Positron emission tomography imaging in evaluation of MS pathology in vivo

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Abstract

Positron emission tomography (PET), gives an opportunity to quantitate the expression of specific molecular targets *in vivo* and longitudinally in brain, and thus enhances our possibilities to understand and follow up MS-related pathology. For successful PET imaging, one needs a relevant target molecule within the brain, to which a blood-brain barrier-penetrating specific radioligand will bind. TSPO-binding radioligands have been used to detect activated microglial cells at different stages of MS, and remyelination has been measured using amyloid PET. Several PET ligands for detection of other inflammatory targets besides TSPO have been developed but not yet been used for imaging MS patients. Finally, synaptic density evaluation has been successfully tested in human subjects, and gives opportunities for evaluation of the development of cortical and deep gray matter pathology in MS. This review will discuss PET imaging modalities relevant for MS today.

Abbreviations

2-AG	2-arachidonoylglycerol
A2A	adenosine receptor A2A
AD	Alzheimer's disease
ALS	amyotrophic lateral sclerosis
APP	β amyloid precursor protein
ATP, ADP, AMP	adenosine triphosphate/diphosphate/monophosphate
BBB	blood brain barrier
CB	cannabinoid receptor
CNS	central nervous system
COX	cyclooxygenase
EAE	experimental autoimmune encephalomyelitis
ECS	endocannabinoid system
FR	folate receptor
GM	grey matter
iNOS	inducible nitric oxide synthase
M1	classically activated or proinflammatory microglia
M2	alternatively activated or anti-inflammatory microglia
MMP	matrix metalloproteinase
MS	multiple sclerosis

1	nAChR	nicotinic acetylcholine receptors
2	NAWM	normal appearing white matter
3	2X	ionotropic purinergic receptor
4	P2Y	metabotropic purinergic receptor
5	PD	Parkinson's disease
6	PET	positron emission tomography
7	ROS	reactive oxygen species
8	RRMS	relapsing remitting MS
9	SPMS	secondary progressive MS
10	SV2A	synaptic vesicle glycoprotein 2A
11	TSPO	18-kDa translocator protein

12

13 **Introduction**

14 The diagnosis and follow-up of multiple sclerosis (MS) is mostly based on conventional
15 MRI including T2-, T1-weighted and post-gadolinium T1-weighted images.[1, 2] The
16 technique is, however, unspecific and unable to fully differentiate between
17 abnormalities such as inflammation, demyelination, ischemia and neural damage.[3]
18 Thus, more specific *in vivo* molecular imaging techniques are needed for better
19 understanding of the disease pathology.

20

Positron emission tomography (PET) imaging enables targeted, quantitative and non-invasive imaging of physiological and pathological processes *in vivo*. The technique is based on radiolabeled ligands which accumulate into target tissues and structures. The radiolabels are radioisotopes with short half-life, such as ^{11}C, ^{15}O and ^{18}F. The ligands on the other hand are molecules which bind specific targets expressed during processes of interest. The hallmarks of MS pathology and thus the processes in focus are inflammation, gliosis, demyelination, and degeneration. Using PET techniques, it is possible to image the heterogeneity of the MS lesions and inflammatory changes in normal appearing white matter (NAWM) and grey matter (GM), but also functional changes of the brain. Brain PET imaging in MS has focused on imaging the reactive immune cells of the brain-resident innate immune system, that is, the microglia and macrophages. Other targets for MS-relevant PET imaging include reactive astrocytes, neurons, and myelin. In this review, we discuss the present status of ligand development and the practical experience in imaging of the various MS-relevant targets.

Microglia as an imaging target

Microglia are resident immune cells of the central nervous system (CNS). They are very much like peripheral macrophages as they are motile and capable of phagocytosis, and of secreting cytokines, chemokines, free radicals, and neurotrophic factors.[4] Microglia have two important functions in the CNS: defense and maintenance. As part of the innate immune system, they take care of the immune defense of the CNS. They also

maintain homeostasis by contributing to neuronal proliferation and differentiation, and modulation of synaptic connections.[5]

In a healthy CNS the morphology of microglia is ramified, and they are considered as "resting". Resting is somewhat misleading as even in this stage the microglia are actively gathering information about their microenvironment and can thus be considered rather as surveying than resting.[6] Change in the homeostasis of CNS causes resting microglia to be activated. Previously, the distinction of microglia between resting and activated was considered as on–off situation. However, this process seems more complex and dependent on the activation mechanism. Classically activated or pro-inflammatory microglia (M1-like) are activated by interferon ɣ, and they secrete reactive oxygen species (ROS) and inflammatory cytokines.[7] Alternatively activated or anti-inflammatory microglia (M2-like) are divided to three subclasses (M2a, M2b and M2c) according to their function and molecules stimulating the activation.[8] Microglial activity is induced in several neurodegenerative pathologies including MS, Alzheimer's (AD), and Parkinson's diseases (PD), and amyotrophic lateral sclerosis (ALS). Thus, it has become a major target of PET brain imaging of neuroinflammation and neurodegeneration.

