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Interaction of the amyloid imaging tracer FDDNP with hallmark Alzheimer's disease pathologies

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Abstract

The distinctive cortical uptake of the tracer ¹⁸F-FDDNP (2-(1-{6-[(2-fluoroethyl(methyl)amino]-2-naphthyl)ethylidene)malononitrile) in Alzheimer's disease (AD) is believed to be due to its binding to both neurofibrillary tangles (NFTs) and highly fibrillar senile plaques (SPs). We therefore investigated the binding of a tracer concentration of ³H-FDDNP to brain sections containing AD hallmark pathologies. Semi-adjacent sections were labelled with ³H-PIB (Pittsburgh Compound-B, 2-[4'-(methylamino)phenyl]-6-hydroxybenzothiazole) and ¹⁴C-SB13 (4-N-methylamino-4'-hydroxystilbene) for comparison. Neocortical sections containing widespread SPs, and cerebrovascular amyloid angiopathy (CAA), produced a sparse and weak labelling following incubation with ³H-FDDNP. Furthermore, in sections containing NFTs there was no overt labelling of the pathology by ³H-FDDNP. In contrast, sections labelled with ³H-PIB displayed extensive labelling of diffuse plaques, classical plaques, CAA, and NFTs. ¹⁴C-SB13 produced a broadly similar binding pattern to PIB. Radioligand binding assays employing in vitro generated amyloid-beta peptide fibrils produced a ~10-fold reduced affinity for ³H-FDDNP (85.0±2.0 nM) compared with ³H-PIB (8.5±1.3 nM). These data provide an alternative mechanistic explanation for the observed low cortical uptake of ¹⁸F-FDDNP in AD; in that the ligand is only weakly retained by the hallmark neuropathology due to its low affinity for amyloid structures.

Keywords

Alzheimer's disease; amyloid; imaging; amyloid beta; PIB; FDDNP; SB13

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Introduction

A range of amyloid PET tracers are currently undergoing extensive trials for the differential diagnosis of dementias and as potential pharmacodynamic endpoints for disease modifying therapeutics in AD (Lockhart, 2006; Nordberg 2008 for recent reviews). Of the five tracers (^{18}F -FDDNP (Shoghi-Jadid et al 2002), ^{11}C -PIB (Klunk et al 2004), ^{11}C -SB13 (Verhoeff et al., 2004), ^{18}F -BF227 (Kudo et al 2007) and ^{18}F -AV1-BAY94-9172 (Rowe et al 2008)) that have so far progressed into humans only PIB has received an extensive and robust biochemical profiling of its binding to a range of in vitro amyloid filaments (Klunk et al 2003; Lockhart et al 2004; Fodero-Tavoletti et al 2007; Ye et al 2008) and, more importantly, neuropathological lesions (Lockhart et al 2007; Fodero-Tavoletti et al 2007; Ye et al 2008; Ikonomic et al 2008). Such studies are critical to understanding not only the molecular basis of tracer binding to amyloid but also interpreting the disease specific patterns of retention and potential changes associated with drug intervention (Kadir et al 2008).

The tracer FDDNP is distinct from the other amyloid probes in terms of its chemotype, in vitro binding properties and in vivo cortical uptake, the latter in terms of both the magnitude of the signal and its neuroanatomical pattern (Shoghi-Jadid et al 2002; Small et al 2006; Shin et al 2008; Braskie et al 2008; Small et al 2009). Data from these imaging studies indicates that the maximal cortical uptake of the tracer in AD cases is at most ~20 % higher when compared with non-demented control cases; in contrast ^{11}C -PIB demonstrates a 100-200 % higher cortical uptake in AD cases relative to non-demented controls (Klunk et al 2004; Pike et al 2007;). Importantly the differences in both the pattern and magnitude of cortical uptake were confirmed in a recent study in which subjects were sequentially scanned with ^{18}F -FDDNP and ^{11}C -PIB (Shin et al 2008).

Critically, the highest relative area of FDDNP uptake is associated with the medial temporal lobe, an area prone to NFT pathology and conversely, consistently associated with a lower uptake of PIB (Ikonomic et al 2008). This difference has provided circumstantial evidence that in vivo FDDNP binds to NFTs as well as highly fibrillar forms of SPs (i.e. classical plaques, CPs) (Shoghi-Jadid et al 2002; Small et al 2006). In vitro evidence supporting this pattern of interaction is sparse and based on studies either employing high μM concentrations of unlabelled FDDNP (Agdeppa et al 2001; Bresjanac et al 2003; Smid et al 2006) or low-magnification tissue autoradiography employing ^{18}F -FDDNP with no supporting high-magnification histology to definitively identify SPs and/or NFTs (Agdeppa et al 2001). Additionally, there is data (Ye et al 2005; Suemoto, et al 2004) contradicting the reported high (i.e. low nM) affinity of FDDNP for A β fibrils (Agdeppa et al 2001).

