT mice. Autoradiographic and immunohistochemical ex-vivo studies confirmed the strong link between increased microglial activation, upregulation of TSPO, and amyloid deposition in this AD model. Whether [^{11}C]PBR28 is as sensitive in detecting early microglial activation in AD models characterized by a milder inflammatory response should be further explored.

Overall, the results of in vivo TSPO studies in AD animal models suggest that although second-generation TSPO radioligands appeared more sensitive, increased TSPO expression could be detected only at late stages when microglial activation was more intense, independently of the radiotracer used. Interestingly, however, they provide evidence that TSPO expression is mainly associated to microglial activation and amyloid pathology

Among the other NI targets a particular the attention has been focused on CB_2R. The CB_2R is expressed by activated microglia and mediate the inflammatory response in vitro and in vivo even in AD as reported in the paragraph "Molecular PET probes for NI". Increased specific binding of [^{11}C]A836339, a selective CB_2R PET radiotracer, has been recently demonstrated in the brain of 12 months old APPswe PS1-dE9 Tg mice as compared to age-matched WT. Quantitative immunohistochemistry analysis revealed that activated microglia showed the maximum intensity of CB2R expression as compared to activated astroglia. [73]

The role of astrocytes in the NI response in preclinical models of AD is less known and less explored, because there are no radiotracers and molecular targets selective for astrocytes. Recently, using [^{11}C]-deuterium-L-deprenyl ([^{11}C]DED), a marker of astrocyte activation [74] increased cortical and hippocampal radiotracer binding was reported in APPswe mice compared to age-matched WT mice, more marked at 6 months than at 8–15 months or 18–24 months. In agreement with results from human studies [33] these findings suggested that astrocytes activation is an early response that may precede amyloid load. However, in vitro autoradiography failed to reveal significant [^{3}H]-L-deprenyl differences between APPswe and WT mice and immunohistochemistry showed more prominent astrocytes activation in the hippocampus at 18–24 than at 6 months in APPswe mice. These discrepancies deserves further investigation. Although the in vivo detection of NI with microPET in neurodegenerative models of AD remains challenging due to the relative mild regional changes of the neuroinflammatory response, taken together all data reported above highlight the potential usefulness to confront PET imaging of NI, through different molecular targets, and other biomarkers such as β-amyloid load, Tau deposition and the integrity of neurotransmission pathways in different available models of AD in order to better understand the time-course of pathological processes and the effect of potential therapeutic approaches.

Other Neurodegenerative disorders

Amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by a progressive loss of motor neurons (MN) in the spinal cord, brainstem nuclei and motor cortex. [75] NI, and specifically activated microglia are neuropathological features of ALS and might be involved in MN degeneration processes in ALS. Different genetic mutations were identified in familial and sporadic ALS allowing the generation of a number of new transgenic rodent models of disease. The mutation of the gene encoding the enzyme Cu2+/Zn2+ superoxide dismutase (SOD1) is the most frequently found and well characterized. SOD1 transgenic mice is the model that best describes ALS phenotypes.[76] In particular it displays NI and neurodegenerative features similar to those reported in ALS patients. Therefore, SOD1^{G93A} mice provide a useful tool for investigating

ported in ALS patients. Therefore, SOD1^{G93A} mice provide a useful tool for investigating NI in vivo using molecular imaging. PET and TSPO radiotracers such as [^{11}C]PK11195 and [^{18}F]DPA714 have been first used in patients with sporadic ALS and provided evidence of increased binding in different cerebral regions including motor cortex, prefrontal cortex, thalamus.[1,13] MicroPET studies of NI in animal models of ALS might contribute to validation of new PET radioligands, to a better understanding of pathological mechanism involved, to monitor disease progression and therapy. Gargiulo *et al.*[13] were the first to demonstrate that significant increase in TSPO expression could be measured in vivo with [^{18}F]DPA-714 and microPET/CT in the brainstem of symptomatic SOD1^{G93A} mice aged 117 ± 12.7 days, compared to age-matched transgenic mice over-expressing the non-mutated wild-type human SOD1 (Figure 1.C). Importantly, immunohistochemistry revealed that the TSPO expression was increased in the brainstem motor nuclei (trigeminal, facial, ambiguous and hypoglossal nuclei), known to be site of neurodegeneration, and was colocalized with Iba1 immunoreactivity. It is noteworthy however that the small size of the spinal cord prevented in vivo accurate evaluation of TSPO changes despite immunohistochemistry evidence of increased TSPO expression and activated microglia in all spinal tracts. Moreover, the possible contribution of activated astrocytes to the PET-TSPO signal was not evaluated and deserves further investigation. Using the same animal model, Brownell *et al.*[77] investigated in a small number of animals the contribution of altered glutamatergic function to NI in ALS at different stages of symptomatic disease using PET and [^{18}F]FPEB, a metabotropic glutamate receptor subtype 5 (mGluR5) radiotracer and [^{11}C]PBR28, a second-generation high affinity TSPO radioligand. The results revealed that both [^{18}F]FPEB binding potential and [^{11}C]PBR28 accumulation were increased during the progression of disease severity. Immunohistochemical analyses showed increased Iba1 staining in brain and spinal cord regions where increased expression of mGluR5 was observed. However, the TSPO expression was not assessed in ex-vivo studies. These results suggested that excessive glutamate may contribute to NI in ALS pathology. Overall, although the results of these studies require a further validation in a greater number of animals, they strengthen the high relevance of microPET studies of TSPO sites for the characterization of microglial activation in SOD1G93A mice model of ALS and of possible mechanisms implicated in the ALS pathology.