18-kDa translocator protein

Most of the PET studies on MS have focused on imaging activated microglia using the 18-kDa translocator protein (TSPO) as a target. TSPO is a protein expressed on outer membrane of mitochondria of activated microglia, and its high expression is linked to neuroinflammation and neuronal injury.[9] Recent evidence from *in vitro* and animal studies also indicates that the increased expression of TSPO in neuroinflammation might be specific for proinflammatory M1-like microglia.[10] TSPO expression has however, also been described in macrophages and in some astrocytes.[11]

The oldest TSPO-ligand is [^{11}C]PK11195. Due to the shortcomings of [^{11}C]PK11195 characteristics, such as relatively low blood-brain barrier (BBB) penetration and high non-specific binding, new second- and third-generation TSPO ligands have been developed. These, however, show heterogeneous binding to TSPO due to genetic polymorphism (rs6971), which complicates interpretation of the results. For [^{11}C]PBR28[12] and [^{18}F]FEPPA[13] this has been investigated in detail, but similar differences in the binding affinity between subjects has been observed for all second- and third-generation TSPO ligands. The sensitivity of only one second-generation tracer, namely [^{11}C]ER176, has been shown to be sufficient enough for the quantification of all three binding affinity types.[14] For all other tracers, low-affinity binders have too low specific radioligand binding to allow reliable quantification.

Despite the challenges, TSPO imaging has provided valuable information about microglial activation in MS *in vivo*. Using [^{11}C]PK11195, investigators have demonstrated that microglial activation is not only increased in MRI-detected focal inflammatory lesions in MS patients with active disease, but also in the NAWM and at the rim of chronic active lesions in patients with secondary progressive MS (SPMS), compared to relapsing-remitting multiple sclerosis (RRMS) and healthy controls. Importantly, the level of TSPO binding correlates with clinical disability.[15, 16] Figure 1 demonstrates the TSPO binding patterns related to demyelination in an MS patient. We have recently reviewed TSPO imaging in detail in this journal and elsewhere.[17, 18]

Cannabinoid receptor CB2

Cannabinoid receptors are G-protein coupled receptors of two subtypes, cannabinoid receptor 1 (CB1) which is mainly expressed in the CNS and cannabinoid receptor 2 (CB2) which is found particularly in the immune system, but also in certain parts of the CNS.[19] CB2 is expressed in neurons and microglia, and the expression is increased upon microglial activation.[20] There is growing evidence suggesting that CB2 receptor expression and activity contributes to the shift of M1-like microglia towards M2-like microglia. This makes CB2 an interesting and potential target of *in vivo* imaging of neuroinflammation.[21, 22] Interestingly, microglia themselves produce cannabinoid receptor ligand 2-arachidonoylglycerol (2-AG) in response to adenosine triphosphate

(ATP) stimulation,[23, 24] and the production has been linked to another receptor of interest, purinergic receptor P2X7,[24] discussed later in this review.

In MS, endocannabinoid system (ECS) is disrupted in multiple ways, and the expression of CB receptors, the production of endocannabinoids, and the expression of enzymes metabolizing them is altered.[19] The CB2 has been the main focus of interest as its levels are increased in neuroinflammation and during microglial activation while the expression level of CB1 is unaltered. The activity of CB1 has however been shown to reduce the severity of experimental autoimmune encephalomyelitis (EAE) in mice, which suggests a protective role for CBs.[19] Together with the recent report showing an early increase in CB2 following ischemia,[25] these findings suggest that CB2 expression might indicate an early stage of activation, or "primed" state of microglia. This might be related to an early neuroprotective response to injury. Altogether, the changes in ECS have made it a potentially interesting *in vivo* imaging target. To date the most promising, and the only tracer tested in human subjects, is [^{11}C]NE40.[26, 27]

The role of purinergic receptors during neuroinflammation

The purinergic system includes a heterogenous group of purinergic receptors facilitating important signaling pathways of the nervous and immune system.[28] Extracellular purine and pyrimidine nucleosides and nucleotides, especially ATP, are important signaling

molecules in the brain, and responsible for neuron-to-neuron and neuron-to-glia communication.[29] ATP is a direct ligand of purinergic receptors, but its effects are also mediated through extracellular ecto-nucleotidases which produce extracellular adenosine diphosphate (ADP), adenosine monophosphate (AMP), and adenosine and thus control the ligand availability of nucleotide and adenosine receptors.[30, 31] Nucleotides act through ionotropic (P2X) and metabotropic (P2Y) purinergic receptors, whereas adenosine activates G protein–coupled receptors adenosine A1 receptor (A1), adenosine A2A receptor (A2A), adenosine A2B receptor (A2B), and adenosine A3 receptor (A3).[32]

Adenosine A2A receptor

Among the four types of adenosine receptors, A2A signaling has been particularly described as a potent regulator of inflammation.[33] A2A receptors (A2ARs) are most abundant in the striatum, and in pathological conditions their expression has been demonstrated also in other CNS areas and cell types, such as microglia and astrocytes.[34] Most of all, A2AR has been shown to be involved in glial activation and neuroinflammation.[35] Development of several PET tracers (table 1) have enabled imaging of A2AR and revealed its increased and spreading expression in neuroinflammatory and neurodegenerative diseases including MS.[35, 36] In AD, the astrocytic expression of A2AR has been recently shown,[34] but the identity and role of

A2AR-expressing cells in MS remains to be seen. For further reading, we have recently reviewed imaging of A2AR in more detail elsewhere.[35]

Purinergic P2X7 receptor (P2X7R)

