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ARTICLE

New molecular targets for PET and SPECT imaging in neurodegenerative diseases

Marcel Benadiba,[1,2,3] Gert Luurtsema,[1] Lauro Wichert-Ana,[3,4] Carlos Alberto Buchpigel,[3,5] Geraldo Busatto Filho[2,3]

[1] Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, Netherlands
[2] Laboratory of Psychiatric Neuroimaging (LIM 21), Department of Psychiatry, Faculdade de Medicina, Universidade de São Paulo, Brazil
[3] Núcleo de Apoio à Pesquisa em Neurociência Aplicada (Center for Interdisciplinary Research on Applied Neurosciences: NAPNA), Brazil
[4] Department of Internal Medicine, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil
[5] Center of Nuclear Medicine, Department of Radiology, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brazil

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Abstract
The pathophysiology of neurodegenerative diseases (ND) such as Alzheimer's disease (AD) and Parkinson's disease (PD) has not yet been completely elucidated. However, in the past few years, there have been great knowledge advances about intra-and extracellular proteins that may display impaired function or expression in AD, PD and other ND, such as amyloid beta (Aß), α-synuclein, tau protein and neuroinflammatory markers. Recent developments in the imaging techniques of positron emission tomography (PET) and single photon emission computed tomography (SPECT) now allow the non-invasive tracking of such molecular targets of known relevance to ND *in vivo*. This article summarizes recent findings of PET and SPECT studies using these novel methods, and discusses their potential role in the field of drug development for ND as well as future clinical applications in regard to differential diagnosis of ND and monitoring of disease progression.

Corresponding author: Prof. Dr. Geraldo Busatto Filho. Rua Dr. Ovídio Pires de Campos, S/Nº - 2º andar; CMN – Centro de Medicina Nuclear; CEP 05403-010 São Paulo, SP, Brasil. Phone: (+55 11) 2661-8132; Fax: (+55 11) 3082-1015. E-mail: labbneuro@hcnet.usp.br.

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Introduction

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) are *in vivo* imaging techniques that allow the non-invasive tracking of brain pathophysiological processes underlying various neurological and psychiatric disorders. These techniques have also been successfully used in various aspects of drug development, including the understanding of the mechanism of action of pharmacological agents in the central nervous system (SNC), their dosage regimens and thresholds for clinical response and emergence of side-effects.[1]

Several studies over the past decades have shown that PET and SPECT methods can reliably map neurochemical processes of interest in the brain, including the density and affinity of postsynaptic receptors for neurotransmitters such as dopamine, serotonin and others, as well as presynaptic transporters for these transmitters, precursors such as L-DOPA and transmitter degrading enzymes. Such approach has provided invaluable information about neurochemical abnormalities involved in psychiatric and neurological disorders, as well as helping to elucidate the mechanism of action of the pharmacological agents commonly used to treat these conditions.

More recently, technological advances have enabled the use of PET and SPECT to probe a number of other intra- and extra-cellular proteins that may display impaired function or expression in brain diseases. Such advances have moved the neurological and psychiatric uses of PET and SPECT from a strict neurochemical imaging role to a much more flexible and comprehensive profile of applications, providing knowledge about molecular brain mechanisms that may be much closer to the pathophysiological essence of neurological and psychiatric disorders than superficial neurotransmitter changes.

One of the most promising applications of such new PET and SPECT methods regards to the investigation of pathophysiological aspects of neurodegenerative disorders (NDs). This is of great relevance given the large prevalence of NDs such as Alzheimer's disease (AD) and Parkinson's disease (PD) in elderly life, as well as the fact that a greater knowledge about the pathophysiology of these disorders may help in the development of novel pharmacological treatments capable of interfering with their molecular pathological substrate. Taken those issues into account, this review will focus on perspectives for new PET and SPECT tracers developed to allow the mapping of intracellular and extracellular mechanisms of particular relevance to AD, PD and other NDs.

Molecular brain imaging with PET and SPECT: basic principles

In order to allow the efficient visualization, characterization and quantitative measurements of relevant biological processes in the brain, PET and SPECT techniques demand the development of suitable probes that can be labeled with a positron emitting isotope (in the case of PET) or photon emitting isotope (in the case of SPECT). Importantly, because of their limited spatial resolution, the use of computed tomography (CT) or magnetic resonance imaging (MRI) is often required. Functional and structural techniques can be easily fused using special software, by creating parametric images. However, the development of hybrid systems where functional techniques are fully integrated with structural cross-sectional methods also helped to attenuate the lack of anatomical resolution of PET and SPECT. These parametric images give both anatomical and functional information, allowing the identification of regions which exhibit differences in the uptake of labeled compounds. Anyway, the most employed radioisotopes for labeling PET probes are carbon-11 (^{11}C) and fluorine-18 (^{18}F), differing basically in their half-lives and maximum energy. The first (^{11}C) must be produced by an on-site cyclotron located near to the PET imaging facility due to the very short physical half-life (20 minutes). However, the longer half-life of ^{18}F (110 minutes) allows the delivery of ^{18}F-labeled ligands to a broader list of PET facilities located in the same town, or even in neighborhood cities. For SPECT imaging, probes can be labeled with iodine-123 (^{123}I) or technetium-99m (^{99}mTc);[2] these isotopes have much longer half-lives than those used in PET imaging, avoiding the need for a nearby cyclotron.