Parkinson's disease (PD)

Parkinson's disease (PD) is characterized by the relatively selective death of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) leading to a striatal dopamine deficit. It is mainly sporadic and is usually accompanied by motor symptoms such as bradykinesia, rigidity,

postural instability and resting tremor, although a rising occurrence of non-motor symptoms is now recognized. One neuropathological feature of PD is the occurrence of neuroinflammatory processes which manifests particularly through the activation of microglial cell and astrocytes in the SNc [78] Regulating NI appears therefore to be a relevant therapeutic approach for PD, and PET molecular imaging in animal models can bring valuable information in this context. A wide range of preclinical models of PD are to date available, and among them those developed in rodents are well-adapted to PET exploration. In this field, the most extensively used are the toxin-based models, in particular through the systemic injections of 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) in mice and stereotaxic delivery of 6-hydroxydopamine (6-OHDA) into the forebrain or in the striatum of rats.[79] These neurotoxins lead to the loss of DA neurons, activation of microglia and astrocytes and T-cells infiltration.[80] Inflammatory models of PD have also been produced through infusion in the SNc and striatum of the prostaglandin J2 (PGJ2) in mice and LPS in rats, mimicking progressive loss of DA neurons, and activation of astrocytes and microglia. [81] Besides these toxin-based models, rodent models of PD with genetic mutations have been generated, and among them the transgenic mice expressing α-synuclein has gained particular attention since aggregation of this misfolded protein is a feature of most cases of PD. [82] Several PET radioligands have already been used in rodent models of PD in particular for monitoring the density of dopamine transporters (DAT) and D2-receptors.[83] However, there are to date few PET studies investigating neuroinflammatory response associated to PD neurodegeneration in rodent models. Cicchetti *et al*. [84] have monitored in a 6-OHDA-lesioned rat model the degeneration of the DA system using the DAT tracer [^{11}C]CFT and have evaluated the microglial response by [^{11}C]PK11195 at 3 weeks post-lesion. Reduced [^{11}C]CFT uptake paralleled with increased [^{11}C]PK11195 binding in the striatum and SNc, indicative of a microglial activation, as confirmed by OX42 immunostaining. These findings encouraged further investigation about the relationships between NI and progressive DA neurons degeneration in PD using PET imaging. In their recently developed mouse model of PD induced by the SNc/striatum injection of PGJ2, Shivers *et al*. [81] have evaluated the neuroprotective effect of pituitary adenylate cyclase-activating polypeptide (PACAP). In vivo PET imaging with the TSPO ligand [^{11}C]PK11195 confirmed that PGJ2 induced a local microglia activation that was not significantly modified by the treatment. These in vivo results were also in agreement with in vitro Iba1 immunostaining. Very recently, Fricke *et al*. [85] have longitudinally studied the neurodegeneration, NI and neurogenesis in an intranigral 6-OHDA-induced PD mouse model. The microglial activation was evaluated using the TSPO tracer [^{18}F]DPA-714 which showed an increased accumulation at the direct lesion site but not at the striatal level. These results were also confirmed by in vitro Iba1 signal, demonstrating that in this

model the degeneration of dopaminergic cell bodies was accompanied with NI. It is interesting to note that when 6-OHDA is administered at the level of dopaminergic nerve endings, i.e. the striatum, the NI is observed both in this primary lesion site and in the SNc. [84, 86] Overall, although few data yet still available, it appears that in vivo PET imaging of NI in parallel to neurodegeneration in rodent models of PD can be useful to monitor the relationships between both processes and then evaluate different innovative neuroprotective approaches aiming at slow down the course of the disease.

Huntington's disease (HD)

Huntington's disease (HD) is a genetic disorder characterized by mutant huntingtin protein, that results in progressive loss of GABAergic medium spiny neurons (MSNs) in the striatum and in cortical areas leading to muscle coordination problems and cognitive impairment.[87] Several studies have hypothesized a role of NI, and in particular the activation of microglia, in the pathogenesis of HD. Studies on pre-symptomatic HD patients showed microgliosis in brain regions affected, that correlate with the clinical severity. Mutant huntingtin stimulates microglia to release cytokines, acting as proinflammatory factors that further induce microglial self-activation, followed by neuronal dysfunction and death. [88] Rodent models of HD have been developed and are classified in genetic and nongenetic. Prior to the transgenesis of huntingtin, rodent models of HD were based on intrastriatal injection of neurotoxins such as quinolinic acid (QA) and kainic acid (KA), or systemic injection of 3-nitropropionic acid (3-NP), 3-nitropropionic acid (3-NP) and malonic acid (MA), producing a predictable cell death in striatal neurons.[89] Most of the typical features of HD in humans are reproduced in these models, including neuronal loss, reactive glyosis and microglia activation. These models, and in particular the QA-induced one, have been recently reconsidered only on the light of the excitotoxic hypothesis of HD that involved a deregulation of the kynurenine pathway. [90] Genetically modified mouse and rat lines expressing a form of the mutant HTT (mHTT) gene might more representative of the HD progression and pathology. The R6/1, R6/2 and YAC transgenic mice models are the most widely used HD models and are characterized by a progressive striatal neuronal atrophy. In particular, knock-in mouse models are considered most representative because they develop moderate striatal pathology and exhibit an increased astrogliosis in the striatum at 24 months of age. [89] Moreover, regarding the study of the brain immune system, the use of transgenic animals may be even more interesting because it has been shown that the expression of mHTT in microglia promotes cell-autonomous pro-inflammatory transcriptional activation via myeloid lineage-determining factors and induce neuronal death in the presence of sterile inflammation.[91] There is no available HD–specific biomarkers for monitoring