P2X7 receptors (P2X7R) are ATP-gated non-selective ion channels which are abundant in microglia, and they are also expressed in other glial cells such as astrocytes and oligodendrocytes. Neuronal expression of P2X7 has been somewhat controversial[37, 38] but most of the evidence indicates its expression on presynaptic terminals[28]. The receptor activity in immune cells indicates a role for P2X7 in immune functions and inflammatory responses, and its activity has been shown to promote microglial activation and proliferation.[39] Most of all, the activity of P2X7 is a key event in activation and recruitment of the inflammasome,[40] which is needed for processing and release of proinflammatory cytokines interleukin (IL)-1β and IL-18.[41] The interest in P2X7 as an imaging target for PET rises from its expression in microglia and increased activity during pathologies including neuroinflammation.[28] Recent reports indicate that P2X7 could be used in discriminating the proinflammatory M1-like microglia from M2-like microglia.[42]

Increased expression of P2X7 in activated microglia has been reported in MS,[42, 43] although a recent study found that the P2X7 receptor is expressed yet inhibited in peripheral monocytes during acute phases of relapsing-remitting MS (RRMS).[44] This

report further shows an increase in the expression of P2X7 in the astrocytes in the frontal cortex of SPMS patients. Several ligands targeting P2X7 have been evaluated preclinically (table 2) but only a few have shown potential to proceed to studies in humans.[45, 46] The existence of multiple splice variants and genetic polymorphism of the P2X7 gene[47, 48] issue a challenge to research as some of them affect the function of the receptor, and thus may also lead to heterogeneity in binding potential of the tracer.

In addition, another purinergic receptor P2Y12 has been of interest as it is expressed particularly in resident M2-like microglia, and not in peripheral macrophages.[49] In line, its expression has been reported to be altered in pathologies such as AD, ALS, and MS.[50-52] However, more research is needed before P2Y12 can be considered as a valid PET imaging target.

Imaging myelin

Destruction of the myelin sheath wrapped around axons in the CNS is one of the hallmarks of MS. Remyelination can however restore the axonal function lost upon the demyelination process, and enhancing remyelination is a potential therapeutic approach. Hence, the ability to measure the remyelination process *in vivo* is of great interest both for designing therapeutic studies, and for evaluation of natural disease evolution. Thioflavine T derivatives (Pittsburgh Compound-B (PiB), flutemetamol, florbetabir and florbetaben) are ligands that bind to fibrillar amyloid Aβ deposits and have mainly been

used for the detection of cortical gray matter amyloid pathology in AD and related disorders. However, since these ligands also bind avidly to myelin in the white matter, with β amyloid precursor protein (APP) being the proposed binding site and playing a role in the processes of demyelination and remyelination, there has been growing interest for amyloid PET imaging in MS.[53] Indeed, the degree of demyelination in MS can be measured using [^{11}C]PiB showing not only decreased binding within demyelinated lesions, but also increasing binding with dynamic remyelination correlating to decreasing disability in follow-up.[54, 55] In addition, other promising PET tracers for myelin imaging have also been developed[56] and used in animal models of MS. Table 3 summarizes the key ligands and studies related to PET imaging of myelin in MS.

Imaging grey matter pathology – focus on neuronal synapses

Recent neuropathological studies have revealed over 50 % reduction in synaptic density in both lesioned and normal-appearing cortical grey matter in patients with late RRMS or SPMS.[57] A novel PET ligand binding to a synaptic vesicle glycoprotein 2A (SV2A),[58, 59] [^{11}C]UCB-J, now enables imaging of synapses in the living human brain.[59] PET studies using this ligand will likely increase our understanding of the temporal development of grey matter pathology in MS and the association of white matter pathology to the developing neurodegeneration. The ability to image the synaptic

density *in vivo* will likely have enormous potential in clarifying the mechanisms leading to progression of the disease.

Other potential PET imaging targets of neuroinflammation

There are several other potential PET imaging targets which have been used only in preclinical research or have shown potential also in clinical research but are still missing the final proof of their usability. We summarize shortly some of these targets and the pathology behind them in table 4.

Future perspectives

TSPO-binding radioligands have formed the cornerstone of PET imaging of neuroinflammation, but the field is still struggling with challenges related to TSPO-ligands, such as non-specificity of ligand binding, and heterogeneity of analysis methods.[18] New TSPO ligands have been developed to overcome these problems, but genetically determined differences in the binding affinities of the second- and third-generation TSPO ligands have brought more variables into the already complex picture. Better targets than TSPO are needed for imaging neuroinflammation, and ligands binding to such varied CNS targets as P2X7 receptor, A2A receptor and CB2 receptor are already in human investigational use. Other aspects of MS pathology, such as remyelination and loss of synapses can be evaluated using PET imaging and radioligands binding to amyloid protein and SV2A, respectively (Figure 2). Strong

collaboration is needed between neuroimmunologists, neurobiologists, neuropathologists, radiochemists, and PET imagers for optimal detection of the most relevant CNS targets for *in vivo* imaging of MS disease-relevant pathology and for the development of PET ligands binding to these targets. Longitudinal PET imaging has the potential to elucidate kinetics of certain aspects of MS pathology, such as astrogliosis, remyelination, and microglial activation. It can be used to evaluate the treatment effects of new and existing drugs on various aspects of MS pathology at different stages of MS.[60-62] Finally, PET studies may provide new predictive imaging biomarkers to detect those patients most at risk of disease progression. Due to the complex nature of the technique and high costs involved, PET imaging can never replace MR imaging in the evaluation of MS pathology, but it can excellently complement MR imaging by bringing molecular specificity to *in vivo* evaluation of MS brain pathology.

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Declaration of Conflicting Interests

The Authors declare no conflict of interest.