The cerebral concentrations of ligand attained in imaging studies is typically in the low nM range (Mathis et al., 2004; Rowe et al., 2007) and the aim of the present study was to determine the histologic structures labelled by FDDNP at these tracer concentrations. An autoradiography study employing tritium labelled preparations of FDDNP (^3H -FDDNP) and PIB (^3H -PIB) was performed which permitted the comparative analysis of near adjacent sections for ligand binding to key amyloid containing neuropathological lesions. A third amyloid tracer, the stilbene derivative SB13 (labelled with carbon-14, ^{14}C -SB13), was also evaluated on a subset of the sections providing additional corroborative data.

Experimental Procedures

Materials

Radiolabelled preparations of ^3H -PIB (71 Ci/mmol, 2.63 TBq/mmol), ^3H -FDDNP (13 Ci/mmol, 0.48 TBq/mmol) and ^{14}C -SB13 (53mCi/mmol, 1.96 GBq/mmol) were prepared as

custom syntheses by GE Healthcare Life Sciences (Little Chalfont, UK). Hyperfilm-3H (RPN535B: early availability batch) was obtained from GE Healthcare Life Sciences. Thioflavin T (Sigma, Poole, UK) was prepared as an aqueous 10 mM stock and stored at -20°C. A β (1-40) peptide was obtained from California Peptide Research (Napa, California, USA).

Serial cryosections were obtained from the Brain Donation Program at Sun Health Research Institute (Beach et al 2008) as described (Lockhart et al 2007). Commercial sections were obtained from AMS Biotechnology (Europe) Ltd (Abingdon, UK).

Radiolabelling of sections and analysis of films

All sections were treated in the same manner using the methods described in Lockhart et al 2007. Non-displaceable binding was performed using 50 μ M Thioflavin T as the cold competitor ligand. Films were developed and digitised as described in Lockhart et al 2007. No manipulations other than optimization of lighting conditions (for image capture), image cropping and re-sizing were performed except for Figure 2B where the brightness and contrast levels were altered to provide increased image contrast.

Nomenclature and classification of pathological features

The identification of SPs, CAA and NFTs were based on data from two separate staining techniques, combined with morphological appearance, as detailed in Lockhart et al 2007. SP is used as a generic term to encompass both diffuse plaques (DPs) and CPs. CPs were not further differentiated into neuritic plaques and cored plaques. Briefly SPs and CAA were identified using 6E10 antibody (CHEMICON International, Temecula, USA) and Thioflavin S (Sigma, Poole, UK). NFTs were identified using Gallyas stain and Thioflavin S.

Radioligand binding assays

Amyloid beta (A β 1-40) peptide fibrils were prepared as described previously (Lockhart et al, 2004). Radioligand binding assays were performed as described (Lockhart et al, 2004) using a fixed concentration of A β fibrils (500 nM) and a range of ^{3} H-FDDNP or ^{3} H-PIB concentrations as detailed in the Results section. The specific binding signals for both radioligands were determined in the presence of 50 μ M Thioflavin T. Initial attempts to define the specific binding signal for ^{3} H-FDDNP using the non-steroidal anti-inflammatory drug, naproxen (Agdeppa et al 2003), were unsuccessful due to its poor ability to displace bound radiotracer and are discussed further in the Results section. Data were analyzed using Grafit (Erithacus Software Limited, Horley, Surrey, UK) to obtain the apparent dissociation constant (Kd) and the maximum number of binding sites (Bmax) using the single-site ligand binding module.

Results

Comparative autoradiography studies: FDDNP and PIB

The majority of the tissues selected for characterisation were the subject of an earlier autoradiography study using ^{3} H-PIB (Lockhart et al 2007). For the current study, adjacent cryosections were obtained from the same blocks of tissue used in Lockhart et al 2007. Consequently the underlying amyloid neuropathologies of these sections were well characterised and identical case numbers are used (Table 1). The following categories of pathology were examined: Mixed amyloid pathology (SPs, NFTs and CAA) using superior parietal lobe (SPL) from Case B2; CAA predominant pathology using occipital lobe (OCL) from Case C2; and, NFT predominant pathology using entorhinal cortex (ECX) from Case D1. Additionally, we investigated the binding of ^{3} H-FDDNP and ^{3} H-PIB to hippocampal (HIP) sections obtained from a commercial supplier. Only one of these sections, H1,

demonstrated a significant radiolabelling and was the subject of further neuropathological investigation which demonstrated the presence of extensive NFT and SP pathologies (Table 1).