disease progression and assess experimental treatments. Therefore, biomedical research is interested to investigate PET targets in HD focusing on the role of NI, using TSPO selective radiotracers. PET studies using TSPO radioligands, such as [^{11}C]PK11195, [^{11}C]GE180, [^{11}C]PBR28 identified increased activated microglia in HD patients, and have correlated striatal and extrastriatal neuronal dysfunction with increased microglial signal.[87] Limited PET data on microglial activation in HD are available except from AMPA- and QA - induced models that, for the high and reproducible presence of activated microglial, have been widely used for the evaluation and comparison of new TSPO radiopharmaceuticals. In addition, as previously stated, these experimental models based on the induction of striatal excitotoxicity reproduce the progressive loss of spiny neurons as well as other pathological. [92-95] Moresco *et al.*[96] for the first time quantified the time course of neurodegeneration in dopaminergic cells of striatum and NI in unilateral intrastriatal QA-injected rats. They showed a clear time-dependent reduction in the [^{11}C] Raclopride binding in QA treated animals, and neuronal loss was confirmed by evaluating the reduction of a neuronal nuclei marker (NeuN) and of D2 and adenosine A2A receptors densities by immunolabeling in the same animals. Moreover, they found and increase of [^{11}C]PK11195 binding in striatal and extrastriatal regions of the QA injected side. The signal peaked at 8 days and then progressively declined. [^{11}C]PK11195 data were confirmed by immunohistochemical analyses using the microglial marker OX-42 (CD11). Similar results were reported by Arlicot *et al.* [97] using the SPECT ligand CLINDE. TSPO expression was evaluated also in a longitudinal PET studies on cynomolgus monkeys QA model indicating a progressive increase of [^{18}F]DPA-714 binding, that culminated at 21 days but still present at 91 days. Immunohistochemistry revealed an early microglial and a delayed astrocytic activation. TSPO staining showed a stronger colocalization with CD68 microglia than with GFAP-activated astrocytes.[94] Some evidences indicate that TSPO ligands exert a neuroprotective activity, an effect that involves the modulation of microglial cells. In the QA model, Leaver *et al.* [98] showed that PK11195 and three other TSPO ligands increased neuronal survival, an effect that was associated with reduction of microglial activation, but not of astrogliosis. Unfortunately, this study is the only example of the use of TSPO radiotracers to investigate in vivo the effects of microglia modulation on illness progression and neuronal loss.

CONCLUSIONS

PET molecular imaging of NI in a number of preclinical models strongly contributes to improve our knowledge on cellular inflammatory populations involved in different CNS diseases. In addition, this approach brings highly relevant information on the in vivo spatio-temporal dynamics of inflammatory cell types/processes and on their contribution to pathogenic mechanisms. Preliminary data also support the potential role of preclinical PET on detecting the effects of treatments in reducing NI. It should also be stressed the important contribution of preclinical studies in the validation of new PET radioligands by assessing their in vivo brain kinetics, specificity, sensitivity and selectivity before application to human studies. To date, radioligands targeting TSPO sites were mostly developed and used. Although the results obtained in rodents cannot be always directly translated to humans and other limitations such as the PVE should be considered, overall these results demonstrated that TSPO expression was mostly related to increased microglial activation. This response could be detected and characterized especially in stroke and EAE models, while in models of neurodegenerative diseases, it appears more challenging but still feasible. Future studies are needed to explore in more detail the contribution of microglial activation to clinical outcome and disease progression, and to evaluate the effects of therapies. The issue of TSPO human polymorphism and the consequent different binding affinities of the second-generation radioligands, make however complex the use of these radiotracers in human studies. Thus TSPO radiotracers less sensitive to polymorphism are under evaluation. Moreover, interesting alternative radioligands targeting microglia and other NI processes have been proposed or are under development. Future effort should be done for the identification of selective PET biomarkers of microglia, macrophages, monocytes, B and T cells and astrocytes. In this respect, a particular interest is emerging for selective imaging of "neurotoxic" and "neuroprotective" microglial phenotypes, that might be simultaneously present in injuried tissues and more intriguingly, change over time the type and localization. [99] The design of such kind of radioligands is challenging, nevertheless promising probes are under investigation.

In this general context, microPET imaging in rodent models appears to be a strong opportunity to follow-up the neuroinflammatory processes along the pathological course. Moreover multitracers microPET studies may provide new insights on the relationships between NI and other specific hallmarks of brain disorders, such as neuronal integrity (through exploration of receptors or transporters) or abnormal protein aggregates in neurodegenerative diseases. If these studies will be performed in well-characterized animal models of NI and designed for a precise objective, they

formed in well-characterized animal models of NI and designed for a precise objective, they could represent a unique tool with high translational relevance.

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References

1. Jacobs AH, Tavitian B, INMind Consortium. Noninvasive molecular imaging of neuroinflammation. Journal of Cerebral Blood Flow & Metabolism 2012; 0:1–23.
2. Ory D, Celen S, Verbruggen A, Bormans G. PET Radioligands for In Vivo Visualization of Neuroinflammation. Curr Pharm Des 2014; 20: 5897-913.
3. Rosenblat JD, Cha DS, Mansur RB, McIntyre RS. Inflamed moods: a review of the interactions between inflammation and mood disorders. Prog Neuropsychopharmacol Biol Psychiatry 2014; 53: 23–34.
4. Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. Br J Pharmacol. 2006; 147: 232-40.
5. Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. Cell 2010;140: 918-934.
6. Wee Yong V. Inflammation in neurological disorders: a help or a hindrance? Neuroscientist 2010; 16: 408-20.
7. Waerzeggers Y, Monfared P, Viel T, Winkeler A, Jacobs AH. Mouse models in neurological disorders: applications of non-invasive imaging. Biochim Biophys Acta 2010; 1802: 819-39.
8. Schwab C, Klegeris A, McGeer PL. Inflammation in transgenic mouse models of neurodegenerative disorders. Biochim Biophys Acta. 2010;1802:889-902.
9. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, *et al*. Neuroinflammation in Alzheimer's disease. Lancet Neurol 2015; 14: 388-405.