Having crossed the blood-brain barrier (BBB) after intravenous injection, the radiolabeled compound accumulates in certain parts of the brain, depending on the biological process being tracked. Both PET and SPECT are equipped with distinct radiation detectors that are placed in close proximity to the head after injection of the radioligand, and the data collected by such detectors are transformed to generate three-dimensional tomographic maps displaying the regional distribution of radioactivity emitted by the brain. In order to be suitable for *in vivo* brain imaging with PET or SPECT, a radiopharmaceutical compound (also called radiotracer, due to its sub-pharmacological dose) needs to be able to bind specifically to its target (binding potential of a drug) (Figure 1), otherwise the accuracy of the imaging information obtained may be impaired. By definition, binding potential (BP) is a pivotal measure in the use of PET to

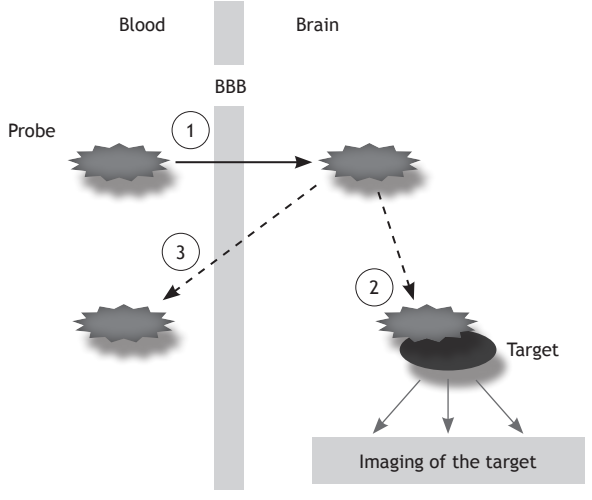

Figure 1 The basic requirements for suitable target-imaging agents include: (1) prompt crossing of the blood-brain barrier; (2) selective binding to the target molecules; and (3) clear and contrasting signals between target and non-target molecules.[4]

measure the density of "available" receptors, e.g. to assess the occupancy by drugs or to characterize abnormalities in receptor distribution in association with neuropsychiatric disorders. Thus, BP is a combined measure that depends on receptor density as well as on drug-receptor affinity.[3]

Amyloid imaging tracers

Extracellular senile plaques are protein aggregates formed by the misbalance between the production and clearance of proteins or peptides in brain tissue of AD patients. Amyloid beta (Aß), released from the cleavage of the amyloid precursor protein (APP), is the most important constituent present in these plaques and represents the main hallmark that characterizes the neuropathological diagnosis of AD. The cleavage of APP can be performed by several proteases or peptidase proteins. Among these, secretases, especially gamma (which contains presenilins, nicastrin, anterior pharynx defective-1, and presenilin enhancer-2) and beta (ß-site APP cleaving enzyme 1, BACE1), are the most important enzymes, with their activity being responsible for the excessive release of the highly amyloidogenic 42 amino acid variant (Aß42) peptide. In contrast, APP cleavage promoted by the α-type secretases disintegrin and metalloproteinase (ADAM) 10 and 17 contributes to the formation of soluble neuroprotective fragments known as Sα-APP.[5]

The great advances in the knowledge about the molecular basis of AD, described above, have led to enormous interest in the development of PET and SPECT tracers that could be useful for *in vivo* imaging of Aß plaques in the human brain. The first PET tracer developed to bind specifically to fibrillar Aß plaques was the ^{11}C-labeled Pittsburgh Compound-B ([^{11}C]PIB). Up until now, this has been the best characterized and most widely used PET tracer for the study of amyloid deposits in the human brain, both in AD and in other NDs. The several potential roles of *in vivo* amyloid imaging techniques in AD are outlined in Table 1.

Since a definite diagnosis of AD can only be confirmed by post-mortem neuropathological examination, diagnostic tools that can be used to give support to a suspicion of AD in an individual with memory deficits and other features of cognitive decline are highly valuable. Several studies have shown a marked degree of [^{11}C]PIB retention in the association cortex of mild AD patients compared to healthy controls[6-9] (Figure 2).