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Table 1: Currently available A2A-receptor binding radioligands.

Radioligand	Model organism	Key results	References
[^{11}C]TMSX	Human PD, *in vivo* Human SPMS, *in vivo*	Increased uptake in putamen among PD patients with dyskinesia. Increased uptake of the tracer in NAWM of SPMS patients.	Mishina et al., 2011[63] Rissanen et al., 2013[36]
[^{18}F]MNI-444	Healthy human subjects, *in vivo*	Useful for imaging A2A in the human brain, good in vivo brain kinetic properties and test-retest variability.	Barret et al., 2015[64]
[^{11}C]SCH442416	Rat, *ex vivo* and non-human primate, *in vivo*	Good kinetic properties, high nonspecific binding.	Moresco et al., 2005[65]
	Healthy human subjects, in vivo	Complex tracer kinetics, low specific binding.	Grachev et al., 2014[66]
[^{18}F]-FESCH*	Rat, *in vivo*	Tracer kinetics similar to [^{11}C]Preladenant, fluorinated compound and therefore provides more flexibility.	Khanapur et al., 2017[67]
[^{11}C]Preladenant	Rat, *in vivo*	Good kinetic properties, high striatal uptake, low extrastriatal binding. Radiometabolites in the brain.	Zhou et al., 2017a[68]
	Non-human primate, *in vivo*	High striatal uptake, specific binding.	Zhou et al., 2017b[69]
	Healthy human subjects, *in vivo*	Specific binding, pharmacologically safe. Possibly some radiometabolites in the brain.	Sakata et al., 2017[70]

Abbreviations: PD – Parkinson's disease, SPMS – secondary progressive MS, NAWM – normal appearing white matter
* Analog of SCH442416

Table 2: P2X7 receptor-binding radioligands in development.

Radioligand	Model organism	Key results	Reference
[^{3}H]A-74003	Rat, *in vivo* Rat (EAE) and human MS post-mortem tissues	Marginal uptake. Binding to pro-inflammatory microglia.	Janssen et al. 2014[71] Beaino et al. 2017[42]
[^{18}F]EFB	LPS-induced rat model, *in vivo*	Low penetration through the BBB but still detectable.	Fantoni et al 2017[72]
[^{11}C]JNJ-54173717	Humanized rat and non-human primate, *in vivo*	Good penetration through the BBB, indications of specific binding.	Ory et al. 2016[45]
[^{11}C]-GSK1482160	LPS-induced mouse model, *in vivo* EAE rat model, *in vitro* and non-human primate, *in vivo*	Favorable kinetics, specific binding. Good penetration through BBB. Binding correlates with the number of activated microglia in EAE rat model.	Territo et al 2017[73] Han et al 2017[74]
[^{11}C]SMW139	Rat, *in vivo and ex vivo*, and human AD post-mortem tissues Human MS, *in vivo*	High metabolic stability and receptor binding. No significant difference in binding between controls and AD patients, and no correlation with immunostaining. Clinical studies in MS patients ongoing.	Janssen et al. 2018[75] INMIND report, 2017[46]
[^{18}F]IUR-1601	-	Successful synthesis, preliminary biological evaluation demonstrates good binding affinity.	Gao et al. 2018[76]

Abbreviations: EAE - experimental autoimmune encephalomyelitis, LPS – lipopolysaccharide, BBB – blood-brain barrier, AD – Alzheimer's disease,

Table 3: Radioligands utilized in imaging of demyelination and remyelination in MS.

Radioligand	Model organism	Key results	Reference
[^{11}C]PiB	Focal lesional rat model, *in vivo*	High uptake in WM and cerebrum but low in cerebellum.	de Paula Faria et al., 2014 a[77]
	Non-human primate and human MS, *in vivo*	Specific binding to myelin in WM, binding in humans quantitable using SUV.	Stankoff et al., 2011[54]
	Human MS, *in vivo*	Reduced binding in MS lesions, dynamic remyelination inversely correlated with clinical disability.	Bodini et al., 2016[55]
		Reduced uptake in lesional WM and NAWM associated with decreased visuospatial performance in late MS.	Zeydan et al. 2017[78]
[^{11}C]MeDAS	Focal lesional rat model, *in vivo*	Radioligand distribution and uptake correlate well with myelin density in focally induced lesions. Higher uptake in healthy WM, cerebellum and brain stem when compared to [^{11}C]PiB.	de Paula Faria et al., 2014a[77]
	Focal lesional and EAE rat models, *ex vivo* and *in vivo*	Changes in uptake correlate well with associated myelin loss in the spinal cord.	Wu et al. 2013[79]
[^{11}C]CIC	Focal lesional rat model, *in vivo*	Ligand vulnerable to photoisomerizaton. Slow kinetics, homogeneous brain uptake, less suitable for *in vivo* imaging	de Paula Faria et al., 2014a[77]
[^{18}F]3-F-4-AP	Demyelination and focal lesional mouse and rat models, *ex vivo* and *in vivo*, non-human primate, *in vivo*	Targets axonal voltage-gated K-channels exposed upon demyelination. Binding increases with demyelination. Good properties for brain imaging; fast entry into brain, slow to moderate washout, highest binding in GM and lowest in WM.	Brugarolas et al. 2018[80]

Abbreviations: PiB = Pittsburgh compound B, WM – white matter, SUV = standardized uptake value, NAWM – normal appearing white matter, EAE - experimental autoimmune encephalomyelitis, GM = gray matter

Table 4: Other potential PET imaging targets for neuroinflammation and MS under development.