10. Winkeler A, Boisgard R, Martin A, Tavitian B. Radioisotopic imaging of neuroinflammation. J Nucl Med 2010; 5: 1-4.

11. Lehnert W, Gregoire MC, Reilhac A, Meikle SR. Characterisation of partial volume effect and region-based correction in small animal positron emission tomography (PET) of the rat brain. Neuroimage 2012; 60: 2144-57.

12. Vicidomini C, Panico M, Greco A, Gargiulo S, Coda AR, Zannetti A, *et al*. In vivo imaging and characterization of [^{18}F]DPA-714, a potential new TSPO ligand, in mouse brain and peripheral tissues using small-animal PET. Nucl Med Biol 2015; 42: 309-16.

13. Gargiulo S, Anzilotti S, Coda AR, Gramanzini M, Greco A, Panico M, *et al*. Imaging of brain TSPO expression in a mouse model of amyotrophic lateral sclerosis with ^{18}F-DPA-714 and micro-PET/CT. Eur J Nucl Med Mol Imaging 2016; 43: 1348-59.

14. Toth M, Doorduin J, Haggikvist J, Varrone A, Amini N, Halldin C, et al. Positron Emission Tomography studies with [11C]PBR28 in the Healthy Rodent Brain: Validating SUV as an Outcome Measure of Neuroinflammation. PLoS One. 2015; 10: e0125917.

15. Janssen B, Vugts DJ, Funke U, Molenaar GT, Kruijer PS, van Berckel BN, *et al*. Imaging of neuroinflammation in Alzheimer's disease, multiple sclerosis and stroke: Recent developments in positron emission tomography. Biochim Biophys Acta. 2016; 1862: 425-41.

16. Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, *et al*. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. Nat Rev Drug Discov 2010; 9: 971-88.

17. Owen DR, Yeo AJ, Gunn RN, Song K, Wadsworth G, Lewis A, *et al*. An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. J Cereb Blood Flow Metab 2012; 32: 1–5.

18. Tiwari AK, Ji B, Yui J, Fujinaga M, Yamasaki T, Xie L, *et al*. [18F]FEBMP: Positron Emission Tomography Imaging of TSPO in a Model of Neuroinflammation in Rats, and in vitro Autoradiograms of the Human Brain. Theranostics 2015; 5: 961-69.

19. Lavisse S, Guillermier M, Hérard AS, Petit F, Delahaye M, Van Camp N, *et al*. Reactive astrocytes overexpress TSPO and are detected by TSPO positron emission tomography imaging. J Neurosci. 2012; 32: 10809-18.

20. Choi SH, Aid S, Bosetti F. The distinct roles of cyclooxygenase-1 and -2 in neuroinflammation: implications for translational research. Trends Pharmacol Sci. 2009; 30:174-81.

21. Minghetti L. Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases. J Neuropathol Exp Neurol. 2004; 63: 901-10.

22. Wu C, Li F, Niu G, Chen X. PET Imaging of Inflammation Biomarkers. Theranostics 2013; 3: 448-66.

23. Shukuri M, Mawatari A, Ohno M, Suzuki M, Doi H, Watanabe Y, *et al*. Detection of Cyclooxygenase-1 in Activated Microglia During Amyloid Plaque Progression: PET Studies in Alzheimer's Disease Model Mice. J Nucl Med. 2016; 57: 291-96.

24. Ashton JC, Glass M. The Cannabinoid CB2 Receptor as a Target for Inflammation-Dependent Neurodegeneration. Curr Neuropharmacol. 2007; 5: 73-80.

25. Ramírez BG, Blázquez C, Gómez del Pulgar T, Guzmán M, de Ceballos ML. Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. J Neurosci 2005; 25: 1904-13.

26. Ahmad R, Postnov A, Bormans G, Versijpt J, Vandenbulcke M, Van Laere K. Decreased in vivo availability of the cannabinoid type 2 receptor in Alzheimer's disease. Eur J Nucl Med Mol Imaging 2016 [Epub ahead of print]

27. Horti AG, Gao Y, Ravert HT, et al. Synthesis and biodistribution of [11C]-A-836339, a new potential radioligand for PET imaging of cannabinoid type 2 receptors (CB2). Bioorg Med Chem 2010; 18: 5202-207.

28. Monif M, Burnstock G, Williams DA. Microglia: Proliferation and activation driven by the P2X7 receptor. The International Journal of Biochemistry & Cell Biology 2010; 42: 1753-56

29. Ory D, Celen S, Gijsbers R, Van Den Haute C, Postnov A, Koole M, et al. Preclinical evaluation of a P2X7 receptor selective radiotracer: PET studies in a rat model with local overexpression of the human P2X7 receptor and in non-human primates. J Nucl Med. 2016 [Epub ahead of print].

30. Zinnhardt B, Viel T, Wachsmuth L, Vrachimis A, Wagner S, Breyholz HJ, *et al*. Multimodal imaging reveals temporal and spatial microglia and matrix metalloproteinase activity after experimental stroke. J Cereb Blood Flow Metab 2015; 35:1711-21.

31. Martín A, Szczupak B, Gómez-Vallejo V, Domercq M, Cano A, Padro D, et al. In vivo PET imaging of the α4β2 nicotinic acetylcholine receptor as a marker for brain inflammation after cerebral ischemia. J Neurosci. 2015; 35: 5998-6009.

32. Gulyás B, Pavlova E, Kása P, Gulya K, Bakota L, Várszegi S, *et al*. Activated MAO-B in the brain of Alzheimer patients, demonstrated by [11C]-L-deprenyl using whole hemisphere autoradiography. Neurochem Int 2011; 58: 60-68.