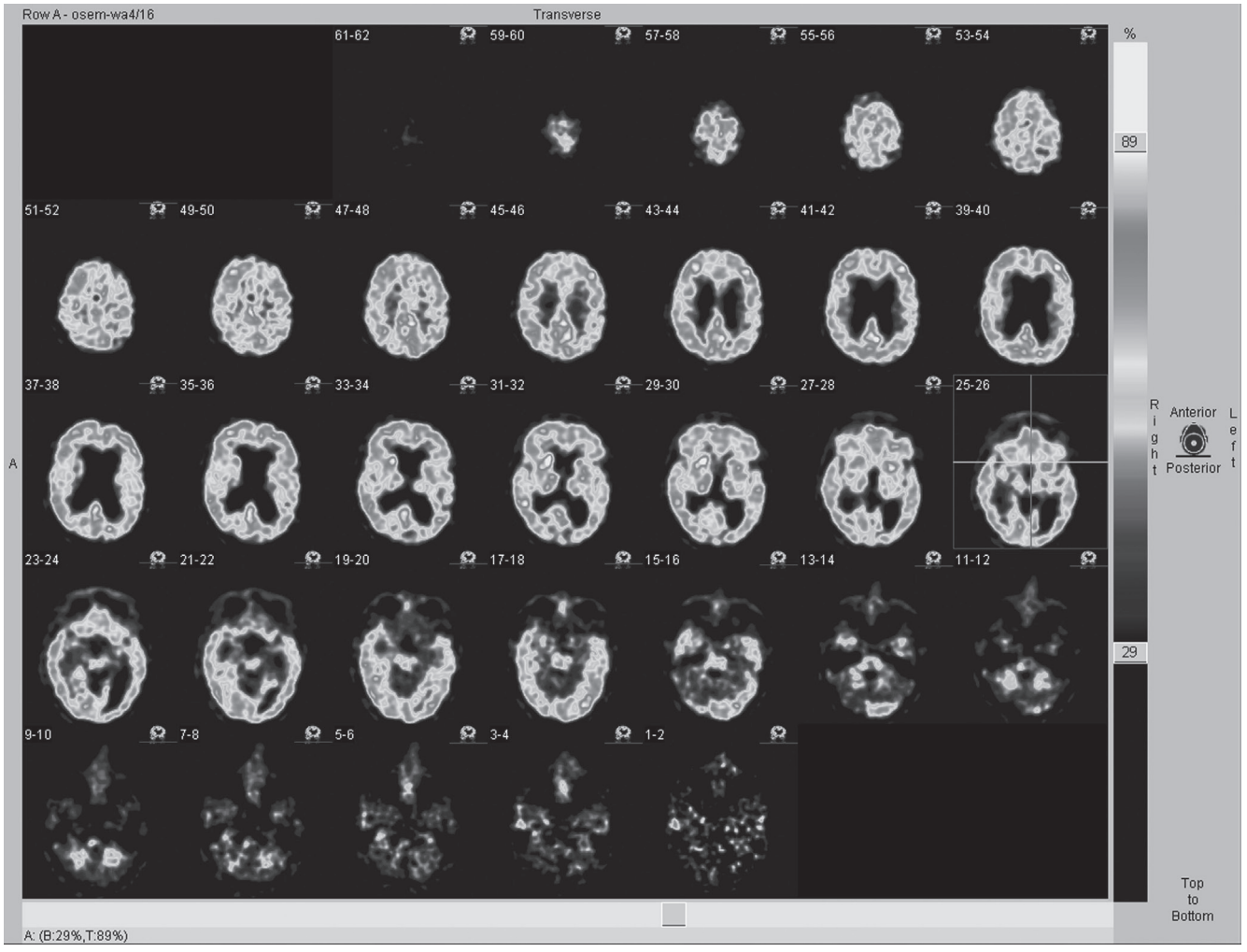

Figure 2 PET images obtained after intravenous injection of 11C-labeled Pittsburgh Compound B (PiB) in a patient with probable Alzheimer's disease, revealing amyloid deposition in the brain. Warmer colors (e.g. red and yellow) indicate greater concentrations of amyloid deposits, while the blue color indicates the absence of these deposits.

Table 1 Uses of amyloid imaging with PET in neurodegenerative disorders

Research applications
• Elucidation of pathophysiological aspects of Alzheimer's disease (AD), minor cognitive impairment and other disorders that involve amyloid deposition in the brain
• Mapping of the progression of brain pathological changes over time
• Evaluation of disease-modifying properties of novel treatments
Potential clinical applications
• Ruling out of Alzheimer's disease in cases of suspected cognitive decline
• Differential diagnosis between Alzheimer's disease and frontotemporal dementia
• Differential diagnosis between dementia with Lewy bodies and Parkinson's disease

These findings established PET imaging with [^{11}C]PIB as a useful imaging tool to aid in the diagnostic confirmation of early AD.[9,10] However, it should be noted that amyloid deposition is not pathognomonic of AD, being for instance found in a proportion of cognitively healthy elderlies. Nevertheless, a negative [^{11}C]PIB PET result is highly informative to rule out the diagnosis of AD.

The usefulness of [^{11}C]PIB imaging with PET for assessing the progression of AD has not been as well established. For instance, an interesting 2-year follow-up study of AD patients revealed that there was no significant increase in [^{11}C]PIB uptake over time, although individually some patients showed clear increases.[8] Such pattern of results indicates Aß deposition plateaus when clinical dementia is already established. PET investigations with [^{11}C]PIB are also clinically useful to aid in the distinction between AD and other dementias. Most notably, patients with frontotemporal dementia (FTD) have generally normal [^{11}C]PIB uptake (although occasional FTD patients may display increased brain uptake).[11,12]

Individuals with objective cognitive decline not severe enough to fulfill the criteria for established dementia receive the diagnosis of minor cognitive impairment (MCI).[13] Subjects diagnosed as suffering from MCI have a high risk of developing dementia, with an estimated rate of conversion to AD of approximately 12% per year.[13] A number of PET studies have shown that a subpopulation of subjects with MCI shows increased levels of [^{11}C]PIB uptake to the same degree as seen in patients with AD.[7,14,15] In addition, recent investigations demonstrate that increased [^{11}C]PIB uptake in the brain of MCI patients is highly predictive of subsequent conversion to AD.[16]

Also noteworthy, most patients with dementia with Lewy bodies (DLB) demonstrate increased [^{11}C]PIB uptake in the brain.[17] Recent reports have shown that [^{11}C]PIB holds promise to help in the discrimination of DLB patients from those with PD, PD with dementia (PDD), PD with mild cognitive impairment (PD-MCI), and healthy control subjects (HCS).[17,18] However, [^{11}C]PIB retention did not differ across the diagnoses of PDD, PD-MCI, PD, and HCS.[18] Importantly, one study reported that the increased [^{11}C]PIB retention in the brains of DLB patients is largely attributable to the binding of [^{11}C]PIB to Aß plaques and not to α-synuclein, the primary structural component of Lewy body fibrils.[19]

