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REVIEW ARTICLE

RECENT ADVANCES IN POSITRON EMISSION TOMOGRAPHY IMAGING OF BRAIN

N. Seneca

Imaging Sciences, a division of Translational Research Sciences, Pharma Research and Early Development (pRED), F. Hoffmann-La Roche, Ltd., Basel, Switzerland

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SUMMARY

Positron emission tomography (PET) has been widely used to noninvasively study various functions of the brain, the pathophysiology of disease and aid in drug development. PET and selective radiolabeled molecules allow imaging of certain critical components of the functioning of healthy and diseased brain. The shortage of available PET radioligands for studying the functioning of the brain is a major limiting factor for expanding their use in research and implementation in a clinical setting. Thus, the development of novel PET radioligands for further understanding brain functions is a key factor for advancement in the field. This article provides an overview of recent developments of novel PET radioligands for imaging various functions of healthy and diseased brain, highlighting the recent development of novel PET radioligands for a monoamine transporter, describing the use of pharmacological challenges combined with PET imaging to measure neurotransmitter release and potential imaging biomarkers of disease pathology. In summary, the article reviews recent developments in the field of PET imaging of the brain.

INTRODUCTION

The field of nuclear medicine is continuously growing, incorporating advancements in the understanding of biology, the pathophysiology of disease, technical instrumentation and molecular imaging. Molecular imaging technologies such as positron emission tomography (PET) are increasingly applied to understand the biological functioning of healthy and diseased brains. In addition, PET is applied more and more as a tool in drug development to assess the in vivo distribution and pharmacological properties of a drug (1, 2). PET utilizes radiolabeled entities labeled with relatively short-lived positron-emitting radionuclides, such as ^{18}F ($t_{1/2}$ = 110 minutes) or ^{11}C ($t_{1/2}$ = 20 minutes). PET radioligands are used to understand the biological functioning of the brain, which can measure functional information, i.e., brain metabolism ([^{18}F]-FDG) or regional blood flow ([^{15}O]-water), or to measure the functioning of specific targets, such as receptors, transporters, various proteins involved in second messenger systems, β-amyloid plaques and activated microglia. Further development of novel PET radioligands used to measure the functioning of specific targets in healthy and diseased brain is a key factor for advancement in the field.

The power of PET imaging is its potential to provide a noninvasive, quantifiable, repeatable and localized measure of molecular processes in the living body. PET imaging measures the in vivo distribution of a PET radioligand after i.v. administration over time. The PET outcome measure is calculated from time–activity curve data, which is a measure of the concentrations of radioactivity uptake in a region of interest over time. By applying a kinetic model to the time–activity curve data, the PET outcome measures are primarily defined as regional values of volumes of distribution (V_T) or binding potentials (BP_{ND}) (3, 4). After the injection of a dopamine transporter PET radioligand, the resulting PET outcome measure is a reflection of the density of the transporter in a particular brain region, such as the striatum. For example, changes in the PET outcome measure may be a reflection of dopaminergic degeneration in a particular disease state (e.g., Parkinson's disease) or after administration of a stimulant such as methylphenidate (5-8). A reduction in the PET outcome measure would most likely be found in both examples. In Parkinson's disease, the reduction in the PET outcome measure would reflect the loss of dopaminergic neurons, whereas in the latter example, the effects of administration of methylphenidate are related to the percentage of transporter sites occupied by the drug and transporter occupancy is reflected as the percent change in the PET outcome measure determined under the drug treatment conditions compared to that under baseline measures. Thus, PET imaging provides a quantifiable measure of the brain uptake of radiolabeled entities, which can be measured in healthy and diseased brains and provide estimations of receptor or transporter occupancy of well-characterized and novel drug candidates.

This review discusses recent developments of PET radioligands for imaging various functions of healthy and diseased brain. The development of a novel monoamine transporter PET radioligand is

Correspondence: Nicholas Seneca, PhD, F. Hoffmann-La Roche, Ltd., Building 663/3113, Hochstrasse 16, 4070 Basel, Switzerland. E-mail: Nicholas.Seneca@roche.com.

described and the limitations for future progress. The use of pharmacological challenges combined with PET imaging to measure neurotransmitter release are also discussed. Finally, potential imaging biomarkers of disease pathology, such as imaging β-amyloid plaques and activated microglia, are reviewed. The PET radioligands described in this article have applicability in testing brain functions of animal, first-in man and diseased brain to ensure proper validation of the target in a research setting and potential implementation into a clinical setting.

RECENT ADVANCES: THE PAST 10 YEARS

Targets

Development of radioligands for the norepinephrine transporter

Molecular imaging of the dopamine (DAT), serotonin (SERT) and norepinephrine transporters (NET) provides valuable insight into the in vivo function of these transporters in various disorders (e.g., depression and attention deficit hyperactivity disorder [ADHD]), as well as potential markers for neurodegenerative diseases (e.g., Parkinson's disease) (9). Furthermore, molecular imaging studies using PET radioligands selective for these transporters have provided valuable information regarding the estimation of transporter occupancy of well-characterized and novel drug candidates (5, 10-14). The majority of molecular imaging studies have focused on DAT and SERT, whereas evaluation of NET has lagged (Fig. 1). An alteration in monoamine transporters in various disorders is not limited to only DAT and SERT, increasing evidence suggesting a critical role for NET. NET has been implicated in several indications, such as depression (15), stress (16) and stimulant addiction (17), and a functional loss of NET in genetic knockout mice results in profound neurobiological, physiological and behavioral changes (18-20). Thus, the development of selective PET radioligands for NET would provide valuable insight into the in vivo function of this monoamine transporter.

Evaluation of analogues of reboxetine as potential PET radioligands has led to the most promising in vivo binding to NET (21, 22). Both [^{11}C]- and [^{18}F]-labeled analogues of reboxetine have been prepared and evaluated as in vivo PET radioligands for NET in rodent, monkey and human brain (21-23). In vitro autoradiography using radiolabeled analogues of reboxetine (e.g., the *O*-methyl analogue MeNER, also known as MRB, defined in this review as MeNER/MRB, and the di-deuterated *O*-fluoromethyl analogue FMeNER-D_2) has demonstrated selective and specific binding to NET in rodent and postmortem human brain (24, 25). Initial evaluation of the *O*-[^{11}C]-methyl analogue of reboxetine (i.e., [^{11}C]-MeNER/MRB) demonstrated moderate to low specific binding to NET in the nonhuman primate brain (26, 27). These initial animal experiments were followed by several studies evaluating the selectivity and specificity of [^{11}C]-MeNER/MRB binding in healthy human brain (28-31). After these initial studies in man, recent NET occupancy studies have been reported in nonhuman primate and healthy control subjects (30, 32, 33). Estimations of NET occupancy for clinically effective doses of

