We then radiolabeled PBB2 and PBB3 with ^{11}C to conduct autoradiographic and PET assays using PS19 mice. *In vitro* autoradiography using frozen tissue sections showed binding of these radioligands to the brain stem of PS19 mice and neocortex of AD patients (Fig. 6A). As expected from their lipophilicities, [^{11}C]PBB3 yielded high-contrast signals with less nonspecific labeling of myelin-rich white matter than did [^{11}C]PBB2, and the accumulation of [^{11}C]PBB3 in pathological regions was nearly completely abolished by the addition of nonradioactive compounds. Similarly, *ex vivo* autoradiographic studies demonstrated that intravenously administered [^{11}C]PBB3 selectively labeled the brain stem and spinal cord of PS19 mice harboring neuronal tau inclusions, while tau-associated [^{11}C]PBB2 radiosignals were less overt due to a considerable level of nonspecific background (Figs. 6B and S6C–F). Finally, *in vivo* visualization of tau lesions in PS19 mouse brains was enabled by a microPET system using these two tracers (Figs. 6C and S6A, B). Following intravenous injection, [^{11}C]PBB3 rapidly crossed the BBB, and unbound and nonspecifically bound tracers were promptly washed out from the brain with a half life of ~10 min (left panel in Fig. 6E). The retention of [^{11}C]PBB3 signals in the brain stem of 12-month-old PS19 mice lasted over the imaging time (90 min), producing a pronounced difference from that in age-matched non-Tg WT mice (left panel in Fig. 6E). By selecting the striatum as a reference region lacking tau deposits, the target-to-reference ratio was estimated for the brain stem, with the value in PS19 mice peaking at around 70 min, contrasting with its continuous decrease over 60 min in WT mice (right panel in Fig. 6E). The mean ratio at 45–90 min was increased by 40% in 12-month-old PS19 mice as compared with age-matched WT mice ($p < 0.01$ by t-test). The agreement between localizations of PET signals and tau inclusions in PS19 mice was proven by postmortem FSB staining of brain sections from scanned mice (Fig. 6D). Significantly, the mean target-to-reference ratio in the brain stem quantified by PET correlated closely with the number of FSB-positive inclusions per brain section in the same region of the postmortem sample ($p < 0.001$ by t-test; data not shown). [^{11}C]PBB2 exhibited slower clearance from the brain and higher nonspecific retention in myelin-rich regions than [^{11}C]PBB3 (Fig. S6G), resulting in insufficient contrast of tau-bound tracers in the brain stem of PS19 mice and a small difference in the target-to-reference ratio of radioactivities between PS19 and WT mice (8% at 45–90 min; $p < 0.05$ by t-test; Fig. S6H) relative to those achieved with [^{11}C]PBB3.

As radiolabeling at the dimethylamino group in PBB5 with ^{11}C was unsuccessful, ^{11}C-methylation of a hydroxyl derivative of this compound was performed, leading to the production of [^{11}C]methoxy-PBB5 ([^{11}C]mPBB5; Fig. S5C). PET images demonstrated complex pharmacokinetics of [^{11}C]mPBB5 (Fig. S5D, E), and the difference in the specific radioligand binding between Tg and WT mice was small relative to the [^{11}C]PBB3-PET data (Fig. S5F). After taking all of these findings into consideration, [^{11}C]PBB3 was selected as the most suitable ligand for *in vivo* PET imaging of tau pathology in tau Tg nice and human subjects.