Finally, two other important areas of potential use for amyloid imaging tracers include drug development and monitoring of treatment effects (Table 1). For example, one study of AD patients treated with phenserine, an anticholinesterase compound, showed an improvement in cognition that was not however accompanied by significant changes in mean cortical [^{11}C]PIB retention in the brain.[20]

Given the longer half-life of 18F-labeled probes in comparison to [^{11}C]labeled compounds (110 minutes vs. 20 minutes), there has been a great degree of interest in the past few years in the development of ^{18}F-labeled compounds for brain amyloid imaging with PET, which could be transported from radiopharmaceutical production facilities to other PET imaging sites. A number of such 18F-labeled amyloid imaging tracers, including [^{18}F]flutemetamol (an ^{18}F-labeled derivative of PIB), [^{18}F]AV-45 (florbetapir) and [^{18}F]BAY 94-9172 (florbetaben), are already in an advanced stage of clinical trials.[21-24] Noteworthy, florbetapir has been recently approved by the FDA in the United States for PET imaging of the brain in adults under evaluation for AD and other causes of cognitive decline.[25] Clinical studies indicate that these 18F-labeled amyloid imaging tracers provide good discrimination between AD patients and healthy subjects. However, they still present some technical limitations, including a relatively low degree of specific binding *in vivo*, as well as a high level of white matter binding in healthy human brains which reduces the contrast between cortical and non-cortical specific uptake of the tracer.

Furthermore, two technetium (^{99m}Tc) and rhenium (Re) labeled ligands have been recently synthesized for Aß imaging with SPECT. They are derived from the compounds (2-(1-(6-(dialkylamino)naphthalen-2-yl)ethylidene)malononitrile (DDNP) and 1-(6-(dialkylamino)naphthalen-2-yl) ethanone (ENE). However, these compounds showed low affinities for Aß aggregates and require further refinements in order to improve their diffusion through the BBB.[26] In addition, it is also important to mention that SPECT has lower sensitivity and spatial resolution while compared to PET, and this might also promote possible differences in accuracy between these two techniques.

Tau imaging tracers

In addition to the extracellular Aß plaques mentioned above, intracellular neurofibrillary tangles (NFTs), composed of filaments of microtubule-binding hyper-phosphorylated protein tau, are also an important hallmark of neurodegenerative disorders including AD, being preferentially located in the hippocampus and associative cortical regions.[27,28] Previous neuropathological research suggests that the deposition of NFTs occurs before the manifestation of clinical symptoms in AD and is sufficient to provide a neuropathological diagnosis of AD.[29-31] Thus, *in vivo* imaging of NFTs in conjunction with imaging of Aß plaques would be useful for the early and accurate diagnosis of AD. A quantitative evaluation of tau pathology may also be helpful in tracking the severity of dementia, since the degree of deposition of NFTs correlates well with the clinical severity of dementia. Finally, given that some forms of frontotemporal lobar degeneration are characterized by pathological accumulation of tau protein, tau imaging tracers show also great promise for the diagnosis

of such conditions and their differentiation from AD and psychiatric disorders.[32]

The first radiotracer developed for tau protein imaging in the brain with PET was ^{18}F-FDDNP,[33,34] and this was followed by ^{18}F-FSB[35] and ^{18}F-FP-curcumin.[36] However, all these radioligands bind not only to NFTs but also Aß plaques in the brain.[35,36,37] Therefore, these tracers have limited value for accurately investigating tau-related aspects of AD, or to reinforce the diagnosis of FTD. One additional limitation of [^{18}F]FDDNP is its low signal/noise ratios for PET imaging, due to its reduced specific binding signal and rapid brain uptake of lipophilic metabolites.[37,38]

Recently, however, a series of quinolone derivatives that bind to tau NFTs with higher affinity than ß-amyloid fibrils have been identified.[39] One of these derivatives, 2-(4-aminophenyl)-6-(2-([^{18}F]fluoroethoxy)) quinolone ([^{18}F] THK523), has been evaluated for imaging of tau pathology in the brain with PET.[40] It demonstrated high affinity and selectivity for tau fibrils *in vitro* and *in vivo*. Interestingly, this tracer presented low binding in the brains of transgenic mice overexpressing APP with significant accumulation of cerebral Aß, thus demonstrating its selectivity for tau.[40] Furthermore, auto-radiographic and histofluorescence analyses of human hippocampal serial sections from AD patients exhibited positive THK523 binding that co-localized with immunoreactive tau pathology, while not highlighting Aß plaques. These experiments indicate that [^{18}F]THK523 fulfills the criteria for a proper radioligand that could be used in human imaging trials.

More recently, *in vitro* and *in vivo* studies have also shown that TH2, a novel radioiodinated rhodanine and thiohydantoin (TH) derivative, binds specifically to NFTs and may be suitable for SPECT imaging of tau pathology.[41] One other potential SPECT tracer is the phenyldiazenyl benzothiazole (PDB) derivative 4-[2-(5-methoxy-2-benzothiazolyl) diazenyl]-N, N-dimethyl-benzenamine, which binds to tau aggregates with a two-fold selectivity relative to Aß aggregates.[42] However, biodistribution experiments using normal mice show that PDB derivatives display persistent levels of radioactivity in the brain. This makes them unsuitable for imaging NFTs *in vivo* in humans at the present time, and structural changes to the PDB scaffold may be needed to make these compounds useful for imaging NFTs in the human brain with SPECT.