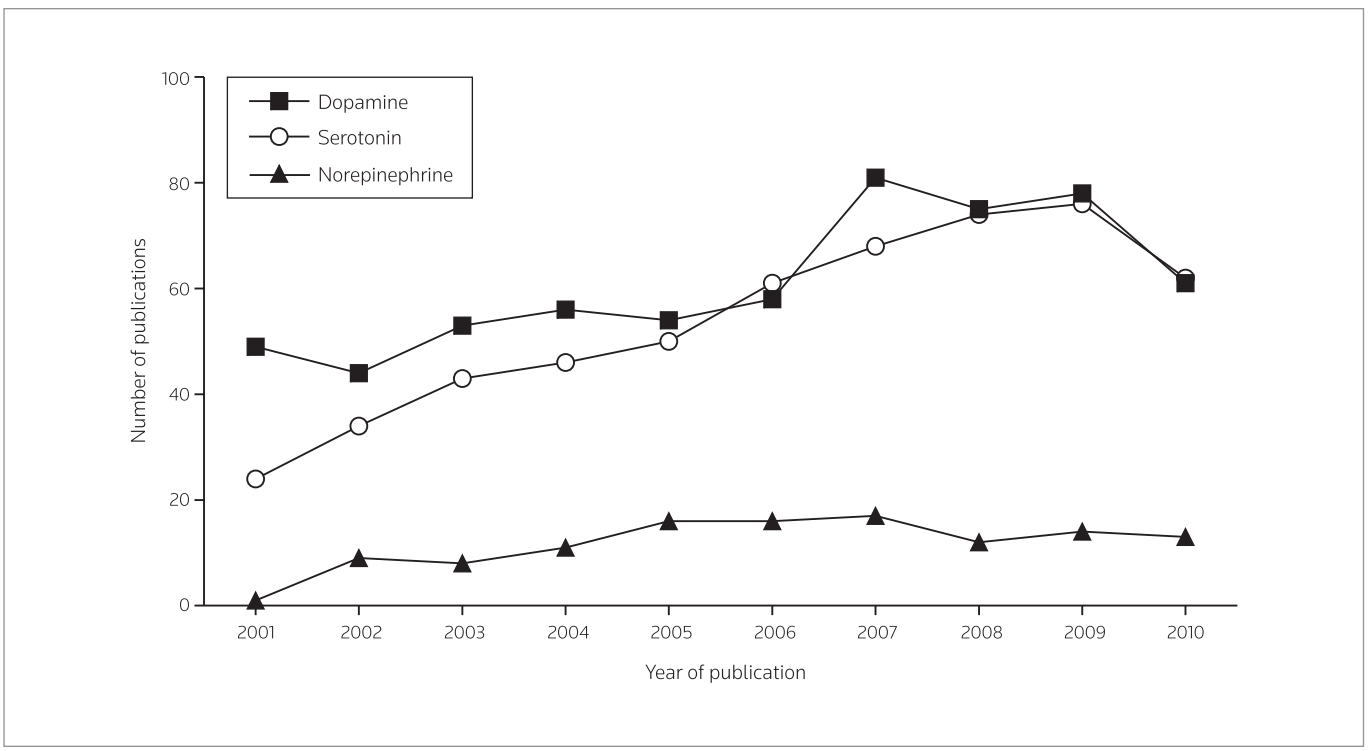

Figure 1. The number of publications per year for monoamine transporter PET radioligands (e.g., dopamine [■], serotonin [○] and norepinephrine [▲]). Results are from searches using ISI Web of Knowledge (www.isiknowledge.com). Keywords used in the search included: brain*positron emission tomography*transporter (e.g., dopamine or serotonin or norepinephrine transporter).

atomoxetine using [^{11}C]-MeNER/MRB were found to be similar to those reported earlier using (*S,S*)-[^{18}F]-FMeNER-D_2 (14, 33, 34). Thus, three different publications have demonstrated a dose-dependent and saturable binding of the [^{11}C]- and [^{18}F]-labeled analogues of reboxetine with increased doses of atomoxetine in monkey but not in human brain (14, 30, 33, 34). Although atomoxetine was not found to occupy NET in human brain, the stimulant methylphenidate was recently found to occupy up to 80% of the NETs in healthy human brain after a single oral dose (32).

Of particular interest is one of the first imaging studies in a patient or target indication using either the [^{11}C]- or [^{18}F]-labeled analogues of reboxetine (35). This study demonstrated that NET is susceptible to an age-dependent reduction, as has been shown for other monoamine transporters. Furthermore, cocaine-dependent subjects showed a significant increase in radioligand binding in brain regions with known NET-rich regions, such as thalamus. The increased radioligand binding in cocaine-dependent subjects compared to healthy controls reflects a potential upregulation of NET. In conclusion, [^{11}C]-MeNER/MRB has been shown to bind selectively and specifically to NET in nonhuman primate and human brain. However, PET radioligand uptake showed that specific binding to NET did not reach maximal values (i.e., equilibrium) during the time of PET measurement and the uptake provided a somewhat noisy signal at later scanning times. These potential limitations are some of the relative deficiencies of [^{11}C]-MeNER/MRB for quantitative studies of NET in brain.

The potential limitations outlined above using the [^{11}C] analogue of reboxetine led to the preparation and evaluation of radiofluorinated analogues of (*S,S*)-MeNER/MRB (24, 36). Initial evaluation of nonhuman primate brain uptake and regional brain distribution indicated that (*S,S*)-[^{18}F]-FMeNER-D_2 may be a useful radiofluorinated ligand for imaging NET (36). A direct comparison of [^{11}C]-MeNER/MRB and (*S,S*)-[^{18}F]-FMeNER-D_2 in the same animal resulted in several advantages for the [^{18}F]- versus the [^{11}C]-labeled analogue of reboxetine: 1) whole brain uptake was found to be higher and washed out faster; 2) brain regions with known high densities of NET, such as thalamus and brainstem, reached specific binding peak equilibrium during the PET measurement; 3) the time–activity curve data showed a lower noise level at later time points (37). However, one major disadvantage of (*S,S*)-[^{18}F]-FMeNER-D_2 compared to [^{11}C]-MeNER/MRB is that the cortical uptake of the radioligand is influenced by high bone uptake with resulting defluorination, which complicates the calculation of the PET outcome measures in those brain regions.