In contrast to the cortical and hippocampal formation sections labelled with ^3H -PIB those incubated with ^3H -FDDNP did not display an overt macroscopic, punctate labelling pattern (Figures 1 and 2). However, higher magnification views of contrast-enhanced images from the case B2 SPL section (Figure 3) revealed intermittent patches of punctate FDDNP-labelling associated with the outer cortical layers. An analogous labelling pattern was present on similarly contrast-enhanced images from FDDNP-labelled OCL section (data not shown). This is consistent with the radiotracer only weakly labelling the largely A β related neuropathology present on these sections.

Case B2 (SPL) was characterised with a predominantly A β peptide-related neuropathology with extensive DPs and CPs, and moderate CAA (Table 1). In particular the outer portion of the cortical surface was characterised by a more or less continuous band of DPs characteristic of subpial diffuse amyloid deposits (Lockhart et al 2007). The absence of ^3H -FDDNP labelling of this ribbon-like A β band indicated that the radiotracer did not significantly label this diffuse form of amyloid. Analysis of the higher magnification, contrast-enhanced images (Figure 3 B and C) allowed some tentative assignment of radiolabelled features with both DPs and CPs, however, this is not absolute given the low level of contrast associated with the autoradiographic images. Higher resolution microautoradiography techniques may be required to address these possibilities further; however, in terms of the significance of these findings for in vivo imaging the present study indicates that at tracer concentration FDDNP interacts weakly with A β peptide related neuropathology.

Case D1 (ECX), containing extensive NFT pathology and no A β related pathologies (Lockhart et al 2007), also displayed no overt labelling with tracer concentrations of ^3H -FDDNP (Figure 2A). An enhanced image, similar to that generated for case B2 (Figure 3B), failed to demonstrate evidence of specifically labelled structures (data not shown). In contrast, the semi-adjacent sections from this case displayed discrete patches of labelling with ^3H -PIB (Figure 2B), replicating the findings from our previous study, where we found that the PIB binding was selectively increased over the NFT clusters of the layer II “stellate cell islands” of the ECX (Lockhart et al 2007).

The hippocampal formation section, associated with Case H1, was also negative for labelling with ^3H -FDDNP (Figure 2D). Although, in contrast-enhanced images a weak labelling was associated with regions containing extensive SP pathologies (as noted above). In contrast, the ^3H -PIB labelled section displayed an extensive labelling which was particularly pronounced in the parahippocampal gyrus and subiculum, although this extended, in finer detail, through the hippocampus and into the adjoining dentate gyrus (Figure 2E). Histological analysis of adjacent sections indicated the presence of extensive DP pathology associated with the presubiculum and parasubiculum as well as the entorhinal and transentorhinal areas. Additionally, there was a lightly-labelled band in the hippocampus CA1 region, associated with numerous NFTs in the same distribution on a semi-adjacent section (Figure 2F).

The areas labelled with ^3H -PIB showed a robust correspondence with the underlying neuropathologies and unequivocally confirms our previous findings that this tracer binds NFTs in addition to A β lesions (Lockhart et al 2007). However, as noted previously and clearly demonstrated with case H1, the intensity of the PIB associated with the NFT pathology is greatly reduced relative to its labelling of A β deposits. The pattern of ^3H -PIB

labelling associated with the parasubiculum and presubiculum closely matches earlier studies identifying these patches of A β deposits as being largely diffuse in nature (Akiyama et al 1990; Mufson et al 1999) and closely associated with the parvopyramidal cell islands of these regions (Kalus et al 1989).

Comparative autoradiography studies: PIB and SB13

The autoradiographic images of the semi-adjacent neocortical sections labelled with ^{14}C -SB13 underscores the detection sensitivity of this method (Figure 1 C and F). Due to the limiting numbers of ECX and HIP sections available for analysis the interaction of SB13 with NFT pathology was not investigated. The data demonstrated that ^{14}C -SB13 macroscopically labelled the sections in a more or less equivalent manner to the ^3H -PIB labelled sections (Figure 1 B and C, E and F). SB13 therefore seems to target identical pools of A β lesions (DPs, CPs and CAA) as PIB which is consistent with their broadly analogous in vivo cortical uptake patterns (Verhoeff et al., 2004).

A further facet that appears to be shared by these two tracers is their behaviour with CAA associated with apolipoprotein E- ϵ 4 (ApoE4) positive cases. Previous studies indicated that ^3H -PIB associated with this specific A β pool was only partially displaceable in labelling reactions containing excess unlabelled ligand (Lockhart et al 2007). Although not as extensively examined in this study both the sections treated with SB13 are from ApoE4 positive cases and these both display areas where there is an incomplete displacement of the radiotracer (see insets to Figures 1C and F), although clearly this is most pronounced on the CAA enriched OCL sections. A non-ApoE4 positive OCL case, containing extensive CAA pathology, labelled with ^{14}C -SB13 displayed complete displacement of the radiotracer (data not shown).