Lewy Body tracers

One other important area of research refers to the development of PET and SPECT radiotracers capable of binding specifically to Lewy bodies. Such compounds may be highly useful for the diagnosis and assessment of therapy and severity of pathological progression of α-synuclein-associated disorders. α-synuclein is the main constituent of Lewy bodies and is known to interact with several proteins also involved in neurodegeneration.[43] Its pathological accumulation may alter mitochondrial function,[44] synaptic rearrangement,[45] microtubule associated-protein like tau function (because it can interact with tubulin),[46,47] neuronal Golgi apparatus behavior and vesicle trafficking,[48] and cell membrane lipid composition and fluidity.[49]

The compound BF227, initially designed as an Aß imaging agent,[50] was recently demonstrated to label to both Aß plaques and Lewy bodies in immunohistochemical/fluorescence analyses of human brain sections of sufferers of AD and PD, respectively. Thus, [^{18}F]BF2^{27} is regarded as a potential non-Aß-selective biomarker for the study of PD.[51] It should be noted that BF2^{27} has been recently shown to stain α-synuclein-containing glial cytoplasmic inclusions in post-mortem tissue. In the same report, PET examinations with carbon-11-labelled BF227 ([^{11}C]BF227) detected α-synuclein deposits in the living brains of patients with multiple system atrophy (MSA).[52] This indicates that [^{11}C]BF227 could be a potential tool to monitor the effectiveness of neuroprotective therapy for α-synucleinopathies. However, further studies are warranted to verify whether Lewy bodies in other α-synucleinopathies as well as glial cytoplasmic inclusions can be detected by [^{11}C]BF227 PET.

Tracers for neuroinflammation

Neuroinflammation is a known ageing-related multifactorial process which is commonly found in earlier stages of NDs, and is directly implicated in the progression of these diseases.[53] Up until now, the most widely used tracer to visualize neuroinflammation in the brain has been [^{11}C]PK11195, which is capable of mapping microglial activation through binding of the 18-kDa translocator protein (TSPO), formerly known as peripheral benzodiazepine receptor (PBR). TSPO is mainly found in the outer mitochondrial membrane and is primarily involved in cholesterol transport for further steroidogenesis. In brain tissue, TSPO expression is relatively low. However, a dramatically up-regulation occurs when microglia is activated, which confers to this protein an important role as a neuroinflammatory biomarker in the brain.[54] [^{11}C]PK11195 has been recently used in several studies of psychiatric disorders, revealing patterns of widespread microglia activation in the brain (Figure 3). In NDs, studies with this tracer have shown that microglial activation is indeed an early pathological event,[55-58] thus providing support to the possible use of anti-inflammatory based therapeutic interventions for NDs. However, it is important to consider that it is also in the early stages of NDs that microglia have protective effects, for example, by promoting amyloid clearance. However, these cells become increasingly dysfunctional at later stages, then contributing to disease progression.[59]

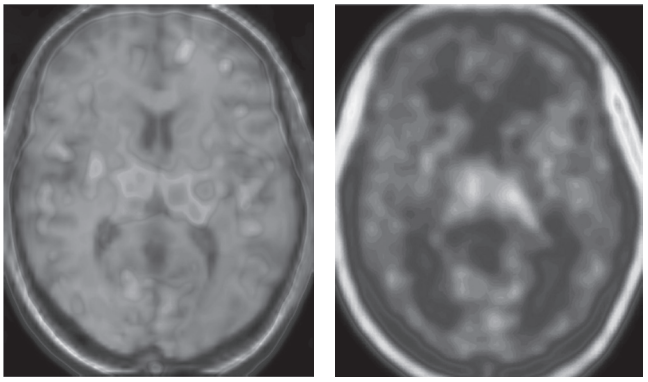

Figure 3 Representative PET parametric image of [^{11}C] PK11195 binding potential (BP) in a healthy individual, superimposed on an MRI (magnetic resonance imaging) template (A); and a [^{11}C]PK11195 PET image from a patient with schizophrenia (B). Note the higher radiotracer uptake in the subject with schizophrenia (in blue color), suggesting the occurrence of neuroinflammatory processes.

Unfortunately, critical issues have limited the use of [^{11}C] PK11195 for measuring neuroinflammation in the brain, including poor bioavailability in brain tissue and high levels of nonspecific binding.[60] That high level of nonspecific activity makes the interpretation of images very complex and cumbersome. More recently, several other TSPO-related PET tracers have been characterized and are being used in preclinical and clinical studies of neuroinflammation in association with NDs (see Ching et al.,[61] for review). Such radiotracers, including [^{11}C] DPA-713 and the 2-Phenylimidazo[1,2-a]pyridineacetamide derivative, [^{11}C]CLINME, may be more suitable for visualizing mild neuroinflammation than [^{11}C]-(R)-PK11195, given that they: are more sensitive for the detection of small amounts of TSPO; have lower levels of non-specific binding; and provide higher signal to-noise ratios. Such properties have been evaluated using infection models, whereby the rate of tracer uptake in infected areas is compared to the uptake in healthy tissues.[62,63] Such superior properties in comparison to [^{11}C]-(R)-PK11195 have also been demonstrated for [^{18}F]PBR111, the fluorinated version of [^{11}C]CLINME,[64] and [^{18}F]DPA-714,[62] with the advantage that these latter tracers are labeled with 18F (110-minute half-life). Noteworthy, recent preclinical TSPO imaging studies have been successfully conducted using models of multiple sclerosis and glioma with [^{18}F]DPA-714.[65,66,67]