The potential advantages mentioned above using (*S,S*)-[^{18}F]-FMeNER-D_2 led to further evaluation in human brain of the uptake and quantification of the PET outcome measures, as well as transporter occupancy studies both in nonhuman primates and healthy control subjects (13, 14, 34, 38-40). Transporter occupancy studies in nonhuman primates demonstrated that clinically effective doses of atomoxetine cause dose-dependent and saturable binding of (*S,S*)-[^{18}F]-FMeNER-D_2 (14, 34), whereas maximal transporter occupancy of approximately 48% was demonstrated in healthy controls who were administered a single dose of the antidepressant nortriptyline (13). Additional studies in man comparing transporter occupancy using other antidepressants, estimates of the variability and reproducibility of the outcome measure (i.e., test and retest studies) and imaging studies in various disorders will determine the utility of using (*S,S*)-[^{18}F]-FMeNER-D_2. In conclusion, the regional uptake and specificity of binding to NET is moderate to low using either (*S,S*)-[^{18}F]-FMeNER-D_2 or [^{11}C]-MeNER/MRB, but to date these are the only available PET radioligands for this monoamine transporter. Despite the moderate to low radioligand binding using these PET radioligands, some of the imaging data acquired to date demonstrate their promise and utility for in vivo imaging of NET in living brain.

Evaluation of compounds other than analogues of reboxetine as potential PET radioligands for NET has been extensively reviewed (41). Briefly, nisoxetine was found to have high affinity for NET in vitro; nevertheless, [^{11}C]-nisoxetine exhibited high levels of nonspecific binding in vivo (42). Recently, another analogue of nisoxetine, [^{18}F]-MFP-3, has been studied as a potential PET radioligand for imaging NET (43). To date, only the radiosynthesis and in vitro evaluation of [^{18}F]-MFP-3 have been presented and future in vivo studies in animal and man will determine the utility of this potential radioligand. In addition, several analogues of cocaine have been radiolabeled for NET, but due to high affinity for DAT and the low density of NET in brain, the radioligands selectively labeled DAT in vivo (44). Finally, evaluation of [^{11}C]-radiolabeled desipramine, talopram and talsupram demonstrated low brain uptake and lack of selective NET binding in vivo, although they exhibited high selectivity for NET over other monoamine transporters (45, 46). Thus, several compounds of different structural and/or chemical classes have been evaluated as potential PET radioligands for NET, but newer compounds will hopefully yield a PET radioligand with more optimal binding properties for NET compared to those currently available.

The development of PET radioligands for NET faces additional challenges compared to those encountered when developing PET radioligands for other monoamine transporters. Several factors need to be considered, such as, but not limited to:

1. Low densities of NET compared to other monoamine transporters in the brain. Density and regional brain distribution vary among DAT, SERT and NET. For example, DAT has the highest density in the brain (B_{max} = 274 pmol/g), followed by lower levels of SERT (B_{max} = 143 pmol/g), and NET having the lowest density in the brain of the three monoamine transporters (B_{max} = 4.4 pmol/g). In addition to having a low density in the brain compared to other monoamine transporters, the brain regions with the highest density of NET are distributed in small brain regions such as the locus coeruleus, anterior ventral nucleus of the thalamus and hypothalamus (25, 47-49). Thus, not only does NET have low densities in brain, but the highest density regions are localized in small brain regions. The development of future compounds as selective NET PET radioligands would require high affinity for the target and severalfold selectivity over other monoamine transporters which have higher densities and broader regional distribution in the brain.

2. Advantages of using a high-resolution PET camera to improve quantifying and localizing radioligand uptake in small brain regions known to contain high densities of NET. The highest densities of NET are distributed in small brain regions such as the locus coeruleus, anterior ventral nucleus of the thalamus and hypothalamus. The locus coeruleus, the highest density region, is difficult to localize and quantify with PET due to its small volume and the lower resolution

of typical clinical PET cameras. However, using a higher resolution PET camera, such as the High Resolution Research Tomograph (HRRT), can improve the accuracy in quantifying and localizing radioligand uptake in small brain regions, such as subdivisions of the thalamus and brainstem. Both the [^{11}C]- and [^{18}F]-labeled analogues of reboxetine have been studied using the HRRT PET camera and it was noted to provide fairly clear localization of radioligand uptake compared to previously published data using a typical clinical PET camera (32-35, 37, 39).

3. The use of the appropriate reference region and kinetic model(s) for quantification of PET outcome measure. Binding potential (BP_{ND}) is one method to quantify radioligand binding, which compares the concentration of a radioligand in receptor-dense to receptor-free regions of the brain. Several different brain regions have been used as a receptor-free region (i.e., a reference region which is a brain region devoid of specific binding) for both the [^{11}C]- and [^{18}F]-labeled analogues of reboxetine. For example, the caudate nucleus, occipital cortex and prefrontal cortex have all been used as reference regions when calculating BP_{ND}. Different receptor-free brain regions have been used to calculate BP_{ND} in several publications. Since radioligand binding may differ among the three different regions used as a receptor-free region, it is difficult to compare the estimates of BP_{ND} between studies. An extensive discussion comparing different reference regions and advanced kinetic modeling techniques used for calculating PET outcome measures using [^{11}C]-MeNER/MRB or (*S,S*)-[^{18}F]-FMeNER-D_2 can be found in Gallezot et al. (33).

Pharmacological manipulation of endogenous neurotransmission in brain

Pharmacological challenges combined with molecular imaging provide an indirect measure of neurotransmitter alterations in living brain. Increased dopamine release induced by a pharmacological challenge (e.g., administration of amphetamine) has been shown to decrease PET radioligand binding to dopamine D_2 and D_3 receptors in animal and human brain (50-52). In addition, molecular imaging studies combined with microdialysis demonstrated a dose-dependent relationship between administration of increasing doses of amphetamine, resulting in increases of extracellular dopamine release which correlated with a dose-dependent and maximal change in PET radioligand binding to dopamine D_2 and D_3 receptors (Fig. 2A and B) (53-57). These in vivo pharmacological challenges can be thought of as an extension of in vitro competition assays, which can be partially explained by the classic occupancy model (52). Briefly, the classic occupancy model predicts that an increase of extracellular dopamine causes a decrease of PET radioligand binding due to competition from endogenous dopamine (i.e., dopamine displacement). That is, PET radioligand binding during baseline conditions reflects the availability of PET radioligand binding to dopamine D_2 and D_3 receptors versus competition from endogenous dopamine. Increases of extracellular dopamine release induced by a pharmacological challenge lead to increased endogenous dopamine competition, resulting in decreased availability of PET radioligand binding to dopamine D_2 and D_3 receptors.