In vitro characterisation of the ^3H -FDDNP preparation using A β fibrils

In an effort to further understand the weak labelling of the ^3H -FDDNP treated sections (relative to both ^3H -PIB and ^{14}C -SB13) we investigated the binding properties of the radioligand preparation for in vitro generated A β fibrils, using a previously developed radioligand binding assay (Lockhart et al 2004; Ye et al 2005). The non-steroidal anti-inflammatory drug, naproxen, was reported to bind with high affinity to the same binding on A β fibrils as FDDNP ($K_i \sim 6 \text{ nM}$, Agdeppa et al 2003) and we initially attempted to use this drug to define the non-specific binding signal. However, in our hands, naproxen did not display a strong dose dependent competition with ^3H -FDDNP and displaced <20 % of the filter bound ^3H -FDDNP when used at a very high concentration (500 μM , data not shown). These findings are in keeping with our previous investigations of FDDNP binding using a fluorescence assay format (Ye et al 2005). Replacing naproxen with Thioflavin T (50 μM) resulted in a specific binding signal of ~60-70 %, which we have found typical for this A β binding assay format (Lockhart et al 2004; Ye et al 2005).

The binding of ^3H -FDDNP (concentration range 300 to 0.0125 nM) was determined using a fixed concentration of A β fibrils (500 nM). The resultant binding isotherm was consistent with a single class of ligand binding sites and a K_d value of $85.0 \pm 2.1 \text{ nM}$ (Figure 4). The B_{max} value associated with the batch of fibrils was $3.8 \pm 0.04 \text{ nM}$, which is equivalent to 1 ligand binding site per ~130 A β peptide monomers. Efforts to detect the two affinity sites reported by Agdeppa et al (2001) were unsuccessful as a two-site fit of the data produced binding constants that were incompatible with the range of ligand concentrations under investigation (data not shown). Characterisation of the same batch of A β fibrils using ^3H -PIB, under identical assay conditions, produced a K_d value of $8.5 \pm 1.3 \text{ nM}$ (data not shown), which is ~10-fold lower (i.e. higher affinity) than that observed for the ^3H -FDDNP

preparation. Consistent with our previous investigations (Ye et al 2005), these data indicate that FDDNP binds with a relatively weak affinity to in vitro generated A β fibrils.

Discussion

The data associated with this study indicate that FDDNP, when utilised at the tracer concentrations associated with in vivo imaging studies, has a low sensitivity for amyloid-containing structures such as SPs and NFTs. This provides an alternative, data driven, mechanism for the observed low cortical uptake of the tracer in AD patient populations. Our conclusions are based in two strands of analysis.

Firstly, there was strong evidence from the tissue autoradiography that 3H-FDDNP did not label to any significant extent cortical sections heavily burdened with amyloid containing pathologies. In comparison near-adjacent sections reacted with either ³H-PIB or ¹⁴C-SB13 demonstrated an extensive and essentially equivalent labelling pattern (at least with respect to labelling A β lesions). These latter findings demonstrate the robustness of the technique in detecting ligand binding irrespective of the radioisotope and associated specific activity. Evidence from the contrast enhanced images from case B2 (Figure 3) indicated that ³H-FDDNP may be labelling a subset of A β peptide related pathologies which are most likely to be highly fibrillar CPs and CAA as these contain the highest densities of ligand binding sites. Further investigation using higher resolution emulsion autoradiography techniques will be required to address this outstanding issue.

The current data do not contradict the findings from earlier studies which reported the binding of FDDNP to a wide range of amyloid containing neuropathological lesions including senile plaques (both diffuse and classical), neurofibrillary tangles, Lewy bodies and prion deposits (Agdeppa et al 2001; Bresjanac et al 2003; Smid et al 2006), as these studies were performed using high μ M concentrations of FDDNP which are >1000-fold in excess of those achieved during imaging scans and associated with the current study. It is difficult to gauge whether the current data conflicts with the single previous tissue autoradiography study, which used an ¹⁸F-labelled ligand preparation, as no detailed microscopic analyses of the treated section or supporting neuropathology from adjacent sections were reported (Agdeppa et al 2001).

Secondly, further support for our conclusions was obtained from the in vitro analysis of ³H-FDDNP binding to A β 1-40 fibrils. These studies demonstrated that the binding affinity of ~85 nM was around 10-fold less than of 3H-PIB for the same preparation of fibrils, and ~80-fold lower than that reported originally for the ligand (Agdeppa et al 2001). PET tracers characteristically have a low nM affinity for their in vivo target(s) and the current finding of a relatively low affinity interaction of FDDNP with A β fibrils, supported by similar findings in two earlier studies (Ye et al 2005; Suemoto et al 2004), may in part explain the low signal-to-noise associated with FDDNP. Importantly, each of these latter studies used different assay formats and methods to prepare the A β fibrils. Although the reason(s) for these discrepancies are still unclear they may revolve around the method used to derive the binding data (Agdeppa et al 2001) and is discussed further in Ye et al (2005). It is of course also conceivable that the differences in the in vitro binding constants observed between the groups are due to subtle variations in the quaternary folding of the assembled fibrils. If this is the case FDDNP must be uniquely sensitive to these potential conformational differences as it has not been observed to any significant degree for the other amyloid tracers. We have not attempted to further dissect the nature of the FDDNP binding site on A β fibrils in this study, but its density and compound selectivity appear similar to those described in our earlier study (Ye et al 2005) and are consistent with the 3H-FDDNP radioligand assays

detecting the low density site, termed BS3, in our previous studies (Lockhart et al 2004; Ye et al 2005).