Several other new potential molecular targets for neuroinflammation have emerged recently. The activity of ß-glucuronidase, a lysosomal enzyme that is released from reactive astrocytes and microglia, has been successfully imaged recently in a HSV-1-induced encephalitis rat model using 18F-FEAnGA68 (Figure 4). Also, the imidazoline2 Binding Site (I_2BS), I(2)R, has been shown to be altered in several brain disorders including ND.[69] Several ligands for I_2BS, including deprenyl, are able to inhibit monoamine oxidase (MAO),[70] whose activity is increased in AD human brain astrocytosis (or astrogliosis, i.e. an abnormal increase in the number of astrocytes due to neurotoxicity or brain injury) measured by [^{11}C]DED[71] (see below). Thus, imaging I_2BS is a promising tool for the study of neuroinflammation in NDs. Recently, the ligand [^{11}C]FTIMD was evaluated with an improved ultra-high specific activity which afforded the detection of small changes in I(2)R expression in the rat brain.[72] Another potentially useful ligand is BU99008, which demonstrates better *in vivo* brain uptake and specificity in comparison with [^{11}C]FTIMD.[73]

Finally, cyclooxigenase (COX) enzymes are widely known as key molecular targets for anti-inflammatory drugs. Recently, [^{11}C]ketoprofen methyl ester was pre-clinically evaluated and proved to be efficient in quantifying COX-1 expression in both neuroinflammation and neurodegeneration models. In addition, it afforded better results than [^{11}C] PK11195 in quantifying neuroinflammation. Therefore, [^{11}C] ketoprofen methyl ester demonstrated to be sensitive for neuroinflammatory processes targeting COX-1 in activated microglia and macrophages.[74]

Moreover, as astrocytosis is a commonly observed phenomenon involved in neuroinflammation and neurodegeneration, this may also be evaluated in the human brain using PET tracers. The most relevant radioligand evaluated for this purpose to date is 11C-deuterium- L-deprenyl or [^{11}C]DED, as mentioned before. Recent studies demonstrated an increase in [^{11}C]DED binding throughout the brain of AD patients that also display high levels of [^{11}C]PIB uptake, suggesting that astrocytosis is an early phenomenon in the development of AD, probably being an intermediate step between amyloidosis and neuronal loss.[75,76]

Finally, there are also promising results in the use of SPECT tracers for neuroinflammation. For instance, [^{123}I] PK11195 has recently been used in a pilot SPECT study with AD patients.[77] In addition, the ^{123}I-radiolabeled compound 6-chloro-2-(4'iodophenyl)-3-(N,N-diethyl)-imidazo[1,2-a] pyridine-3-acetamide or [^{123}I]CLINDE was also successfully tested in preclinical studies. Using different animal models of neuroinflammation, [^{123}I]CLINDE demonstrated good performance to assess TSPO changes related to both astroglial and microglial activation.[78,79] There are also preliminary investigations of [^{125}I]DPA-713 in rats exposed to a seizure-inducing neurotoxicant; these studies revealed increased brain radioactivity in neurotoxicant-treated rats compared with controls, which was completely blocked by administration of PK11195.[80]

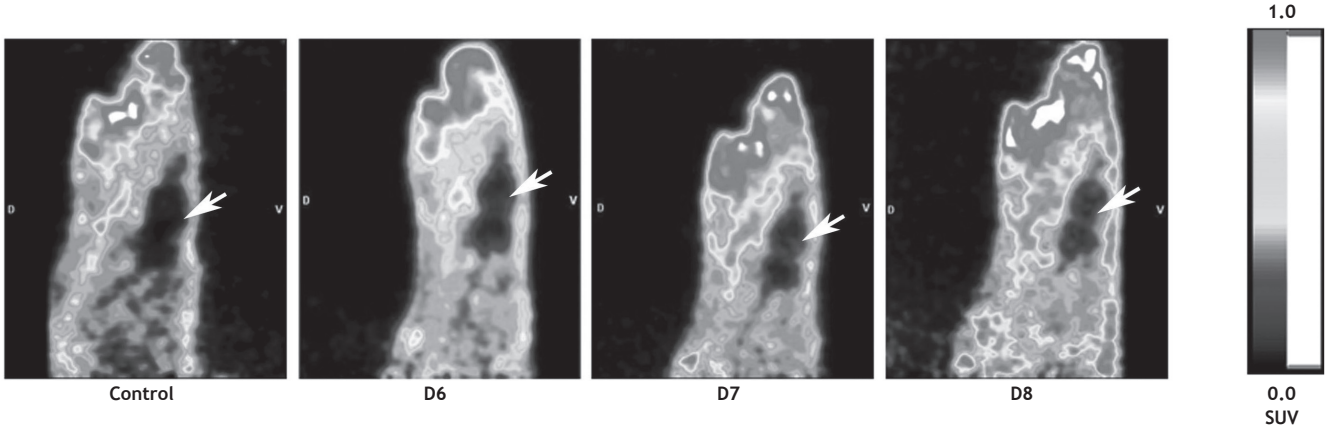

Figure 4 Sagittal view of the head of a control rat (control) and a rat infected with HSV-1 (day 6(D6); day 7 (D7) and day 8 (D8) after virus inoculation). The images represent tracer uptake between 10 and 60 minutes after injection of [^{18}F]FEAnGA. Note the time-dependent microglial activation in the brain (arrows).