It is noteworthy that the hippocampus of many PS19 mice was devoid of overt [^{11}C]PBB3 retention (Fig. 6C), although a pronounced hippocampal atrophy was noted in these animals. This finding is in agreement with the well-known neuropathological features of PS19 mice in the hippocampus, since the accumulation of AT8-positive phosphorylated tau inclusions results in the degeneration of the affected hippocampal neurons prior to or immediately after NFT formation, followed by the clearance of their preNFTs or NFTs that are externalized into the interstitial CNS compartment (Fig. S2). To explore the feasibility of our imaging agents in studies with other tauopathy model mice, we also performed fluorescence labeling with PBBs for brain sections generated from rTg4510 mice (Santacruz et al., 2005, and Supplemental Experimental Procedures). As reported elsewhere (Santacruz et al., 2005), these mice developed numerous thioflavin-S-positive neuronal tau inclusions in the

neocortex and hippocampus, and reactivity of these lesions with PBBs was demonstrated by *in vitro* and *ex vivo* fluorescence imaging (Fig. S7).

Detection of tau pathologies in living brains of AD patients by comparative PET imaging with [^{11}C]PBB3 and [^{11}C]PIB

In order to compare the bindings of [^{11}C]PBB3 and [^{11}C]PIB to tau-rich regions in the human brain, *in vitro* autoradiography was carried out with sections of AD and control hippocampus. A notable difference in labeling between these two radioligands was observed in the CA1 sector and subiculum of the AD hippocampus, where fibrillar tau aggregates predominantly localized to NFTs and neuropil threads (Fig. 7A).

We subsequently conducted an exploratory clinical PET study for patients with probable AD (n = 3) and age-matched cognitively normal control (NC) subjects (n = 3). All AD patients exhibited a marked increase in the retention of [^{11}C]PIB in plaque-rich areas, and all NC were negative for this PET assay. These subjects then received a [^{11}C]PBB3-PET scan, and the [^{11}C]PIB and [^{11}C]PBB3 images were compared in the same individuals. Intravenously injected [^{11}C]PBB3 was delivered to the brain tissue despite its relatively rapid metabolism in humans (Fig. 9A, B). Unlike [^{11}C]PIB, [^{11}C]PBB3 showed minimal nonspecific binding to white matter and other anatomical structures with high myelin content, although it accumulated in dural venous sinuses in control and AD brains (Figs. 7B, 8, 9B). Time courses of regional radioactivity (Fig. 9C, D and S8A, B) and the standardized uptake value ratio (SUVR) to the cerebellum (Fig. S8C, D) demonstrated accumulation of [^{11}C]PBB3 in several brain regions of AD patients as compared to controls (definition of these VOIs is indicated in Fig. S8E). In agreement with autoradiographic findings, binding of [^{11}C]PBB3 to the medial temporal region including the hippocampus contrasted strikingly with the low-level retention of [^{11}C]PIB in this area (Fig. 7B). There was a slight increase in the retention of [^{11}C]PBB3 primarily in the medial temporal region of a control subject with a loss of several points in Mini-Mental State Examination (MMSE) (Subject 3 in Fig. 8), appearing similar to the tau pathology at Braak stage III–IV or earlier (Braak et al., 1991), distinct from the lack of enhanced [^{11}C]PIB signals. Indeed, mild increase of medial temporal SUVR (Fig. 9E) contrasted with unremarkable change in lateral temporal and frontal SUVRs in this subject (Fig. 9G, H). Signals of [^{11}C]PBB3 were also intense mainly in the limbic region of a subject with early AD (Subject 4 in Fig. 8), but profound and moderate increases of SUVRs were also observed in the lateral temporal and frontal cortices, respectively, of this case (Fig. 9G, H), resembling the localization of tau deposits at Braak stage V–VI (Braak et al., 1991). With the further cognitive decline as scored by MMSE (Subjects 5 and 6 in Fig. 8), additional increase in the retention of [^{11}C]PBB3 was found in the medial temporal region, precuneus and frontal cortex (Fig. 9E, F, H). Meanwhile, a substantial decline of [^{11}C]PBB3 binding was noted in the lateral temporal cortex of Subject 6 (Fig. 8, 9G). The SUVRs in the medial temporal region, precuneus and frontal cortex were consequently well correlated with the decline of MMSE scores (Fig. 9E, F, H). In distinction from [^{11}C]PBB3-PET data, there was no overt association between the binding of [^{11}C]PIB and disease severity in AD patients (Fig. 8), consistent with previous observations. These data support the potential utility of [^{11}C]PBB3 for clarifying correlations between the distribution of tau deposition and the symptomatic progression of AD.