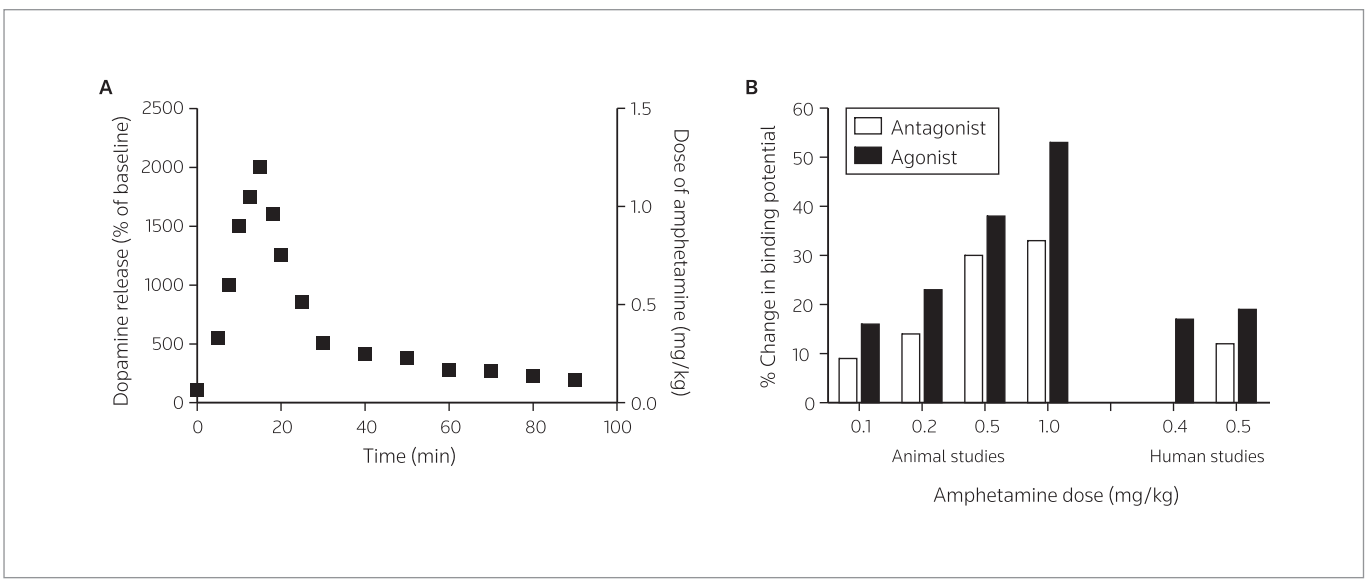

Figure 2. Increases of extracellular dopamine concentrations by administration of amphetamine correlate to a certain percentage change of the PET outcome measure using dopamine D_2 and D_3 PET radioligands. (**A**) Increasing doses of amphetamine cause a dose-dependent increase in the concentration of extracellular dopamine as measured by microdialysis in the striatum of animals (data are the average from the following references: 53-57). (**B**) Administration of increasing doses of amphetamine has been shown to cause a dose-dependent decrease in the PET outcome measure of dopamine D_2 and D_3 PET radioligands. Animal studies have shown that this % change is greater when using an agonist versus an antagonist PET radioligand. To date, only two human studies have assessed the effects of oral amphetamine in decreasing the PET outcome measure of dopamine D_2 and D_3 agonist PET radioligands in healthy controls. The effect of similar doses of amphetamine (i.e., 0.4-0.5 mg/kg) in man was much less than that shown in animal studies. Data included in the bar graph are the average of animal studies, which included data for antagonist (i.e., [^{11}C]-raclopride) and agonist radioligands (i.e., [^{11}C]-NPA, [^{11}C]-MNPA and [^{11}C]-PHNO), from references 56 and 65-67, and human studies from references 68 and 69.

The majority of pharmacological challenge studies combined with molecular imaging of dopamine D_2 and D_3 receptors have been performed with antagonist radioligands, such as [^{11}C]-raclopride. Antagonists of G protein-coupled receptors, such as dopamine D_2 and D_3 receptors, have equal affinity for receptors in the high- (i.e., coupled) or low- (i.e., uncoupled) affinity state (58-61). Since dopamine is the endogenous agonist for dopamine D_2 and D_3 receptors, it would more effectively compete with the binding of an agonist compared to an antagonist radioligand. The recent development of three dopamine D_2 and D_3 receptor agonist radioligands, [^{11}C]-NPA, [^{11}C]-MNPA and [^{11}C]-(+)-PHNO, has made it possible to examine whether these radioligands more effectively compete with changes in endogenous dopamine (62-64). Pharmacological challenge studies in animals have consistently reported a more marked effect using agonist PET radioligands compared to antagonist PET radioligands (Fig. 2B) (56, 65-67). Extrapolation of reported results in animals has been limited to only two healthy control studies (68, 69). At similar doses of amphetamine, the decrease of agonist PET radioligand binding (i.e., [^{11}C]-NPA and [^{11}C]-PHNO) to dopamine D_2 and D_3 receptors resulted in a similar marked effect in healthy control subjects (68). Although a marked effect was shown using these two different agonist PET radioligands in man, the extent of the effect was less than in studies in animals (68, 69). In addition, a direct comparison using both an agonist and antagonist PET radioligand in the same healthy control subjects has been limited to only [^{11}C]-NPA and was not reported for [^{11}C]-PHNO. Further studies are warranted using the dopamine D_2 and D_3 receptor agonist PET radioligands to examine the utility of pharmacological challenge studies in healthy controls and patient populations of interest, such as patients with schizophrenia.

Few studies have combined pharmacological challenges with molecular imaging to measure alterations of neurotransmitter levels in brains of patients with schizophrenia (56, 70). Greater increases in extracellular dopamine release induced by administration of amphetamine led to a more pronounced decrease in PET radioligand binding to dopamine D_2 and D_3 receptors in patients with schizophrenia compared to healthy controls. In addition, administration of amphetamine mediated the expression of psychotic symptoms in patients with schizophrenia, which were positively correlated with a more pronounced decrease in PET radioligand binding to dopamine D_2 and D_3 receptors. These positive results combining pharmacological challenges with molecular imaging provided supporting evidence of the dopamine hypothesis of schizophrenia (i.e., hyperactivity of dopaminergic neurotransmission). Further evaluation of these effects using agonist PET radioligands for dopamine D_2 and D_3 receptors may shed more light on alterations of neurotransmitters in various target patient populations of interest.

Imaging biomarkers for disease pathology

Monitoring microglia activation as a biomarker for disease pathophysiology

Activation of microglia is thought to play an important role in the pathophysiology of various neurological and psychiatric disorders (71-73). Translocator protein (18 kDa; *TSPO*; previously referred to as peripheral-type benzodiazepine receptor, or PBR) is a mitochondrial protein that has been shown to co-localize with activated microglia in postmortem brain samples of diseased brains (72, 74, 75). PET imaging of translocator protein has been performed as a marker for inflammation in brain using [^{11}C]-PK-11195 for nearly 20 years. For example, uptake of [^{11}C]-PK-11195 as an in vivo PET radioligand for translocator protein has been found to be increased in active lesions and normal-appearing white matter in patients with multiple sclerosis (76, 77). However, the uptake of [^{11}C]-PK-11195 has undesirable high nonspecific binding and low specific binding (i.e., to translocator protein), which hampers quantification of the PET outcome measure(s), which reflect the amount of microglia activation (i.e., increased PET outcome measure reflects increased microglia activation). In recent years, significant efforts have been made to develop new PET radioligands with a different chemical structure and more desirable in vivo binding properties to translocator protein compared to [^{11}C]-PK-11195. The development of newer PET radioligands, such as [^{11}C]-PBR-28, [^{11}C]-DAA-1006, [^{18}F]-PBR-111 and [^{11}C]-DPA-713, has demonstrated a greater ratio of specific to nonspecific binding to translocator protein and lower nonspecific binding (78-81). The development of these novel radioligands has been reflected in an increased number of publications per year in studies utilizing PET radioligands selective for translocator protein (Fig. 3). Thus, molecular imaging studies using newer PET radioligands selective for translocator protein may provide a valuable tool to detect the amount of microglia activation in diseased brain.