Tracers for brain lipid metabolism

Arachidonic acid (AA) and docosahexaenoic acid (DHA), an omega-6 and omega-3 polyunsaturated fatty acid (PUFA), respectively, are very important constituents of phospholipids in cell membranes and contribute extensively to cell signaling in the brain. AA can be obtained from the conversion of its precursor linoleic acid obtained from the diet, whereas the brain concentration of DHA depends on dietary DHA content as well as liver synthesis from its precursor, α-linolenic acid.[81,82] The CNS response to injury and to the onset (and progression) of neurodegeneration involves the release of free DHA and AA along with the synthesis of stereospecific docosanoid derivatives and prostanoids, respectively.[82,83] The release of AA in such conditions is mediated by specific phospholipases, e.g. PLA2, which contribute to the conversion of AA into inflammatory molecules such as prostaglandin E2 (PGE2) by the cyclooxygenase (COX) 1 and 2 enzymes. Interestingly, a recent study trying to understand the role of PLA2 in NDs demonstrated that the inhibition of PLA2 in rat brain leads to a decrease in total tau protein.[84] On the other hand, DHA has anti-inflammatory properties and their docosanoid derivative (e.g. neuroprotectin D1) displays a neuroprotective bioactivity in the brain against various insults, including oxidative injury, ischemia-reperfusion, and inflammation. In addition, low concentrations in the brain have been detected in patients with brain disorders such as AD and depression.[83,85,86,87] Importantly, measured rates of AA and DHA incorporation into brain phospholipids represent their respective rates of metabolic consumption, because these PUFAs are not synthesized de novo or converted significantly from their precursors in the brain[82] (see below).

In recent PET investigations, an increase of 26% in the global brain incorporation of AA in AD patients compared with healthy subjects was observed using [^{11}C]arachidonic acid ([^{11}C]AA).[88] Such incorporation was particularly increased in brain regions where neuroinflammation is thought to be present in AD, and [^{11}C] AA could thus be a novel marker of activated microglia to be used in studies of neurodegenerative disorders. Further studies have evaluated the positron-labeled [1-(11)C]DHA tracer to map the incorporation of unesterified plasma DHA into the brain of healthy adult human volunteers. Values of incorporation coefficients k* for DHA were higher in gray than white matter brain regions. For the entire human brain, the net DHA incorporation rate, J_{in}, the product of k* and the unesterified plasma DHA concentration, equaled 3.8 ± 1.7 mg/day. The authors highlighted that this net rate, approximating the net rate of DHA consumption by brain, is less than the recommended human dietary DHA supplementation of 200 mg per day.[89] Thus, with the use of [1-(11)C]DHA, it is possible to quantify regional and global human brain DHA metabolism in relation to health and disease.[90] In addition, a more recent study demonstrated that is possible to measure brain incorporation of plasma DHA *in vivo*. Thus, quantitative imaging of DHA incorporation from plasma into the brain can be used as an *in vivo* biomarker of brain DHA metabolism and neurotransmission.[87] Importantly, this may help to monitor DHA consumption *in vivo* in patients with disorders such as depression and AD, in which DHA supplementation may be helpful.[91-93]

Other new molecular targets for neuroimaging in neurodegeneration

A potent and selective protein kinase C (PKC) inhibitor, Enzastaurin (LY317615), was recently labeled with 11C for PET imaging applications ([^{11}C]Enzastaurin).[94] PKC is an enzyme involved in several cell biology mechanisms and is one of the most important initial elements involved in the induction of the previously mentioned α-secretases, ADAM-10 and 17, which are involved in neuroprotection.[95] Also, a sensitive myelin probe, [^{11}C]MeDAS, was recently synthesized and proved to be effective in detecting myelin changes in the brain. This radiotracer, which can be used as a myelin-imaging marker to early monitor myelin degeneration *in vivo*,[96] is a potentially useful development for the investigation of NDs, since degeneration of neurons, axons, and synapses is clearly present in AD as much as in multiple sclerosis.[97]

Heat shock proteins (HSP) also display important roles in neuroprotection. HSP70, for example, is a known protein found in aggresome of Lewy bodies and is mainly implicated in the degradation of aberrant proteins.[98] Aggresome is a general response of cells which occurs when the capacity of the proteasome (involved in degradation of unusable proteins) is exceeded by the production of aggregation-prone misfolded proteins.[99] Similarly, cathepsins are also critical for the degradation of enzymes that may be implicated in NDs.[100] Thus, impaired function of these proteins may facilitate the progression of NDs. Interestingly, a PET reporter system (i.e. which uses reporter genes) for imaging gene expression in the intact brain was recently used to image and monitor the activation of the heat shock factor 1 (HSF1)/HSP70 transcription factor.[101] In addition, another group recently imaged the activity of the cysteine cathepsin using ^{64}Cu-Z-FK(DOTA)-AOMK and PET. Imaging of these proteins using the more widely available ^{18}F radioisotope tracer may provide a great tool in the future for early diagnosis and monitoring of disease progression of NDs.

Innate immune responses also play an important role in neurodegeneration. For example, it was recently found that monocytes and microglia that are deficient for myeloid differentiation factor 88 (MyD88) (involved in Toll-like receptor signaling pathway) exhibit a functionally impaired phagocytic reaction to Aß.[102] In addition, MyD88 is involved in the dopaminergic neuronal degeneration induced by the neurotoxin MPTP in the enteric nervous system (ENS) of the mouse.[103] However, this neurodegeneration is not a MyD88-dependent mechanism.[104] Thus, more knowledge is needed before PET/SPECT imaging studies can be considered for this new target protein.