Given the consistently small (~20 %) maximal increases in cortical uptake of ^{18}F -FDDNP (Shoghi-Jadid et al 2002; Small et al 2006; Shin et al 2008; Braskie et al 2008) it is worth considering what factors, others than those relating to the present study findings, could explain this phenotypic effect. Although, not addressed by this study concerns have been expressed over the validity of the Logan analysis method (van Berckel and Scheltens, 2007) and the unknown role of polar metabolites in defining plasma input functions (Luurtsema et al 2008). The lack of a fully validated model emphasises that differences in the non-specific binding component in either the reference tissue (pons, Shoghi-Jadid et al 2002 or cerebellum, Small et al 2006) or the region of interest could make significant contributions to the derived cortical uptake values. The medial temporal lobes, which are associated with the highest ^{18}F -FDDNP binding values (Shoghi-Jadid et al 2002; Small et al 2006), display not only extensive NFT and SP pathologies but are also subject to significant volumetric reductions as dementia progresses (reviewed in Ries et al 2008). Disease related pathology could also potentially contribute to alterations in brain uptake and removal and could include subtle changes to blood brain barrier integrity (Zlokovic 2008), although this latter point may equally affect other amyloid tracers.

Of further interest is the data associated with ^{14}C -SB13, which represents the first detailed description of the tissue binding characteristics of a stilbene derivative, and indicates that the ligand has a broadly similar sensitivity for the different pools of A β -related pathologies (e.g. DPs, CPs and CAA). The cortical uptake of ^{11}C -SB13 in vivo appears analogous to that of ^{11}C -PIB (Verhoeff et al., 2004) and this data supports the view that both ligands are markers of A β -related cerebral amyloidosis. Given the high degree of structural homology between SB13 and AV1-BAY94-9172 (Rowe et al 2008) it seems probable that the data apply equally to the latter tracer as well.

Finally, the labelling of the hippocampal formation section (HIP1) with PIB illustrates on a single section the differential sensitivity of the tracer for the hallmark AD pathologies and complements two recent studies, which in part, addressed this issue (Lockhart et al 2007 and Ikonomic et al 2008). It is clear from all of these studies that whilst PIB is able to bind to NFTs, or possibly a subset of extracellular ('ghost') tangles (Ikonomic et al 2008), that this labelling is not as intense as that observed for SPs and CAA, and that in vivo the tracer is a highly specific marker of A β associated lesions (Lockhart et al 2007).

In summary, the primary data associated with this study provide prima facia evidence that the PET imaging agent, FDDNP, when used at tracer concentrations fails to significantly label the hallmark pathologies of AD, namely SPs and NFTs. The findings provide a robust, data focused, explanation for the unique cortical uptake associated with FDDNP and provide a rational basis for the further development of tracers associated with this chemotype class.

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Abbreviations

Aβ	amyloid-beta peptide
AD	Alzheimer's disease
ApoE	apolipoprotein E
AV1-BAY94-9172	4-(N-methylamino)-4'-(2-(2-(2-fluoroethoxy)ethoxy)ethoxy)stilbene
BF227	2-(2-[2-Dimethylaminothiazol-5-yl]ethenyl)-6-(2-[fluoro]ethoxybenzoxazole
CAA	cerebrovascular amyloid angiopathy
CPs	classical plaques
DPs	diffuse plaques
ECX	entorhinal cortex
FDDNP	2-(1-{6-[(2-fluoroethyl(methyl)amino]-2-naphthyl}ethylidene)malononitrile
HIP	hippocampal
NFTs	neurofibrillary tangles
NDB	non-displaceable binding
OCL	occipital lobe
PET	positron emission tomography
PIB	Pittsburgh Compound-B (2-[4'-(methylamino)phenyl]-6-hydroxybenzothiazole)
SB13	4-N-methylamino-4'-hydroxystilbene
SPs	senile plaques
SPL	superior parietal lobe