Several shortcomings of imaging translocator protein as a marker for microglia activation make interpretation of the in vivo imaging data difficult. First, the interpretation of the in vivo data reflected as total translocator protein binding may provide insight into different biological information, depending on the timing of the injury or disease duration. The components of total translocator protein binding in vitro have been shown to have different profiles of microglia versus astrocytes, depending on the timing and duration of injury (Fig. 4A) (82, 83). In addition, the component of binding has been shown to have two different profiles of biological information when assessing the components of microglia versus astrocyte contribution post-injury. For example, in a rodent hippocampal lesion model using the neurotoxin trimethyltin, the component of total translocator protein was shown to reflect an initial increase in microglia activation, but as the duration of injury increased, microglia activation decreased, followed by an increasing contribution of astrocytes (82). Conversely, in a different brain injury model using an ethanol microinjection in rodent striatum, an initial increase in both microglia and astrocytes was detected, followed by a steady decrease of both microglia and astrocytes as time increased post-injury (83). It could be argued that the profiles may have differed for several reasons, not limited to: different drugs were used to induce brain injury (trimethyltin versus ethanol); different brain regions were analyzed (hippocampus versus striatum); different immunohistochemistry stains were used for visualizing microglia (GSI-B4 versus Iba-1); and the assessments were performed for different periods of time post-injury. Regardless, these in vitro results confirm that the binding of translocator protein reflects a component of microglia and astrocytes. These two different profiles of biological function would suggest that, if imaging is performed during an initial stage of an injury or disease, the majority of the PET outcome measure using a translocator protein radioligand would reflect microglia activation. As the duration of injury or disease progresses, translocator protein binding would reflect astro-

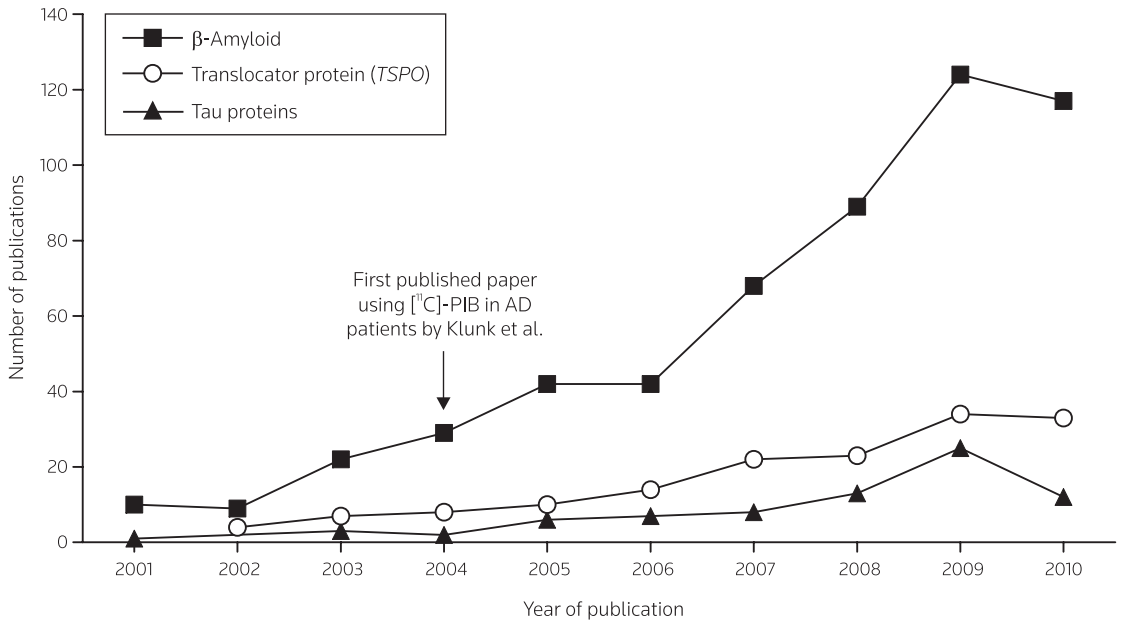

Figure 3. The number of publications per year for PET radioligands used as potential imaging biomarkers for disease pathology (e.g., β-amyloid [■], tau protein [○] and translocator protein [▲]). The development and success of molecular imaging studies using [^{11}C]-PIB to study β-amyloid in patients with Alzheimer's disease have significantly impacted the number of publications in the field since 2004. Results are from searches using ISI Web of Knowledge (www.isiknowledge.com). Keywords used in the search included: brain*positron emission tomography*target of interest (e.g., β-amyloid [■], or tau protein [○] or translocator protein plus peripheral benzodiazepine receptor [PBR] [▲]).

cytes or a combination of the microglia and astrocytes based on the in vitro results described above. Since the biological function of microglia and astrocytes may represent different functions during injury or disease, it would be advantageous to have a PET radioligand selective for one function or the other.

Does the in vitro profile described above affect the interpretation or help explain the PET outcome measure seen in human studies of injury or disease? This in vitro profile could potentially be reflected in two recent examples of human studies using [^{11}C]-PBR-28 (84, 85). For example, an incidental stroke finding identified a 76% increase in [^{11}C]-PBR-28 binding at the site of the infarct (Fig. 4B) (85). The increase in the PET outcome measure would primarily reflect microglia activation based on the in vitro component of binding (i.e., microglia versus astrocytes) described above, since the scan was performed within 12 days of the occurrence of symptoms, whereas in a chronic disorder such as multiple sclerosis, [^{11}C]-PBR-28 binding was not substantially increased in the 11 patients examined, in which some patients had active lesions, undergoing drug treatment and varying duration of illness (84). Of interest was a potential trend towards an increased binding of [^{11}C]-PBR-28 in patients with increased duration of disease and in those patients who had active gadolinium-enhancing lesions (i.e., 3 of the 11 patients examined). Thus, molecular imaging of translocator protein in an acute injury such as stroke may primarily reflect only microglia, which resulted in a significant increase in translocator protein binding, whereas in a chronic disorder such as multiple sclerosis, the contribution of the signal may be mixed (i.e., microglia versus astrocytes), which may have resulted in a moderate increase in translocator protein binding.