Oxidative stress (OS) leading to mitochondrial damage is a major and early phenomenon triggering neurodegeneration.[105,106] Interestingly, a tracer named [^{62}Cu]ATSM, initially designed for the study of tumor hypoxia,[107] was recently used, for the first time, to assess OS in PD. This study demonstrated that striatal OS was enhanced in PD patients compared with controls and increased with the progression of disease severity, particularly in the contralateral striatum.[108] It was further demonstrated that this tracer is very specific for the cells with mitochondrial dysfunction, even under normoxia, thus suggesting that [^{62}Cu]ATSM may indeed be an interesting

tracer for the study of brain disorders involving mitochondrial dysfunction, such as AD and PD.[109]

P-glycoprotein (P-gp) is a known BBB active efflux transporter involved in neuroprotection. Its dysfunction is considered one of the causes of the onset of PD[110,111] and AD.[112] In addition, a correlation between aging and decreased function of this transporter has also been established *in vivo*.[113] Thus, developing methods for imaging P-gp in such diseases is an important challenge nowadays. A number of studies have already been carried out using the radiolabelled P-gp substrate [^{11}C]verapamil in PET studies. However, polar radiolabelled metabolites are formed after injection of this radioligand, and this may result in a non-P-gp-mediated signal as a confounding factor.[114] Therefore, new P-gp tracers for imaging P-gP function in the BBB are needed.

Concluding remarks

The studies reviewed in this article demonstrate the many opportunities to be explored using the already available molecular imaging tracers that map targets of known relevance to NDs, including Aß, α-synuclein, tau protein, and neuroinflammatory markers.

On the other hand, the brain mechanisms underlying NDs have not yet been fully elucidated and other targets of potential relevance to NDs emerge continuously.[115] It is clear the need to develop and use novel molecular imaging compounds for such targets, in addition to those related to Aß, α-synuclein and tau protein, in order to achieve a more complete knowledge about the molecular basis of AD, PD and other NDs. Using such novel molecular imaging compounds, it is expected that PET and SPECT methods will help us to further understand the underlying pathological processes and specific molecular alterations that unfold during early stages of NDs.

With an increasingly larger number of animal research facilities worldwide with access to micro-PET and SPECT technology for preclinical studies, it is expected that pharmacology studies using novel radiotracers will help to identify and validate molecular processes as novel biomarkers to be used as therapeutic targets for treatment, and assess how new drugs are able to modify these biomarkers in animal models of NDs. In this field, one of the most promising strategies should be the use of multi-tracer protocols for the simultaneous evaluation of different molecular targets of relevance to ND. For instance, recent investigations using transgenic mice that express pathologies that characterize both dementia with Lewy bodies (DLB) and AD (DLB-AD mice) have revealed that Aß, tau, and α-synuclein act synergistically to promote the aggregation, phosphorylation, and accumulation of each other, as well as leading to accelerated cognitive decline.[116] Thus, multi-tracer protocols for these three molecular targets must be strongly considered in investigations of NDs.

Finally, it would be highly desirable to translate, in the forthcoming years, some of the above novel findings into direct and objective diagnostic applications in clinical practice. With such developments, PET and SPECT imaging patterns might be used more incisively to improve diagnostic accuracy in doubtful cases, as well as to predict prognoses and treatment response in sufferers of NDs. The larger availability of SPECT and its lower costs may make it the method of choice for such future clinical applications, but a progressively greater access to PET methods across a larger number of hospitals is also expected, particularly in regard to the use of ^{18}F-labeled tracers. The use of any novel radiotracer for clinical applications with PET or SPECT should be weighted against the availability of other promising biomarkers, such as cerebrospinal fluid (CSF) indices of Aß, tau and other pathologies.[117] Large-scale clinical studies should continuously be carried out to ascertain the comparative diagnostic accuracy and cost-benefit of novel PET and SPECT imaging probes and CSF markers, as well as the usefulness of employing such measurements in combination.

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Disclosures

Marcel Benadiba, PhD
Employment: *Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, Netherlands; Laboratory of Psychiatric Neuroimaging (LIM 21), Department of Psychiatry, Faculdade de Medicina, Universidade de São Paulo, Brazil.* **Other:** *Núcleo de Apoio à Pesquisa em Neurociência Aplicada (NAPNA), Universidade de São Paulo, São Paulo, Brazil.*

Gert Luurtsema, PhD
Employment: *Department of Nuclear Medicine and Molecular Imaging, University Medical Center Groningen, University of Groningen, Groningen, Netherlands.*

Lauro Wichert-Ana, PhD
Employment: *Department of Internal Medicine, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil.* **Other:** *Núcleo de Apoio à Pesquisa em Neurociência Aplicada (NAPNA), Universidade de São Paulo, São Paulo, Brazil.*

Carlos Alberto Buchpigel, PhD
Employment: *Center of Nuclear Medicine, Department of Radiology, Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brazil.* **Other:** *Núcleo de Apoio à Pesquisa em Neurociência Aplicada (NAPNA), Universidade de São Paulo, São Paulo, Brazil.*

Geraldo Busatto Filho, PhD
Employment: *Laboratory of Psychiatric Neuroimaging (LIM 21), Department of Psychiatry, Faculdade de Medicina, Universidade de São Paulo, Brazil.* **Other:** *Núcleo de Apoio à Pesquisa em Neurociência Aplicada (NAPNA), Universidade de São Paulo, São Paulo, Brazil.*

** Modest*
*** Significant*
**** Significant. Amounts given to the author's institution or to a colleague for research in which the author has participation, not directly to the author.*

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