33. Carter SF, Schöll M, Almkvist O, Wall A, Engler H, Långström B, *et al*. Evidence for astrocytosis in prodromal Alzheimer disease provided by 11C-deuterium-L-deprenyl: a multitracer PET paradigm combining 11C-Pittsburgh compound B and 18F-FDG. J Nucl Med 2012; 53: 37–46.

34. Rodriguez-Vieitez E, Ni R, Gulyás B, Tóth M, Häggkvist J, Halldin C, et al. Astrocytosis precedes amyloid plaque deposition in Alzheimer APPswe transgenic mouse brain: a correlative positron emission tomography and in vitro imaging study. Eur J Nucl Med Mol Imaging 2015; 42:1119–32.

35. Nag S, Fazio P, Lehmann L, Kettschau G, Heinrich T, Thiele A, *et al*. In Vivo and In Vitro Characterization of a Novel MAO-B Inhibitor Radioligand, 18F-Labeled Deuterated Fluorodeprenyl. J Nucl Med 2016; 57:315-20.

36. Anrather J, Iadecola C. Inflammation and Stroke: An Overview. Neurotherapeutics. 2016; 13: 661-670.

37. Ceulemans AG, Zgavc T, Kooijman R, Hachimi-Idrissi S, Sarre S, Michotte Y. The dual role of the neuroinflammatory response after ischemic stroke: modulatory effects of hypothermia. Journal of Neuroinflammation 2010; 7:74-92.

38. Gelderblom M, Leypoldt F, Steinbach K, Behrens D, Choe CU, Siler DA, *et al*. Temporal and spatial dynamics of cerebral immune cell accumulation in stroke. Stroke 2009;40: 1849-57.

39. Durukan A, Tatlisumak T. Acute ischemic stroke: overview of major experimental rodent models, pathophysiology, and therapy of focal cerebral ischemia. Pharmacol Biochem Behav 2007; 87:179-197.

40. Rojas S, Martin A, Arranz MJ, Pareto D, Purroy J, Verdaguer E, *et al*. Imaging brain inflammation with [(11)C]PK11195 by PET and induction of the peripheral-type benzodiazepine receptor after transient focal ischemia in rats. J Cereb Blood Flow Metab 2007; 27:1975–86.

41. Hughes JL, Jones PS, Beech JS, Wang D, Menon DK, Aigbirhio FI, *et al*. A microPET study of the regional distribution of [11C]-PK11195 binding following temporary focal cerebral ischemia in the rat. Correlation with post mortem mapping of microglia activation. Neuroimage 2012; 59: 2007-16.

42. Martín A, Boisgard R, Thézé B, Van Camp N, Kuhnast B, Damont A, *et al*. Evaluation of the PBR/TSPO radioligand [(18)F]DPA-714 in a rat model of focal cerebral ischemia. J Cereb Blood Flow Metab 2010; 30: 230-41.

43. Boutin H, Prenant C, Maroy R, Galea J, Greenhalgh AD, Smigova A, *et al*. [18F]DPA-714: direct comparison with [11C]PK11195 in a model of cere- bral ischemia in rats, PLoS One 2013; 8: e56441.

44. Vicidomini C, Vinciguerra A, Greco A, Panico MR, S. Gargiulo S, Gramanzini M, *et al*. Imaging of brain TSPO expression in a mouse model of stroke with [18F]DPA-714 and micro-PET. Eur J Nucl Med Mol Imaging 2013; 40: S566–S567.

45. Schroeter M, Dennin MA, Walberer M, Backes H, Neumaier B, Fink GR, *et al*. Neuroinflammation extends brain tissue at risk to vital peri-infarct tissue: a double tracer [11C]PK11195- and [18F]FDG-PET study. J Cereb Blood Flow Metab. 2009; 29: 1216-1225.

46. Walberer M, Jantzen SU, Backes H, Rueger MA, Keuters MH, Neumaier B, *et al*. Schroeter, In-vivo detection of inflammation and neu- rodegeneration in the chronic phase after permanent embolic stroke in rats, Brain Res 2014;1581:80–88.

47. Walter HL, Walberer M, Rueger MA, Backes H, Wiedermann D, Hoehn M, *et al*. Schroeter, In vivo analysis of neuroinflammation in the late chronic phase after experimental stroke, Neuroscience 2015; 292: 71–80.

48. Pappata S, Levasseur M, Gunn RN, Myers R, Crouzel C, Syrota A, *et al*. Thalamic microglial activation in ischemic stroke detected in vivo by PET and [11C]PK11195. Neurology 2000; 55:1052–54.

49. Imaizumi M, Kim HJ, Zoghbi SS, Briard E, Hong J, Musachio JL, *et al*. PET imaging with [11C]PBR28 can localize and quantify upregulated peripheral benzodiazepine receptors associated with cerebral ischemia in rat. Neurosci Lett. 2007; 411: 200-05.

50. Martín A, Boisgard R, Kassiou M, Dollé F, Tavitian B. Reduced PBR/TSPO expression after minocycline treatment in a rat model of focal cerebral ischemia: a PET study using [(18)F]DPA-714. Mol Imaging Biol. 2011;13:10-15.

51. Wang Y, Yue X, Kiesewetter DO, Wang Z, Lu J, Niu G, *et al*. [18F]DPA-714 PET imaging of AMD3100 treatment in a mouse model of stroke. Mol Pharm 2014; 11: 3463–70.

52. Calabrese M, Magliozzi R, Ciccarelli O, Geurts JJ, Reynolds R, Martin R. Exploring the origins of grey matter damage in multiple sclerosis. Nat Rev Neurosci 2015; 16: 147-58.