Another potential limitation of PET radioligands for translocator protein is that a number of subjects have been found to have low uptake in organs with known high densities of translocator protein (defined as nonbinders) compared to the expected high uptake in these organs (defined as binders) (86). Earlier studies using [^{11}C]-PK-11195 had not previously reported significant differences in organ uptake between subjects and the lack of differentiation between nonbinders versus binders may have been due to the low specific and high nonspecific binding in brain and peripheral organs. Conversely, most if not all of the newer PET radioligands for translocator protein, such as [^{11}C]-PBR-28, have been shown to more clearly distinguish subjects as nonbinders and binders. Furthermore, these PET radioligands suffer not only from a distinction between nonbinders and binders, but have been further characterized into mixed-affinity binders (87, 88). Three binding affinity patterns have been found, which has further classified the nonbinders and binders into high-affinity, mixed-affinity and low-affinity binders. In vitro binding assays using human brain tissue (i.e., healthy and diseased brain samples) and peripheral blood samples have shown that PBR-28 has the highest specific binding and differentiation among the three binding affinity patterns, whereas compounds such as PK-11195 cannot differentiate among the three binding affinity patterns.

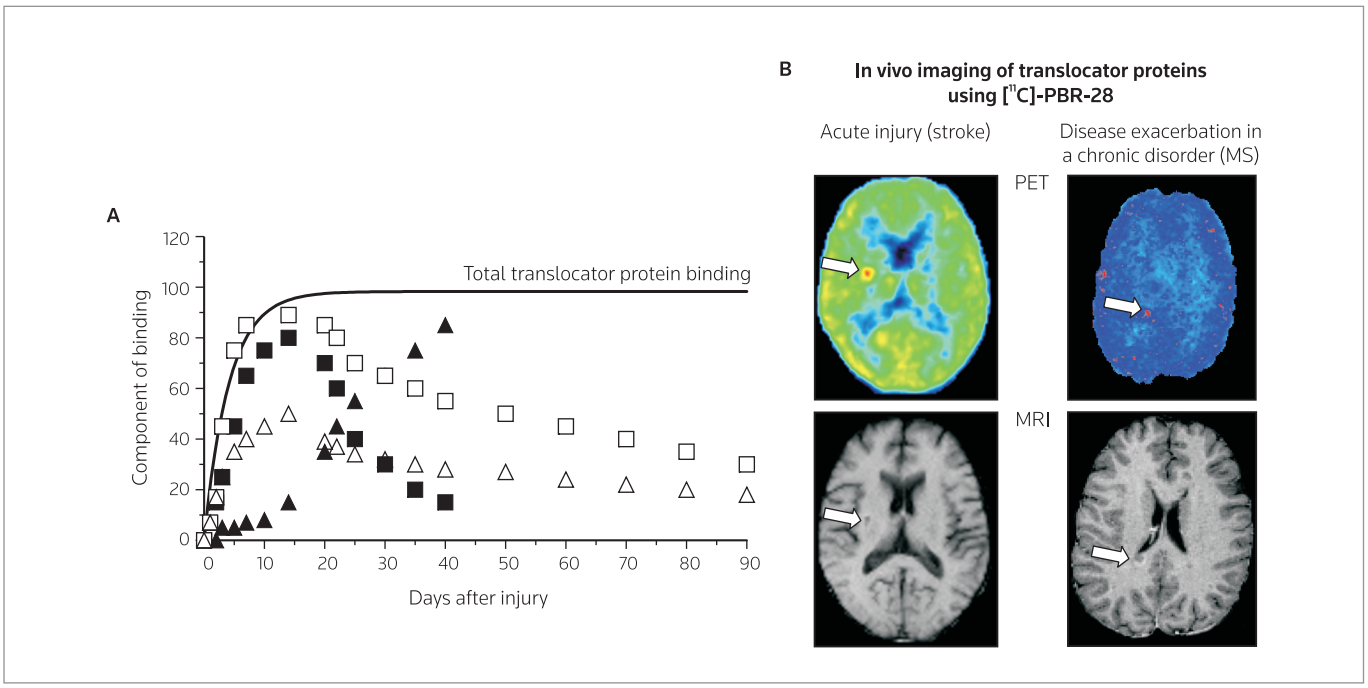

Figure 4. (**A**) Distribution of microglia (■) and astrocytes (▲) assessed by immunohistochemistry after drug-induced injury in rodent brain. The time-dependent distribution of the expression of microglia versus astrocytes differs between two publications. Kuhlmann et al. (2000; closed symbols) and Maeda et al. (2007; open symbols) were both studies showing that directly after injury the majority of translocator protein binding reflects microglia activation. However, the two papers differ post-injury, with one paper showing an increased contribution of astrocytes over time with a corresponding decrease in microglia, whereas the other paper shows a steady decrease of both microglia and astrocytes. Please note that not all time points were assessed post-injury, as displayed in Figure 4A, and additional time points were added to the figure for visual representation. These time-dependent differences of microglia versus astrocytes make interpretation of the in vivo binding of PET radioligands to translocator protein difficult. The total binding seen in the PET images would reflect a percentage of contribution from both microglia and astrocytes, since translocator protein colocalizes. (**B**) [^{11}C]-PBR-28 binding to translocator protein in a subject after a stroke (top left) and in a multiple sclerosis (MS) patient with increased [^{11}C]-PBR-28 binding at the site of some gadolinium-enhancing lesions (top right). Images in the lower portion of the figure are the corresponding magnetic resonance images showing the location of the infarct (bottom left) and the presence of gadolinium contrast-enhanced lesions in the patient with MS (bottom right). Brain images (left) are from Arch Neurol 2009, 66(10): 1288-9, Copyright © 2009 American Medical Association. All rights reserved. The images of the patient with MS were kindly provided by Dr. Steve Jacobson of NINDS, NIH, and Dr. Vasiliki N. Ikonomidou of George Mason University (right).

Further evaluation is warranted to better understand why the different translocator protein PET radioligands bind to three different affinity patterns. Implementation of a peripheral blood assay to prescreen potential imaging subjects into a class of a binding affinity pattern would be essential when performing in vivo PET studies. These tests would help to ensure that research subjects included in an in vivo imaging study are classified by a particular binding affinity pattern.