Reference List

- Agdeppa ED, Kepe V, Liu J, Flores-Torres S, Satyamurthy N, Petric A, Cole GM, Small GW, Huang SC, Barrio JR. Binding characteristics of radiofluorinated 6-dialkylamino-2-naphthylethylidene derivatives as positron emission tomography imaging probes for beta-amyloid plaques in Alzheimer's disease. *J Neurosci*. 2001; 21:RC189. [PubMed: 11734604]
- Agdeppa ED, Kepe V, Petri A, Satyamurthy N, Liu J, Huang SC, Small GW, Cole GM, Barrio JR. In vitro detection of (S)-naproxen and ibuprofen binding to plaques in the Alzheimer's brain using the positron emission tomography molecular imaging probe 2-(1-[6-[(2-[(18F)fluoroethyl(methyl)amino]-2-naphthyl]ethylidene)malono nitrile. *Neuroscience*. 2003; 117:723–730. [PubMed: 12617976]
- Akiyama H, Tago H, Itagaki S, McGeer PL. Occurrence of diffuse amyloid deposits in the presubicular parvopyramidal layer in Alzheimer's disease. *Acta Neuropathol*. 1990; 79:537–544. [PubMed: 2183538]
- Beach TG, Sue LI, Walker DG, Roher AE, Lue L, Vedders L, Connor DJ, Sabbagh MN, Rogers J. The Sun Health Research Institute brain donation program: description and experience, 1987-2007. *Cell Tissue Bank*. 2008; 9:229–245. [PubMed: 18347928]
- Braskie MN, Klunder AD, Hayashi KM, Protas H, Kepe V, Miller KJ, Huang SC, Barrio JR, Ercoli LM, Siddarth P, Satyamurthy N, Liu J, Toga AW, Bookheimer SY, Small GW, Thompson PM. Plaque and tangle imaging and cognition in normal aging and Alzheimer's disease. *Neurobiol. Aging*. 2008 DOI: 10.1016/j.neurobiolaging.2008.09.012.

- Bresjanac M, Smid LM, Vovko TD, Petric A, Barrio JR, Popovic M. Molecular-imaging probe 2-(1-[6-[(2-fluoroethyl)(methyl) amino]-2-naphthyl]ethylidene) malononitrile labels prion plaques in vitro. *J Neurosci*. 2003; 23:8029–8033. [PubMed: 12954864]
- Fodero-Tavoletti MT, Smith DP, McLean CA, Adlard PA, Barnham KJ, Foster LE, Leone L, Perez K, Cortes M, Culvenor JG, Li QX, Laughton KM, Rowe CC, Masters CL, Cappai R, Villemagne VL. In vitro characterization of Pittsburgh compound-B binding to Lewy bodies. *J Neurosci*. 2007; 27:10365–10371. [PubMed: 17898208]
- Ikonomic MD, Klunk WE, Abrahamson EE, Mathis CA, Price JC, Tsopelas ND, Lopresti BJ, Ziolko S, Bi W, Paljug WR, Debnath ML, Hope CE, Isanski BA, Hamilton RL, Dekosky ST. Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease. *Brain*. 2008; 131:1630–1645. [PubMed: 18339640]
- Kadir A, Andreasen N, Almkvist O, Wall A, Forsberg A, Engler H, Hagman G, Larksater M, Winblad B, Zetterberg H, Blennow K, Langstrom B, Nordberg A. Effect of phenserine treatment on brain functional activity and amyloid in Alzheimer's disease. *Ann Neurol*. 2008; 63:621–631. [PubMed: 18300284]
- Kalus P, Braak H, Braak E, Bohl J. The presubicular region in Alzheimer's disease: topography of amyloid deposits and neurofibrillary changes. *Brain Res*. 1989; 494:198–203. [PubMed: 2765920]
- Klunk WE, Wang Y, Huang GF, Debnath ML, Holt DP, Shao L, Hamilton RL, Ikonomic MD, Dekosky ST, Mathis CA. The binding of 2-(4'-methylaminophenyl)benzothiazole to postmortem brain homogenates is dominated by the amyloid component. *J Neurosci*. 2003; 23:2086–2092. [PubMed: 12657667]
- Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, Bergstrom M, Savitcheva I, Huang GF, Estrada S, Aussen B, Debnath ML, Barletta J, Price JC, Sandell J, Lopresti BJ, Wall A, Koivisto P, Antoni G, Mathis CA, Langstrom B. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. *Ann Neurol*. 2004; 55:306–319. [PubMed: 14991808]
- Kudo Y, Okamura N, Furumoto S, Tashiro M, Furukawa K, Maruyama M, Itoh M, Iwata R, Yanai K, Arai H. 2-(2-[2-Dimethylaminothiazol-5-yl]ethenyl)-6-(2-[fluoro]ethoxy)benzoxazole: a novel PET agent for in vivo detection of dense amyloid plaques in Alzheimer's disease patients. *J Nucl Med*. 2007; 48:553–561. [PubMed: 17401091]
- Lockhart A, Ye L, Judd DB, Merritt AT, Lowe P, Morgenstern JL, Hong G, Gee AD, Brown J. Evidence for the presence of three distinct binding sites for the thioflavin T class of Alzheimer's disease PET imaging agents on betaamyloid peptide fibrils. *J Biol Chem*. 2005; 280:7677–7684. [PubMed: 15615711]
- Lockhart A. Imaging Alzheimer's disease pathology: one target, many ligands. *Drug Discov Today*. 2006; 11:1093–1099. [PubMed: 17129828]
- Lockhart A, Lamb JR, Osredkar T, Sue LI, Joyce JN, Ye L, Libri V, Leppert D, Beach TG. PIB is a non-specific imaging marker of amyloid-beta (Aβ) peptide-related cerebral amyloidosis. *Brain*. 2007; 130:2607–2615. [PubMed: 17698496]
- Luurtsema G, Schuit RC, Takkenkamp K, Lubberink M, Hendrikse NH, Windhorst AD, Molthoff CF, Tolboom N, van Berckel BN, Lammertsma AA. Peripheral metabolism of [(18)F]FDDNP and cerebral uptake of its labelled metabolites. *Nucl. Med. Biol*. 2008; 35:869–874. [PubMed: 19026948]
- Mathis CA, Wang Y, Klunk WE. Imaging beta-amyloid plaques and neurofibrillary tangles in the aging human brain. *Curr. Pharm. Des*. 2004; 10:1469–1492. [PubMed: 15134570]
- Mufson EJ, Chen EY, Cochran EJ, Beckett LA, Bennett DA, Kordower JH. Entorhinal cortex beta-amyloid load in individuals with mild cognitive impairment. *Exp Neurol*. 1999; 158:469–490. [PubMed: 10415154]
- Nordberg A. Amyloid imaging in Alzheimer's disease. *Neuropsychologia*. 2008; 46:1636–1641. [PubMed: 18468648]
- Pike KE, Savage G, Villemagne VL, Ng S, Moss SA, Maruff P, Mathis CA, Klunk WE, Masters CL, Rowe CC. Beta-amyloid imaging and memory in non-demented individuals: evidence for preclinical Alzheimer's disease. *Brain*. 2007; 130:2837–2844. [PubMed: 17928318]