53. Peferoen LA, Vogel DY, Ummenthum K, Breur M, Heijnen PD, Gerritsen WH, Peferoen-Baert RM, van der Valk P, Dijkstra CD, Amor S. Activation status of human microglia is dependent on lesion formation stage and remyelination in multiple sclerosis. J Neuropathol Exp Neurol. 2015; 74: 48-63.

54. Gold R, Linington C, Lassmann H. Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. Brain. 2006; 129: 1953-71.

55. Radu CG, Shu CJ, Shelly SM, Phelps ME, Witte ON. Positron emission tomography with computed tomography imaging of neuroinflammation in experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 2007; 104: 1937-42.

56. Buck D, Förschler A, Lapa C, Schuster T, Vollmar P, Korn T, *et al*. 18F-FDG PET detects inflammatory infiltrates in spinal cord experimental autoimmune encephalomyelitis lesions. J Nucl Med. 2012; 53: 1269-76.

57. Abourbeh G, Thézé B, Maroy R, Dubois A, Brulon V, Fontyn Y, et al. Imaging microglial/macrophage activation in spinal cords of experimental autoimmune

encephalomyelitis rats by positron emission tomography using the mitochondrial 18 kDa translocator protein radioligand [^{18}F]DPA-714. J Neurosci 2012; 32: 5728-36.

58. Coda AR, Greco A, Zannetti A, Barba P, Vicidomini C, Panico MR, *et al.* PET Imaging of brain TSPO expression in a mouse model of Experimental Autoimmune Encephalomyelitis using [18F]DPA-714. European Journal of Nuclear Medicine and Molecular Imaging 2014; 41: S322-S323.

59. Mattner F, Staykova M, Berghofer P, Wong HJ, Fordham S, Callaghan P, *et al*. Central nervous system expression and PET imaging of the translocator protein in relapsing-remitting experimental autoimmune encephalomyelitis. J Nucl Med 2013; 54: 291-8.

60. Airas L, Dickens AM, Elo P, Marjamäki P, Johansson J, Eskola O, *et al.* In vivo PET imaging demonstrates diminished microglial activation after fingolimod treatment in an animal model of multiple sclerosis. J Nucl Med. 2015; 56: 305-10.

61. Liu H, Jin H, Yue X, Luo Z, Liu C, Rosenberg AJ, *et al*. PET Imaging Study of S1PR1 Expression in a Rat Model of Multiple Sclerosis. Mol Imaging Biol. 2016 [Epub ahead of print].

62. Martín A, Vázquez-Villoldo N, Gómez-Vallejo V, Padro D, Soria FN, Szczupak B, *et al.* In vivo imaging of system xc- as a novel approach to monitor multiple sclerosis. Eur J Nucl Med Mol Imaging 2016; 43: 1124-38.

63. Venneti S, Lopresti BJ, Wang G, Hamilton RL, Mathis CA, Klunk WE, *et al.* PK11195 labels activated microglia in Alzheimer's disease and in vivo in a mouse model using PET. Neurobiology of Aging 2009; 30:1217–26.

64. Perry VH, Nicoll JA, Holmes C. Microglia in neurodegenerative disease. Nat Rev Neurol 2010; 6: 193-201.

65. Gate D, Rezai-Zadeh K, Jodry D, Rentsendorj A, Town T. Macrophages in Alzheimer's disease: the blood-borne identity. Journal of Neural Transmission. 2010; 117: 961-70.

66. Rapic S, Backes H, Viel T, Kummer MP, Monfared P, Neumaier B, et al. Imaging microglial activation and glucose consumption in a mouse model of Alzheimer's disease. Neurobiol Aging 2013; 34:351–354

67. Maeda J, Higuchi M, Inaji M, Ji B, Haneda, E, Okauchi T, *et al.* Phase-dependent roles of reactive microglia and astrocytes in nervous system injury as delineated by imaging of peripheral benzodiazepine receptor. Brain Res 2007; 1157: 100–111.

68. Maeda J, Zhang MR, Okauchi T, Ji B, Ono M, Hattori S, et al. In vivo positron emission tomographic imaging of glial responses to amyloid-beta and tau pathologies in mouse models of Alzheimer's disease and related disorders. J Neurosci 2011;31:4720-4730.

69. James ML, Belichenko NP, Nguyen TV, Andrews LE, Ding Z, Liu H, *et al*. PET imaging of translocator protein (18 kDa) in a mouse model of Alzheimer's disease using N-(2,5-dimethoxybenzyl)-2-18F-fluoro-N-(2phenoxyphenyl)-acetamide. J Nucl Med 2015;56:311-316.

70. Brendel M, Probst F, Jaworska A, Overhoff F, Korzhova V, Albert NL, *et al*. Glial Activation and Glucose Metabolism in a Transgenic Amyloid Mouse Model: A Triple-Tracer PET Study. J Nucl Med 2016; 57: 954-960.

71. Sérrière S, Tauber C, Vercouillie J, Mothes C, Pruckner C, *et al*. Amyloid load and TSPO in APPswePS1-dE9 mice: a longitudinal study. Neurobiol Aging. 2015; 36:1639-52.

72. Mirzaei N, Tang SP, Ashworth S, Coello C, Plisson C, Passchier J, *et al*. In vivo imaging of microglial activation by positron emission tomography with [(11)C]PBR28 in the 5XFAD model of Alzheimer's disease. Glia 2016; 64: 993-1006.

73. Savonenko AV, Melnikova T, Wang Y, Ravert H, Gao Y, Koppel J, *et al*. Cannabinoid CB2 Receptors in a Mouse Model of Aβ Amyloidosis: Immunohistochemical Analysis and Suitability as a PET Biomarker of Neuroinflammation. PLoS One 2015; 10:e0129618.