Target	Basis for the imaging	Tracers	Key results and limitations	References
Targets with tracers in human studies				
α7 and α4β2 nicotinic acetylcholine receptors (nAChRs)	Involved in synaptic plasticity, neuronal survival and neuroprotection[81]. Expressed mainly in neurons, microglia and astrocytes[82, 83]. Reduced levels in neuroinflammatory and neurodegenerative diseases[84]. Activation of α7 in astrocytes inhibits NF-κB signaling[83].	**2-[^{18}F]-fluoro-A85380**	Reduced uptake in the thalamus and in the occipital cortex. (Human AD, *in vitro*) Reduced uptake in the caudate, putamen and in the thalamus. (Human PD, *in vitro*) Increased binding in the acute phase of cerebral ischemia. (MCAO rat, *in vivo*)	Schmaljohann et al., 2004[85] Schmaljohann et al., 2006[86] Martin et al., 2015[82]
		[^{18}F]flubatine	Favorable kinetics, indications of specific binding. (Healthy humans, *in vivo*)	Sabri et al., 2015[87]
		[^{18}F]XTRA	Good properties for imaging the extrathalamic regions. (Mouse and baboon, *in vivo*) High brain uptake, suitable kinetics and good properties for imaging the extrathalamic regions. (Healthy humans, *in vivo*)	Kuwabara et al., 2017[88] Coughlin et al., 2018[89]
		[^{18}F]nifene	Rapid kinetics compared to other α4β2* tracers. (Healthy humans, *in vivo*)	Betthauser et al., 2017[90] Mukherjee et al., 2018[91]
		[^{18}F]ASEM	High brain uptake, suitable kinetics and biodistribution comparable to in vitro tissue data (Healthy humans, *in vivo*) Minimum 90 minutes scan needed (Healthy human subjects, *in vivo*)	Wong et al., 2014[92] Hillmer et al., 2017[93]
		[^{18}F]DBT-10	Kinetic properties comparable to [^{18}F]ASEM. (Non-human primate, *in vivo*)	Hillmer et al., 2017[93]
Cyclooxygenase 1 (COX-1)	Expressed mainly in microglia and perivascular cells. Facilitates excretion of proinflammatory prostaglandins and is involved in acute and chronic inflammation[94].	**[^{11}C]-KTP-Me**	Penetrates through the BBB, favorable dosimetry and biodistribution. (Healthy humans, *in vivo*) Specific binding in neuroinflammatory areas. (LPS-induced rat and AD mouse model, *in vivo*)	Ohnishi et al., 2014[95] Shukuri et al., 2016[96]

			No difference in binding between healthy subjects and patients. (Healthy and MCI/AD human subjects, *in vivo*)	Ohnishi et al., 2016[97]
Oxidative stress	Mitochondria in activated macrophages, microglia and astrocytes produce oxidizing agents contributing to tissue damage[98]. PET tracers target the metabolic outcome of oxidative stress, such as glucose consumption, or the expression of ROS scavengers and mitochondrial complexes.	**[^{18}F]FDG**	Tracer uptake correlates with ROS production. (Tumor xenografts in mice, *in vivo*) Tracer uptake reduction predicts conversion of MCI to AD. (Human MCI, *in vivo*)	Jung et al., 2013[99] Pagani et al., 2017[100]
		[^{18}F]FASu	Rapid and high tracer uptake. (Tumor xenografts in mice, *in vivo*)	Webster et al., 2014[101]
		[^{11}C]DHQ1	Potential PET tracer for imaging of redox status. (Mouse, *in vivo*)	Okamura et al., 2015[102]
		[^{18}F]F-BCPP-EF	High uptake, suitable kinetics. Specific binding, potential tracer for MC-I imaging. (Ischemic rat model, *in vivo*) Could discriminate the neuronal damaged areas with neuroinflammation. (Ischemic non-human primate model, *in vivo*)	Tsukada et al., 2014a[103] Tsukada et al., 2014c[104]
		[^{62}Cu]ATSM	Tracer accumulation in the striatum enhanced in PD. (Human PD, *in vivo*) Tracer accumulation correlates with disease severity. (Human ALS, *in vivo*)	Ikawa et al., 2011[105] Ikawa et al., 2015[106]
		[^{18}F]NOS	Uptake correlates with myocardial tissue iNOS level. (Human OHT, *in vivo*) Uptake correlates with endotoxin-induced iNOS in lungs. (Healthy humans, *in vivo*)	Herrero et al., 2012[107] Huang et al., 2015[108]
		[^{18}F]ROStrace	High uptake, enhanced tracer accumulation in neuroinflammation. (LPS-induced mouse model, *in vivo*)	Hou et al., 2018[109]
Targets with tracers in preclinical use				
Folate receptor β (FRβ)	Expression strongly elevated in activated macrophages, expression potentially specific for M2-like type [110]. The tracers have been studied mainly in cancer and in peripheral	**[^{68}Ga]/[^{64}Cu]- rf42** (NODAGA-Folate conjugate)	High uptake, suitable kinetics. Specific binding, low background signal. (Tumor xenografts in mice, *in vivo*)	Farkas et al., 2016[111]
		folate-NOTA-Al[^{18}F]	High uptake, specific binding. (Tumor xenografts in mice, *in vivo*)	Chen et al., 2016[112]