- Ries ML, Carlsson CM, Rowley HA, Sager MA, Gleason CE, Asthana S, Johnson SC. Magnetic resonance imaging characterization of brain structure and function in mild cognitive impairment: a review. *J Am Geriatr Soc*. 2008; 56:920–934. [PubMed: 18410325]
- Rowe CC, Ng S, Ackermann U, Gong SJ, Pike K, Savage G, Cowie TF, Dickinson KL, Maruff P, Darby D, Smith C, Woodward M, Merory J, Tochon-Danguy H, O'Keefe G, Klunk WE, Mathis CA, Price JC, Masters CL, Villemagne VL. Imaging beta-amyloid burden in aging and dementia. *Neurology*. 2007; 68:1718–1725. [PubMed: 17502554]
- Rowe CC, Ackerman U, Browne W, Mulligan R, Pike KL, O'Keefe G, Tochon-Danguy H, Chan G, Berlangieri SU, Jones G, Dickinson-Rowe KL, Kung HP, Zhang W, Kung MP, Skovronsky D, Dyrks T, Holl G, Krause S, Friebe M, Lehman L, Lindemann S, Dinkelborg LM, Masters CL, Villemagne VL. Imaging of amyloid beta in Alzheimer's disease with 18F-BAY94-9172, a novel PET tracer: proof of mechanism. *Lancet Neurol*. 2008; 7:129–135. [PubMed: 18191617]
- Shin J, Lee SY, Kim SH, Kim YB, Cho SJ. Multitracer PET imaging of amyloid plaques and neurofibrillary tangles in Alzheimer's disease. *Neuroimage*. 2008; 43:236–244. [PubMed: 18694837]
- Shoghi-Jadid K, Small GW, Agdeppa ED, Kepe V, Ercoli LM, Siddarth P, Read S, Satyamurthy N, Petric A, Huang SC, Barrio JR. Localization of neurofibrillary tangles and beta-amyloid plaques in the brains of living patients with Alzheimer disease. *Am J Geriatr Psychiatry*. 2002; 10:24–35. [PubMed: 11790632]
- Small GW, Kepe V, Ercoli LM, Siddarth P, Bookheimer SY, Miller KJ, Lavretsky H, Burggren AC, Cole GM, Vinters HV, Thompson PM, Huang SC, Satyamurthy N, Phelps ME, Barrio JR. PET of brain amyloid and tau in mild cognitive impairment. *N Engl J Med*. 2006; 355:2652–2663. [PubMed: 17182990]
- Small GW, Siddarth P, Burggren AC, Kepe V, Ercoli LM, Miller KJ, Lavretsky H, Thompson PM, Cole GM, Huang SC, Phelps ME, Bookheimer SY, Barrio JR. Influence of cognitive status, age, and APOE-4 genetic risk on brain FDDNP positron-emission tomography imaging in persons without dementia. *Arch. Gen. Psychiatry*. 2009; 66:81–87. [PubMed: 19124691]
- Smid LM, Vovko TD, Popovic M, Petric A, Kepe V, Barrio JR, Vidmar G, Bresjanac M. The 2,6-disubstituted naphthalene derivative FDDNP labeling reliably predicts Congo red birefringence of protein deposits in brain sections of selected human neurodegenerative diseases. *Brain Pathol*. 2006; 16:124–130. [PubMed: 16768752]
- Suemoto T, Okamura N, Shiomitsu T, Suzuki M, Shimadzu H, Akatsu H, Yamamoto T, Kudo Y, Sawada T. In vivo labeling of amyloid with BF-108. *Neurosci Res*. 2004; 48:65–74. [PubMed: 14687882]
- van Berckel BN, Scheltens P. Getting a grip on Alzheimer's disease: imaging amyloid in the brain. *Lancet Neurol*. 2007; 6:204–206. [PubMed: 17303523]
- Verhoeff NP, Wilson AA, Takeshita S, Trop L, Hussey D, Singh K, Kung HF, Kung MP, Houle S. In-vivo imaging of Alzheimer disease beta-amyloid with [11C]SB-13 PET. *Am J Geriatr Psychiatry*. 2004; 12:584–595. [PubMed: 15545326]
- Ye L, Morgenstern JL, Gee AD, Hong G, Brown J, Lockhart A. Delineation of positron emission tomography imaging agent binding sites on beta-amyloid peptide fibrils. *J Biol Chem*. 2005; 280:23599–23604. [PubMed: 15855161]
- Ye L, Velasco A, Fraser G, Beach TG, Sue L, Osredkar T, Libri V, Spillantini MG, Goedert M, Lockhart A. In vitro high affinity alpha-synuclein binding sites for the amyloid imaging agent PIB are not matched by binding to Lewy bodies in postmortem human brain. *J Neurochem*. 2008; 105:1428–1437. [PubMed: 18221373]
- Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron*. 2008; 57:178–201. [PubMed: 18215617]