74. Rodriguez-Vieitez E, Carter SF, Chiotis K, Saint-Aubert L, Leuzy A, Schöll M, *et al*. Comparison of Early-Phase 11C-Deuterium-l-Deprenyl and 11C-Pittsburgh Compound B PET for Assessing Brain Perfusion in Alzheimer Disease. J Nucl Med 2016; 57:1071-1077.

75. Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, *et al*. Amyotrophic lateral sclerosis. Lancet. 2011; 377: 942–55.

76. McGoldrick P, Joyce PI, Fisher EMC, Greensmith L. Rodent models of amyotrophic lateral sclerosis. Biochimica et Biophysica Acta 2013; 1832:1421–36.

77. Brownell AL, Kuruppu D, Kil KE, Jokivarsi K, Poutiainen P, Zhu A, et al. PET imaging studies show enhanced expression of mGluR5 and inflammatory response during progressive degeneration in ALS mouse model expressing SOD1-G93A gene. J Neuroinflammation. 2015; 12:217.

78. Lee JK, Tran T, Taney MG. Neuroinflammation in Parkinson's disease. J Neuroimmune Pharmacol 2009; 4: 419-29.

79. Blesa J, Przedborski S. Parkinson's disease: animal models and dopaminergic cell vulnerability. Front Neuroanat 2014; 8: e155.

80. Cebrián C, Loike JD, Sulzer D. Neuroinflammation in Parkinson's disease animal models: a cell stress response or a step in neurodegeneration? Curr Top Behav Neurosci. 2015; 22: 237-70.

81. Shivers KY, Nikolopoulou A, Machlovi SI, Vallabhajosula S, Figueiredo-Pereira ME. PACAP27 prevents Parkinson-like neuronal loss and motor deficits but not microglia activation induced by prostaglandin J2. Biochim Biophys Acta. 2014;1842:1707-19.

82. Schwab C, Klegeris A, McGeer PL. Inflammation in transgenic mouse models of neurodegenerative disorders. Biochimica et Biophysica Acta 2010; 1802: 889–902.

83. Inaji M, Okauchi T, Ando K, Mada J, Nagai Y, Yoshizaki T, *et al.* Correlation between quantitative imaging and behavior in unilaterally 6-OHDA-lesioned rat. Brain Res 2005; 1064: 136-145.

84. Cicchetti F, Brownell AL, Williams K, Chen YI, Livni E, Isacson O. Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging. Eur J Neurosci. 2002;15: 991-98.

85. Fricke IB, Viel T, Worlitzer MM, Collmann F, Vrachimis A, Faust A, *et al.* 6-hydroxydopamine-induced Parkinson's disease-like degeneration generates acute migrogliosos and astrogliosis in the nigrostriatal system but no bioluminescence imaging-detectable alteration in adult neurogenesis. Eur J Neurosci 2016; 43: 1352-65.

86. Maia S, Arlicot N, Vierron E, Bodard S, Vergote J, Guilloteau D, Chalon S. Longitudinal and parallel monitoring of neuroinflammation and neurodegeneration in a 6-hydroxydopamine rat model of Parkinson's disease. Synapse 2012; 66: 573-83.

87. Pagano G, Niccolini F, Politis M. Current status of PET imaging in Huntington's disease. Eur J Nucl Med Mol Imaging 2016; 43: 1171–82.

88. Connolly C, Magnusson-Lind A, Lu G, Wagner PK, Southwell AL, Hayden MR, *et al*. Enhanced immune response to MMP3 stimulation in microglia expressing mutant huntingtin. Neuroscience 2016; 14: 74-88.

89. Ramaswamy S, McBride JL, Kordower JH. Animal Models of Huntington's Disease. ILAR Journal. 2007; 48: 356-73.

90. Schwarcz R, Guidetti P, Sathyasaikumar KV, Muchowski PJ. Of mice, rats and men: Revisiting the quinolinic acid hypothesis of Huntington's disease. Prog Neurobiol 2010; 90: 230-45.

91. Crotti A, Benner C, Kerman BE, Gosselin D, Lagier-Tourenne C, Zuccato C, *et al.* Mutant Huntingtin promotes autonomous microglia activation via myeloid lineage-determining factors. Nat Neurosci. 2014; 17: 513-21.

92. Boutin H, Chauveau F, Thominiaux C, Grégoire MC, James ML, Trebossen R, *et al*. 11C-DPA-713: a novel peripheral benzodiazepine receptor PET ligand for in vivo imaging of neuroinflammation. J Nucl Med 2007; 48: 573-81.

93. Damont A, Boisgard R, Kuhnast B, Lemée F, Raggiri G, Scarf AM, Da Pozzo E, *et al*. Synthesis of 6-[^{18}F]fluoro-PBR28, a novel radiotracer for imaging the TSPO 18 kDa with PET. Bioorg Med Chem Lett 2011; 21:4819-22.

94. Lavisse S, Inoue K, Jan C, Peyronneau MA, Petit F, Goutal S, *et al*. [18F]DPA-714 PET imaging of translocator protein TSPO (18 kDa) in the normal and excitotoxically-lesioned nonhuman primate brain. Eur J Nucl Med Mol Imaging. 2015; 42: 478-94.

95. Di Grigoli G, Monterisi C, Belloli S, Masiello V, Politi LS, Valenti S, *et al*. Radiosynthesis and Preliminary Biological Evaluation of [18F]VC701, a Radioligand for Translocator Protein. Mol Imaging. 2015;14. doi: 10.2310/7290.2015.00007.

96. Moresco RM, Lavazza T, Belloli S, Lecchi M, Pezzola A, Todde S, *et al*. Quinolinic acid induced neurodegeneration in the striatum: a combined in vivo and in vitro analysis of receptor changes and microglia activation. Eur J Nucl Med Mol Imaging. 2008; 35: 704-15.