	inflammation.	**folate-PEG$_{12}$-NOTA-Al[^{18}F]**	High uptake, specific binding. Reduced liver uptake. (Tumor xenografts in mice, *in vivo*)	Chen et al., 2017[113]
		[^{18}F]fluoro-PEG-folate	High uptake, specific binding (macrophages). (Arthritic rat model, *in vivo*) High uptake, specific binding. Binding reduced in treated rats. (Arthritic rat model, *in vivo*)	Gent et al., 2013[114] Chandrupatla et al., 2017[115]
		[^{68}Ga]NOTA-folate	High uptake, specific binding. Relatively low kidney uptake and very low liver uptake. (Tumor xenografts in mice, *in vivo*)	Brand et al., 2017[116]
Matrix metalloproteinases (MMPs)	Induced during inflammation. MMP-1, -2, -3, -7 and -9 described in macrophages, microglia, leucocytes and astrocytes in MS. MMP-9 linked to myelin degradation, elevated levels detected in the CSF of MS patients[117]. MMP-9 and -12 expressed in oligodendrocytes and might regulate their maturation and other processes[117].	**[^{18}F]-BR-351**	Potentially targets the activated forms of MMP-2, -8, -9, and -13. High lipophilicity. (Mouse, *in vivo*) Time-dependent increase different from TSPO tracer uptake after ischemia. Uptake associates with increased MMP-9 expression. (MCAO mouse, *in vivo*) High uptake into tumors. Binding colocalized to MMP-2 and -9 expressions. (Mouse model of human glioma, *in vivo*)	Wagner et al. 2009[118] Zinnhardt et al., 2015[119] Zinnhardt et al., 2017[120]
Cyclooxygenase 2 (COX-2)	Inducible cyclooxygenase. Expression increased in inflammation in oligodendrocytes and in immune cells during demyelinating processes in MS[121].	**[^{11}C]-Celecoxib**	Only non-specific binding. (Cerebral ischemia mouse model, *in vitro* and *in vivo*)	Ji et al., 2013[122]
		[^{11}C]-Rofecoxib	Homogeneous tracer uptake. Increase in COX-2 expression not detected. (Inflammatory rat model, *in vivo*) Specific binding in vitro, only non-specific binding in vivo. (Cerebral ischemia mouse model, *in vitro* and *in vivo*)	De Vries et al. 2008[123] Ji et al., 2013[122]

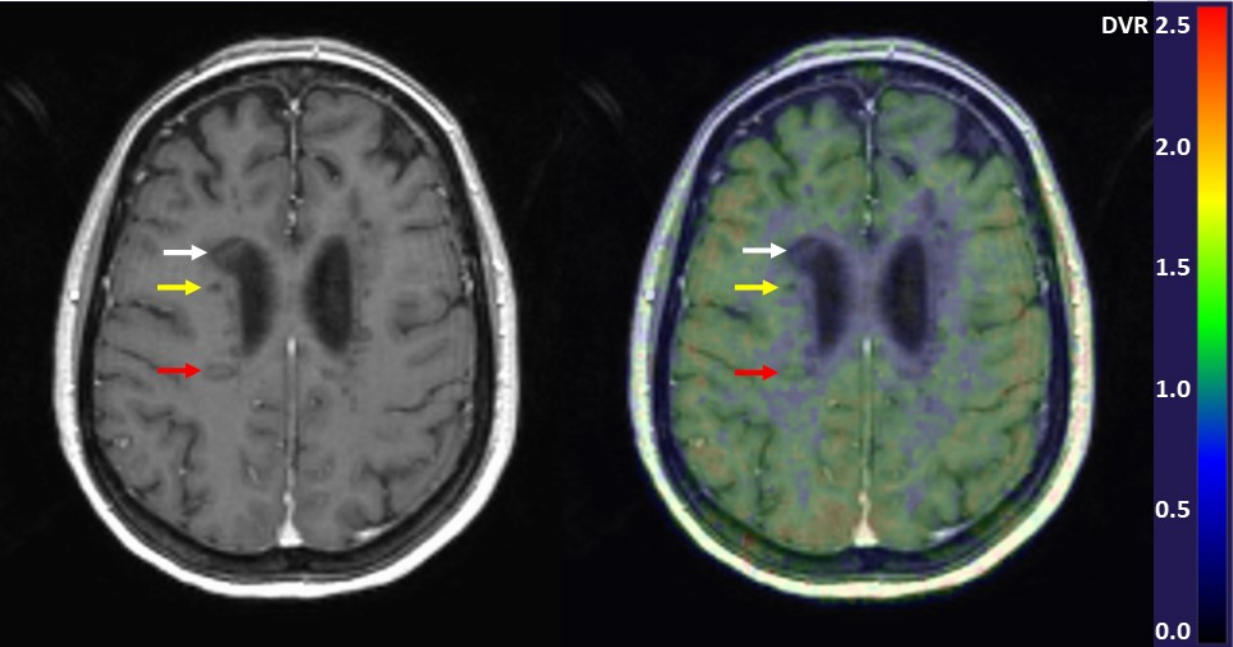
Abbreviations: AD – Alzheimer´s disease, PD – Parkinson´s disease, MCAO - Cerebral ischemia rat model, BBB – blood-brain barrier, LPS – lipopolysaccharide, MCI - mild cognitive impairment, ROS – reactive oxygen species, MC-I – mitochondrial complex I, ALS - amyotrophic lateral sclerosis, OHT – orthotopic heart transplantation,

Figure legends

Figure 1. TSPO binding patterns in MS brain in an SPMS patient (49-year-old female, EDSS 7.5, disease duration 17 years, no immunomodulatory treatment at the time of imaging). Axial views of gadolinium enhanced T1-weighted 1.5T MRI (left), and the same MRI image overlaid with parametric [^{11}C]PK11195 image (right), where the level of [^{11}C]PK11195 binding is visualized as distribution volume ratio (DVR) in each voxel denoted by the color scale bar. The images show an active lesion with slightly increased gadolinium enhancement in the center of the lesion and increased [^{11}C]PK11195 binding correspondingly (red arrows), a chronic active T1 hypointense lesion (yellow arrows) with increased binding in the perilesional area and in the adjacent NAWM, and a large chronic inactive lesion (white arrows) with negligible radioligand binding within the lesion and surrounding it.