Since translocator protein has been found to co-localize with both activated microglia and astrocytes in diseased brains, interpretation of the specific binding using PET radioligands selective for translocator protein may be complicated. Microglia are the resident macrophages of the brain that serve both glial and immune-related functions, which are implicated in the pathophysiology of various neurological and psychiatric disorders (71, 72). Activation of microglia is a bit misleading, since macrophages exist in numerous functionally distinct subpopulations that develop in response to signals within their microenvironment (89, 90). For example, a brain injury or disease can activate microglia and, depending on the activated subpopulation, the resulting macrophage activity can exacerbate disease pathology or support tissue repair (91). On the other hand, the biological function of astrocytes was once thought to be limited to a few functions in the brain (92). Recent evidence suggests that the functions of astrocytes are much broader than initially thought, which has been reviewed in detail elsewhere (93). Briefly, astrocytes play a primary role in synaptic function, contribute to central nervous system metabolism and the regulation of fluid balance and transmitter homeostasis (92-94). Furthermore, astrocytes respond to brain injury or disease by a process of reactive astrogliosis, which involves molecular expression and functional changes of astrocytes, and in severe injury or disease results in persisting scar formation (95, 96). Although newer PET radioligands for translocator protein have demonstrated higher specific and lower nonspecific binding to translocator protein compared to [^{11}C]-PK-11195, the PET outcome is a reflection of not only the activated microglia but also astrocytes. Thus, based on the variety of biological functions of activated microglia and astrocytes, PET radioligands with selectivity for each component of binding would be advantageous for interpreting the PET outcome measure reflected in diseased brains.

Despite the limitations described above, in vivo assessment of inflammatory cell activity in patients with various neurological or psychiatric disorders may provide a quantifiable measure of a patient's immune system activation and alteration during drug treatment. Monitoring the alteration of inflammation (i.e., activation of microglia or astrocytes) during and after drug treatment may provide valuable insight into the molecular mechanism of action of drug treatment and assessment of the status of inflammatory cell activity in diseased brains.

Molecular imaging of β-amyloid in patients with Alzheimer's disease

Molecular imaging studies using PET radioligands such as [^{11}C]-PIB, [^{18}F]-FDDNP, [^{11}C]-SB-13, [^{11}C]-MeS-IMPY, [^{11}C]-BF-227 and [^{18}F]-AV-45 have demonstrated the feasibility of imaging β-amyloid in patients with Alzheimer's disease (AD) (97-102). Since accumulation of β-amyloid has been proposed as one of the underlying pathophysiologies of AD, in vivo quantification of β-amyloid may provide a useful tool to diagnose the disease, monitor the effects on the concentrations of β-amyloid by novel therapeutics, and explore the role of β-amyloid in disease. Therefore, several PET radioligands have been developed to image β-amyloid and are reviewed in detail elsewhere (103-105). Briefly, one of the first in vivo reports of a PET radioligand that detected binding to β-amyloid in the brain of patients with AD was using [^{11}C]-PIB (100) (Fig. 3). Among the PET radioligands listed above, the degree to which these radioligands bind to β-amyloid varies in terms of the percent increase in the PET outcome measures (i.e., binding potential [BP_{ND}] or distribution volume [V_T]) in the brains of patients with AD compared to those of age-matched healthy controls, who would likely have little β-amyloid in brain. A percent increase of less than 20% in the PET outcome measures has been shown using [^{11}C]-SB-13, [^{11}C]-MeS-IMPY and [^{11}C]-BF-227 in brain regions known to contain high densities of β-amyloid in patients with AD. This moderate to low increase in the PET outcome measure is much less compared to the "gold standard" [^{11}C]-PIB, with a percent increase of about 130% in the brains of patients with AD compared to age-matched healthy controls. Thus, molecular imaging studies have confirmed the potential for in vivo quantification of β-amyloid, which may provide a useful tool for diagnosing AD (106, 107).

To explore the role of β-amyloid during disease progression, longitudinal follow-up studies of [^{11}C]-PIB binding to β-amyloid during disease progression and normal aging have shown minimal changes in the PET outcome measure to brain β-amyloid in the brains of patients with AD versus age-matched healthy controls (108-110) (Fig. 5). The follow-up PET scans during these longitudinal studies showed both increased and decreased binding in the brains of these subjects during follow-up imaging, which are within the reported test–retest of [^{11}C]-PIB (111). Additional studies are needed to assess why longitudinal changes have not been found, or longer duration studies are needed, which may demonstrate an increased amyloid load in further disease-progressing patients. Future studies monitoring the alteration of the concentration of β-amyloid by novel therapeutics will determine the utility of molecular imaging of β-amyloid in the brain of patients with AD. A recent example assessed changes of β-amyloid in the brain of mild to moderate AD patients after 78 weeks of treatment with a humanized anti-β-amyloid monoclonal antibody, bapineuzumab (112). A greater reduction in [^{11}C]-PIB binding (i.e., imaging results can be interpreted as reduced β-amyloid load in brain) was found in AD patients treated with bapineuzumab compared to placebo. Unfortunately, the reduction of β-amyloid load in the brains of AD patients was not correlated with a measurable clinical benefit or other biomarker endpoints. Finally, the reduction of [^{11}C]-PIB binding in patients treated with bapineuzumab is somewhat difficult to interpret since the binding of [^{11}C]-PIB reflects a contribution of binding to both cerebrovascular amyloid plus parenchymal β-amyloid (113-119).

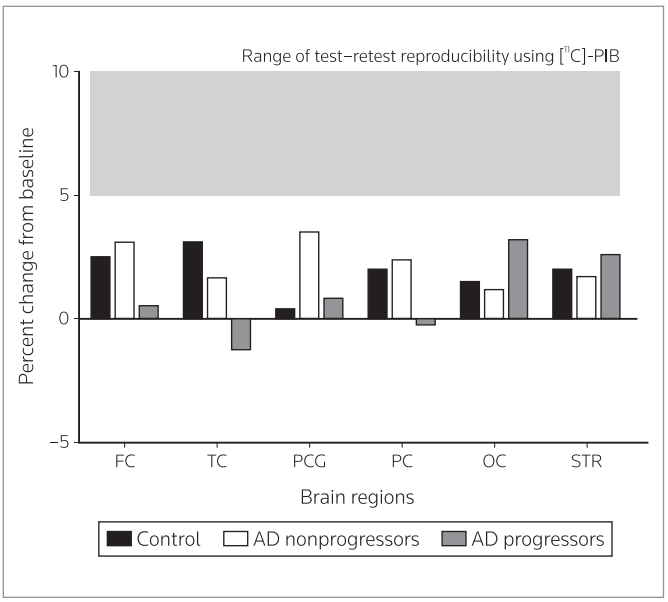

Figure 5. Minimal longitudinal changes in [^{11}C]-PIB binding have been found in normal aging individuals versus Alzheimer's disease patients (i.e., patients who progressed versus nonprogressors) during a 2-year follow-up. Despite cognitive decline, significant cortical atrophy and reduced glucose metabolism in the majority of the patients, the amount of β-amyloid in brain detected by [^{11}C]-PIB did not seem to increase. The percent change in the PET outcome in normal aging individuals versus patients with Alzheimer's disease was within the test–retest reproducibility of [^{11}C]-PIB (shown by a grey square from 5 to 10%). Data included in the figure are the average changes shown in publications (108-110) and the 5-10% estimated test–retest reproducibility is from (111). FC, frontal cortex; TC, temporal cortex; PCG, posterior cingulate gyrus; PC, parietal cortex; OC, occipital cortex; STR, striatum.