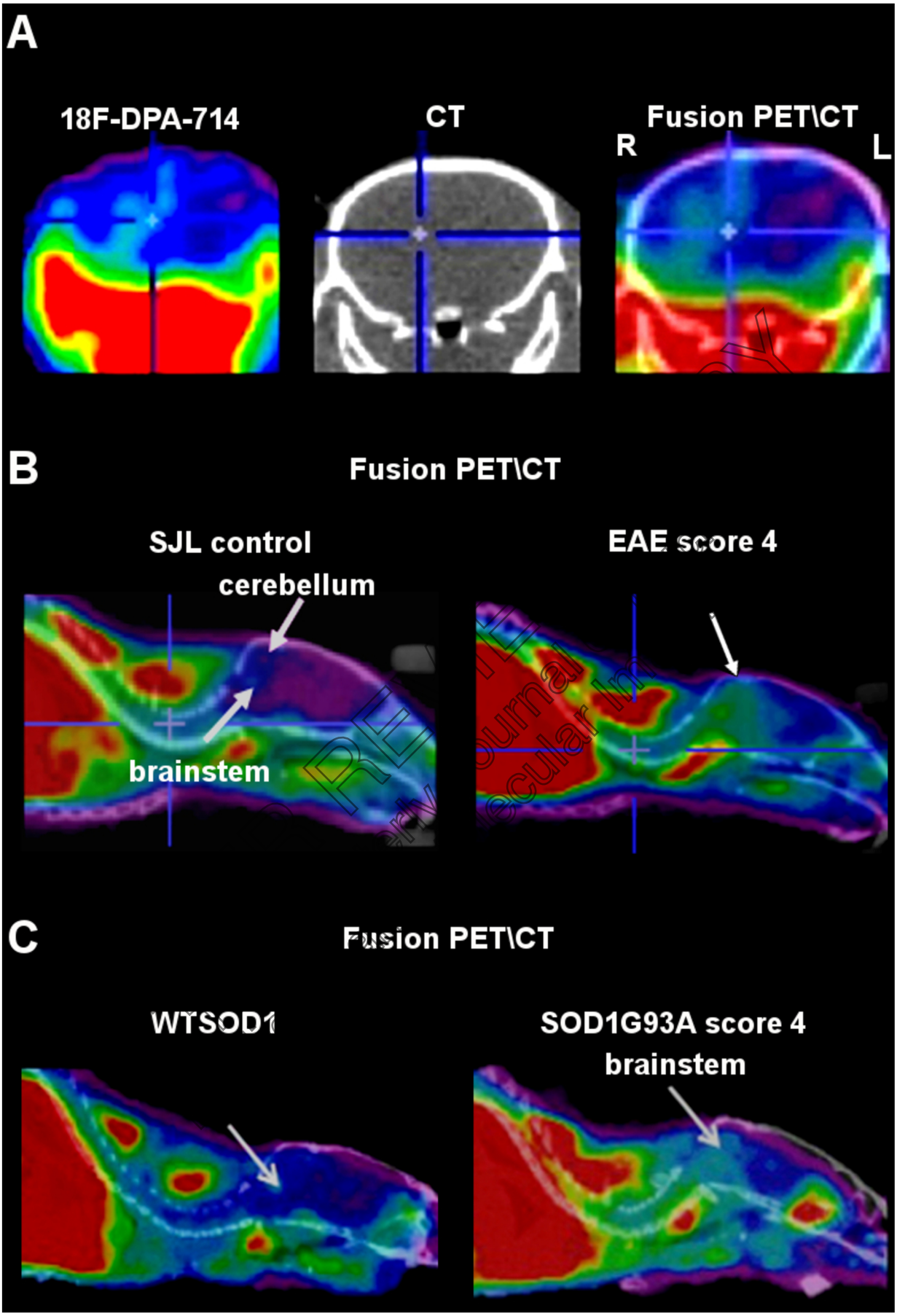
97. Arlicot N, Tronel C, Bodard S, Garreau L, de la Crompe B, Vandevelde I, *et al*. Translocator protein (18 kDa) mapping with [125I]-CLINDE in the quinolinic acid rat model of excitotoxicity: a longitudinal comparison with microglial activation, astrogliosis, and neuronal death. Mol Imaging 2014; 13: 4-11.

98. Leaver KR, Reynolds A, Bodard S, Guilloteau D, Chalon S, Kassiou M. Effects of translocator protein (18 kDa) ligands on microglial activation and neuronal death in the quinolinic-acid-injected rat striatum. ACS Chem Neurosci 2012; 3: 114-9.

99. Benakis C, Garcia-Bonilla L, Iadecola C1, Anrather J. The role of microglia and myeloid immune cells in acute cerebral ischemia. Front Cell Neurosci. 2015; 8: e461.

Figures

Figure 1. A. [^{18}F]DPA-714 PET/CT in a mouse model of MCAO, 7 days after 20 minutes of right transient occlusion: transaxial PET images acquired between 20 and 50 minutes after tracer injection showed increased [^{18}F]DPA-714 uptake in the ischemic side (ref. 44). **B**. PET sum images 20-50 minutes co-registered to CT: sagittal sections showed that [^{18}F]DPA-714 uptake is increased in EAE mice with respect to the control in the brainstem and cerebellum. This increase is higher in the mouse clinically more affected (score 4) (ref. 58). **C**. PET sum images 20-50 minutes co-registered to CT: sagittal sections showed that [^{18}F]DPA-714 uptake is increased in symptomatic SOD1^{G93A} mice (score 4) with respect to the WT SOD1 in the brainstem (white arrow) (ref. 13).


A
18F-DPA-714
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