Figure 2: PET imaging targets relevant for evaluation of MS pathology *in vivo,* and examples of radioligands already used for human CNS imaging. Microglia are shown in orange and astrocytes are shown in green. TSPO (1) is located on the outer membrane of mitochondria in activated microglia and is the most common PET imaging target in evaluation of neuroinflammation in MS. A more comprehensive list of TSPO-ligands in development can be found for example in Alam et al. 2017[124]. Other ligands targeting glial cells include [^{11}C]TMSX and [^{18}F]MNI-444 (A2AR ligands) (2), [^{11}C]SMW139

and [^{11}C]GSK1482160 (P2X7 ligands) (3) and [^{11}C]NE40 (CB2 ligand) (4). Synaptic density can be measured using radioligand [^{11}C]UCB-J which binds to the synaptic vesicle glycoprotein 2A (SV2A) (5). [^{11}C]PiB is a tracer used for myelin imaging (6).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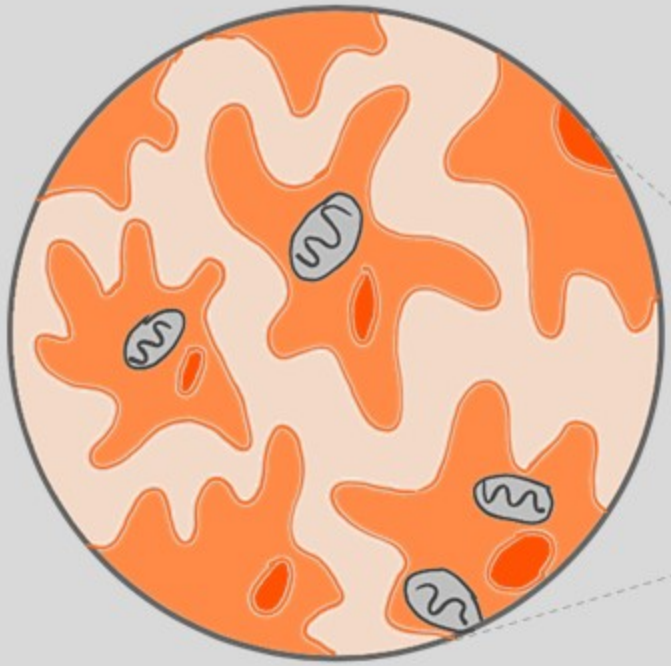
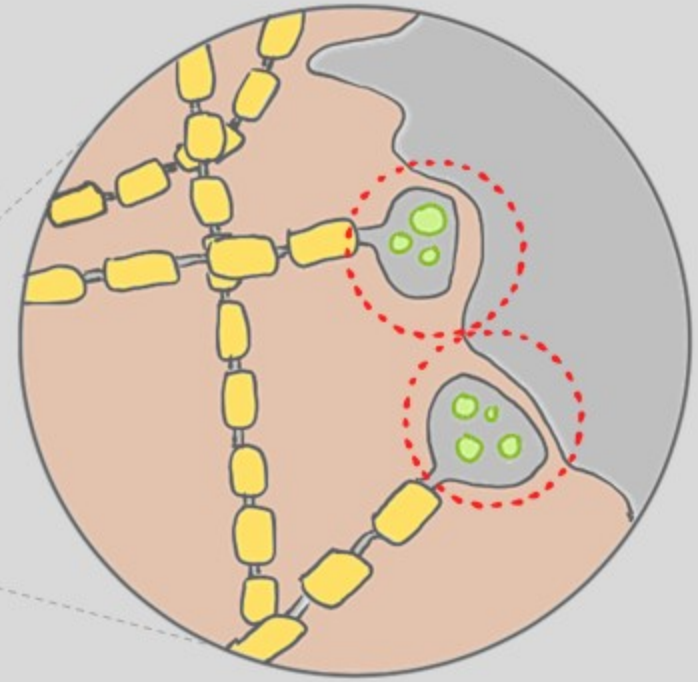
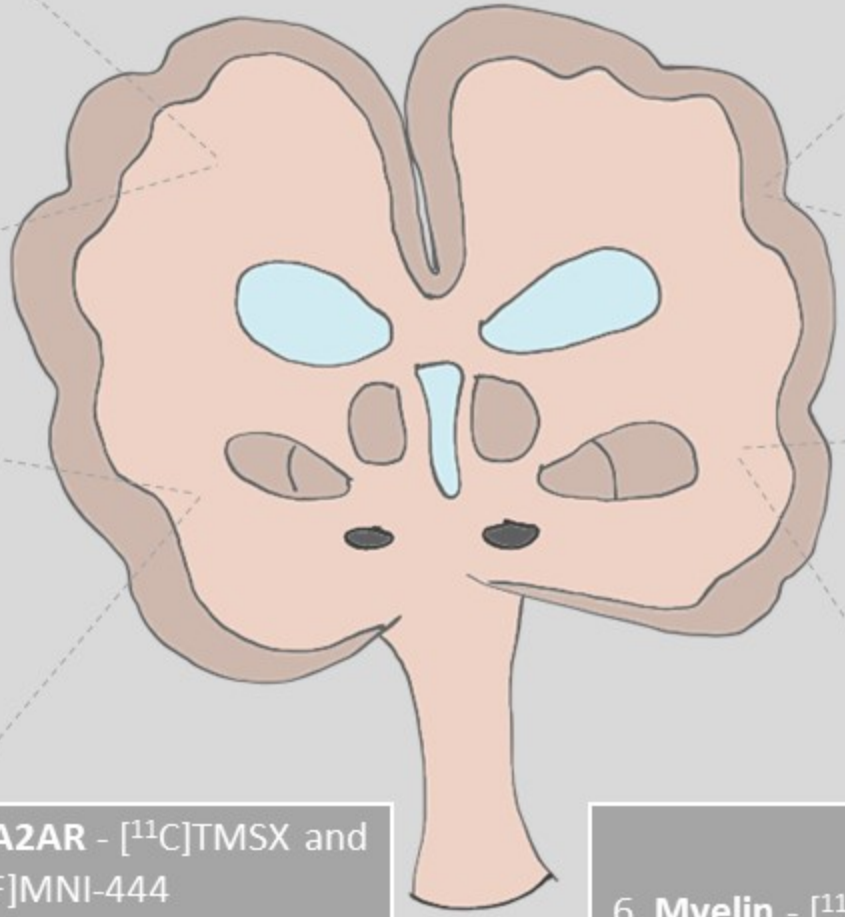
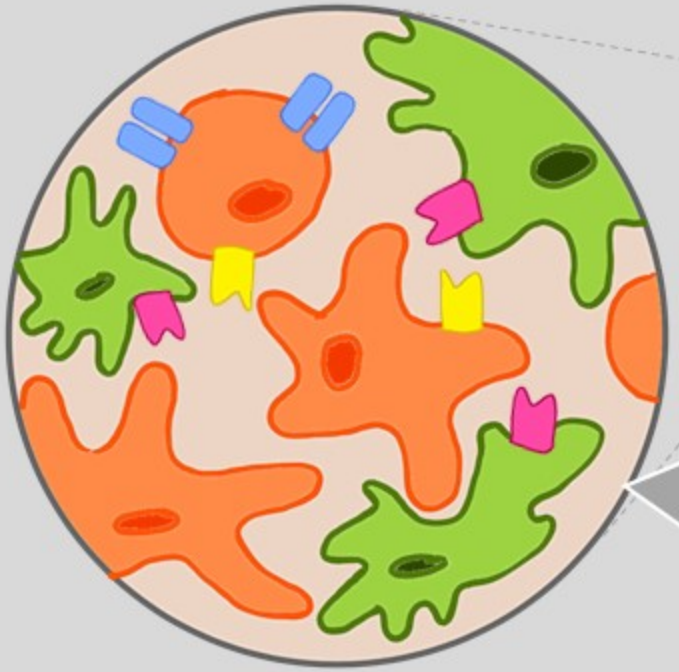