Figure 1.

Neocortical sections labelled with ^3H -FDDNP (A and D), ^3H -PIB (B and E) and ^{14}C -SB13 (C and F). SPL sections from case B2 (A-C) and OCL sections from case C2 (D-F). Insets to pictures represent sections labelled for NDB. Boxed area I is discussed further in Figure 2. Scale bar represents 1 mm.

Figure 2.

Hippocampal region sections labelled with ^3H -FDDNP (A and D), ^3H -PIB (B and E) and Gallyas stain (C and F). A to C are ECX sections from case D1 and D to F are HIP sections from case H1. Insets to pictures (A, B and D) represent sections labelled for NDB. The high magnification images of the Gallyas stained sections demonstrates the presence of NFTs and their approximate correspondence to the radiolabelled sections is indicated by the white boxes. Scale bar represents 1 mm.

Figure 3.

Higher magnification images of SPL section from case B2 labelled with ^3H -FDDNP corresponding to boxed area I (on Figure 1A). A Non-contrast enhanced image demonstrating the sparse labelling of section by the radiotracer. B and C Enhanced, higher magnification images corresponding to Boxes II and III, respectively, from Part A demonstrating a more discrete series of punctate radiolabelled areas associated with this section. D and E Corresponding A β immunohistochemistry of the semi-adjacent section demonstrating presence of diffuse and classical plaques, which are marked with black thick and thin arrows, respectively. The approximate correspondence of these lesions on the FDDNP labelled sections is indicated by the white arrows. Scale bar represents 1 mm.

Figure 4. Binding isotherm for the ligand ³H-FDDNP with in vitro generated Aβ(1-40) fibrils. Data was fitted by non-linear regression to derive K_d and B_{max} values of 85.0±2.1 nM and 3.8±0.04 nM, respectively. The inset figure shows FDDNP concentrations displayed on a logarithmic scale and emphasises that the data provides strong evidence that only a single class of binding sites is detectable.

Neuropathological characteristics of sections. Pathology scores:+++ frequent. ++, moderate. +, sparse. -, absent. Note that the neuropathological diagnosis for each case was based on a full pathological examination according to Consortium to Establish a Registry for AD criteria (Mirra et al., 1991), *not applicable, only vendor supplied clinical diagnosis available. Note that no ApoE genotyping data was available for case H1.

Table 1

Case	Brain Region	Pathology scores of sections used in study				ApoE status	Neuropathological diagnosis
		DP	CP	NFT	CAA		
B2	SPL	+++	+++	+	++	4/4	Definite AD
C2	OCL	-	-	-	+++	4/4	Probable AD
D1	ECX	-	-	+++	-	2/3	Not AD
H1	HIP	+++	++	++	+	-/-	AD*