As *in vitro* fluorescence staining indicated that PBB3 was reactive with not only tau lesions but also several types of senile plaques, particularly dense core plaques, density of binding sites and affinity of [^{11}C]PBB3 for these sites were quantified by autoradiographic binding assays with hippocampal and neocortical sections of AD brains enriched with NFTs and senile plaques, respectively. These analyses demonstrated that specific radioligand binding sites were primarily constituted by high-affinity, low-capacity binding components in NFT-rich regions and low-affinity, high-capacity binding components in plaque-rich regions (Figs

S9A, B). A subsequent simulation for radioligand binding in an area containing these two types of binding sites at a ratio of 1:1 indicated that the selectivity of [^{11}C]PBB3 for NFTs versus plaques may be inversely associated with concentration of free radioligands (Fig. S9C). In a range of free concentration in the brain achievable at a pseudoequilibrium state in human PET imaging (< 0.2 nM), [^{11}C]PBB3 is presumed to preferentially bind to tau lesions relative to *in vitro* autoradiographic (~1 nM) and fluorescence (> 100 nM) labeling.

We also estimated contribution of [^{11}C]PBB3 bound to dense core plaques to total radiosignals in the neocortical gray matter of AD patients, by conducting autoradiography and FSB histochemistry for the same sections. Radiolabeling associated with dense cored plaques accounted for less than 1% and 3% of total gray matter signals in the temporal cortex and precuneus, respectively (Fig. S9D–H). Moreover, fluorescence labeling of adjacent sections with PBB3 demonstrated that approximately 2% and 5% of total gray matter fluorescence signals were attributable to PBB3 bound to dense core plaques in the temporal cortex and precuneus, respectively. Hence, dense cored plaques were conceived to be rather minor sources of binding sites for [^{11}C]PBB3.

Finally, PET scans with [^{11}C]PBB3 and [^{11}C]PIB were conducted for a subject clinically diagnosed as having corticobasal syndrome. Retention of [^{11}C]PIB stayed at a control level, but notable accumulation of [^{11}C]PBB3 was observed in the neocortex and subcortical structures (Fig. 9I), providing the first evidence for *in vivo* detection of tau lesions in plaque-negative tauopathies. Interestingly, right-side dominant [^{11}C]PBB3-PET signals in the basal ganglia was consistent with laterality of atrophy in this area (Fig. S8F). These findings may also be associated with right-side dominant decrease in cerebral blood flow and left-side dominant motor signs in this patient.

DISCUSSION

Here, we report our efforts to develop BBB-penetrant ligands that are capable of binding to and visualizing intracellular tau aggregates in AD and non-AD tauopathies. These compounds may accordingly be useful for the differential diagnosis of neurological conditions in elderly subjects on the basis of the distribution of tau lesions, thereby opening up novel avenues for research toward elucidating mechanisms of tau-mediated neurodegeneration as well as tau-focused biomarkers and therapies.

Despite numerous efforts to develop imaging ligands to visualize tau pathologies in the brains of patients with AD and related tauopathies, the urgent need for these tau biomarkers remains largely unmet. To address this significant challenge, we also took advantage of a multimodal imaging system, which facilitates a quick and label-free validation of candidate compounds in terms of their transfer to the brain and retention in tau-rich regions. In addition, subcellular-resolution imaging optics exemplified by two-photon laser scanning microscopy provided proof of the rapid transfer of intravenously administered potential tau pathology imaging agents from plasma to the CNS extracellular matrix and subsequently to the cytoplasm of neurons where they can bind to intracellular tau inclusions. Based on these encouraging preliminary data using non-labeled compounds, a subset of these compounds was radiolabeled for use in PET imaging of Tg mice that model tau pathology, and a radioligand yielding the best visualization of tau lesions in these Tg mice was selected for further testing in human AD patients and NC subjects as well as a patients with probable CBD. This stepwise strategy enabled us to identify and advance the most promising PET probe for the visualization and quantitative assessment of tau pathology in the CNS of living human subjects. It is also of great interest that another research group has recently reported development of ^{18}F-labeled PET ligands for tau lesions mostly through assessments of binding to brain tissues but not recombinant tau assemblies (Zhang et al., 2012; Chien et al.,