1. **TSPO** - [^{11}C]PK11195, [^{11}C]PBR28, [^{18}F]PBR111, [^{18}F]GE180

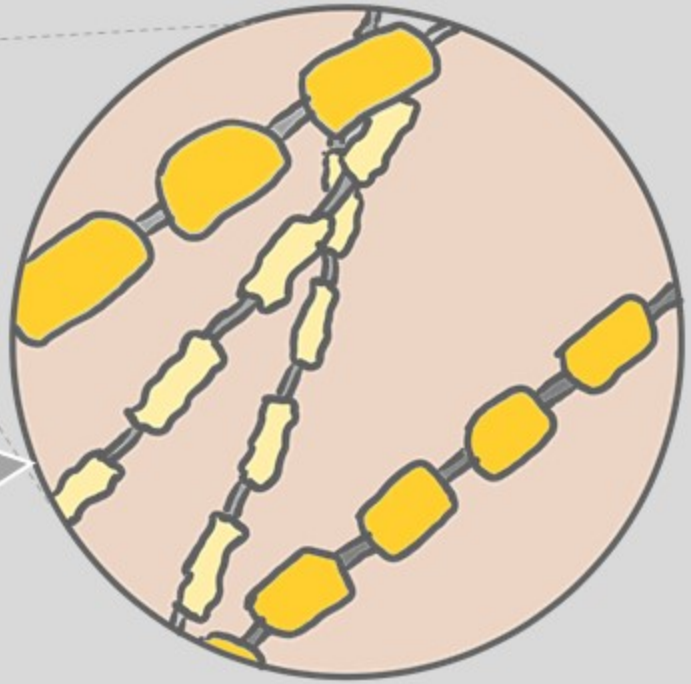
5. **SV2A** - [^{11}C]UCB-J







2. **A2AR** - [^{11}C]TMSX and [^{18}F]MNI-444
3. **P2X7R** - [^{11}C]SMW139 and [^{11}C]GSK1482160
4. **CB2R** - [^{11}C]NE40



6. **Myelin** - [^{11}C]PIB