In conclusion, molecular imaging in a disease as complex as AD may yield useful information to aid in further understanding progression, diagnosis and differentiation of disease, as well as exploring the role of β-amyloid in the disease. Development of novel PET radioligands selective for disease pathology markers other than β-amyloid plaques, such as tau proteins, integrity of the blood–brain barrier and/or markers of inflammatory cell activity may provide further insight into AD.

FUTURE PROSPECTS: THE NEXT FIVE YEARS

The future of any technical field such as molecular imaging relies on the advancement of technology (e.g., the integration of multi-modalities into one machine such as nuclear and magnetic resonance imaging [MRI]), accessibility of the technology for use in a research and clinical setting, and availability of various PET radioligands for use in target patient populations. Advances in imaging technology, such as the integration of PET and MRI modalities, may have a significant impact on research and clinical imaging studies performed in various disease areas (e.g., oncology and neurology) (120-122). The ability to perform dual-modality imaging in the same subject at the same time may provide a unique path for understanding different processes in the brain. The combination of the modalities provides a unique way to acquire more than one imaging outcome measure at once. In addition, dual-modality imaging would decrease stress for the patient, since only one camera and setup would be needed, decrease the logistics of scheduling scan time on two different machines and nearly eliminate inaccurate modality matching by positioning the subject in one camera for both modalities. Thus, advancements in imaging technology may provide a unique way to measure various functions of the brain simultaneously, but it has yet to be shown whether combining these two modalities will have a greater impact on research and/or clinical imaging studies.

The availability of PET radioligands for various molecular processes in the brain poses a limiting factor for the expanded use of molecular imaging in research and clinical settings. The development of novel PET radioligands is crucial to enable further understanding of these processes in healthy and diseased brains. In recent years, significant efforts have been dedicated to developing novel PET radioligands for specific molecular targets (123-129) and/or imaging biomarkers of disease pathology (130-133) (Table I). While this table does not represent all of the recent novel PET radioligands under development, it does demonstrate that all of these particular targets have not been evaluated in healthy and diseased human brain (please note that this statement is based purely on access to publicly available published papers in peer-reviewed journals). Many of these novel PET radioligands may show promising results from in vitro and in vivo functional assays and preliminary in vivo imaging studies in preclinical animal models of human disease, but need validation in human brain. One avenue to more rapidly validate these novel PET radioligands would be to utilize regulatory processes, such as the Radioactive Drug Research Committee (RDRC) and exploratory Investigational New Drug (expIND). These two examples of U.S. regulatory processes may allow for more rapid validation of these and other novel PET radioligands in human brain. Finally, the development of novel target versus disease pathology PET radioligands will enable researchers and clinicians to answer different types of questions. For example, the development of a target-specific PET radioligand can have significant impact on a drug development program and allow further understanding of the target-specific expression in various patient populations, whereas imaging biomarkers of disease pathology can be used to monitor the development of disease and the outcome of therapeutic intervention.

The development of novel PET radioligands as imaging biomarkers of disease pathology may yield the greatest impact for broader implementation of molecular imaging in a clinical setting. Since the disease pathology of various neurological and psychiatric disorders is not well established, the development of specific imaging biomarkers for disease pathology could be challenging. Regardless, one example of an imaging biomarker of disease pathology has been the development of PET radioligands for imaging β-amyloid in patients with AD. PET imaging of β-amyloid in patients with AD using [^{11}C]-PIB has impacted the molecular imaging field in a positive way in terms of further understanding of β-amyloid load in a normal aging brain versus patients with mild cognitive impairment (MCI) versus patients with various severities of AD. These studies also contributed to increased media exposure, have a potential impact to aid in drug development and as a potential tool for use in a routine clinical setting for the diagnosis of AD. These advances have only come to fruition after evaluation of numerous candidate PET radioligands, the establishment of an imaging initiative, known

Table I. *Promising PET radioligands for studying the functioning of healthy and diseased brain.*

			Development status				
Molecular target	Radioligand	Possible disease(s)	Radiochemistry	In vitro	In vivo preclinical	Human	Ref.
Target(s)							
Fatty-acid amide hydrolase	[^{11}C]-CURB	Pain, anxiety, eating disorders	X	X	X	—	123
NOP receptor	[^{11}C]-(*S*)-10c	Psychiatric, pain and drug abuse	X	X	X	—	124
$mGlu_1$	[^{18}F]-FITM ([^{18}F]4)	Neurological & psychiatric	X	X	X	—	125-127
	[^{11}C]-MMTP		X	X	X	—	
	[^{18}F]-MK-1312		X	X	X	—	
$5\text{-}HT_7$	[^{18}F]-4-FP-3 or -2-FP-3	Mood disorders	X	X	—	—	128, 129
	[^{11}C]-DR4446		X	X	X	—	
Biomarker for disease pathology							
Myelin	[^{11}C]-MeDAS	Multiple sclerosis	X	X	X	—	130-132
	[^{11}C]-BMB		X	X	X	—	
Tau proteins	[^{18}F]-THK-523	Alzheimer's disease	X	X	X	—	133

as the Alzheimer's Disease Neuroimaging Initiative (ADNI), which has acquired data from sites around the world, and after years of research and development. Thus, the full potential of PET radioligands as imaging biomarkers of disease pathology may have a positive impact on the field of molecular imaging, but it has yet to be seen if the initial evaluation of these and future potential tools will be fully implementable in a routine clinical imaging setting. Finally, future development of novel PET radioligands will help to ensure that we have the appropriate tools to assess the in vivo functioning of various molecular processes in healthy and diseased brain.

CONCLUSIONS

The power of PET imaging is its potential to provide a noninvasive, quantifiable, repeatable and localized measure of molecular processes in the living body. PET radioligands selective for various molecular processes have the potential to provide further understanding of the in vivo functioning of these processes in healthy and diseased brains. Further development of novel PET radioligands for disease pathology, receptors, proteins, enzymes, second messenger systems, transporter(s), as well as inflammatory cell activity, is a key factor for advancement in the field. Finally, proper validation of novel PET radioligands will provide an easier path for implementation into the clinical setting.

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DISCLOSURES

The author is an employee of F. Hoffmann-La Roche, Ltd. The views expressed by the author of this article do not necessarily represent those of F. Hoffmann-La Roche, Ltd.

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