2013), as in the present approach. These radioligands have been implied to produce considerably high contrasts for tau pathologies in living AD brains, and relatively long radioactive half-life of ^{18}F would enable delivery of radioligands from a radiosynthesis sites to multiple PET facilities. [^{11}C]PBB3 has distinct advantages over these compounds, as exemplified by affinity for diverse tau lesions including Tg mouse tau aggregates, applicability to multimodal imaging and induction of smaller radioactive exposure than ^{18}F-labeled ligands.

In the present work, we clinically validated the performance of [^{11}C]PBB3 as a tau imaging agent by comparing its distribution with that of [^{11}C]PIB in AD brains. Tau deposits in patients with moderate or severe AD are thought to be distributed extensively in the neocortical and limbic regions (classified as Braak stage V – VI) (Braak et al., 1991), thereby resembling localization of senile plaques except for the predominance of tau aggregates in the hippocampal formation. This rationalizes the use of radioactivity in the medial temporal area as an index to validate an imaging probe for tau pathology versus A β deposits in AD patients from prodromal to advanced stages. Furthermore, our preliminary data suggest that [^{11}C]PBB3 may be capable of capturing the temporospatial spreading of neurofibrillary tau pathologies from the limbic system (Braak stage III–IV or earlier) to neocortical areas (Braak stage V – VI) with the progression of AD (Fig. 8). A considerable subset of tau lesions at Braak stage I–II are composed of phosphorylated tau deposits barely reactive with thioflavin-S (i. e. pretangles), and NFTs are relatively low in number and are confined to the transentorhinal cortex (Braak et al., 1991; 2011). Therefore, detection of these early tau pathologies would be more difficult. Our next stage clinical study with expanded sample size and wider range of MMSE scores is currently ongoing to pursue tau accumulation in normal controls and subjects with mild cognitive impairments and AD at diverse stages, and will bring more compelling insights into the significance of tau PET imaging in early diagnosis and prediction of AD. In addition, alterations of [^{11}C]PBB3 retention were indicated in the transition from mild to moderate AD. Loss of PET signals in the lateral temporal cortex of a patient with moderate AD (Subject 6 in Fig. 8) might not result from atrophy of this region, as the hippocampus of the same subject exhibited strong [^{11}C]PBB3 binding despite marked atrophy. Possible explanations for this change include formation of extracellular NFTs and their envelopment by astrocytes in the degenerating neocortex, profoundly modifying accessibility of these NFTs to exogenous molecules (Schmidt et al., 1988). This notion would need to be examined by combined autoradiogarphic and immunohistochemical assays of different brain regions.

Being able to visualize tau deposits with [^{11}C]PBB3 in non-AD tauopathies such as PSP, CBD and related disorders is also of major importance, as suggested in the present PET data supporting detectability of tau deposition in living CBD brains. Although abundant tau deposits are largely confined to specific neuroanatomical locations of the CNS in tau-positive, plaque-negative illnesses, as exemplified by PSP and CBD (Dickson et al., 2011), the homogenous and low-level background signals of [^{11}C]PBB3 in brain parenchyma indicate the possibility of detecting tau lesions that are less abundant and/or less widespread in these disorders than NFTs and neuropil threads in AD. Following such *in vivo* assessments, a postmortem neuropathological evaluation of scanned subjects would be required as a reference standard for PET assays of non-AD tau pathologies.

It has been documented that [^{11}C]PIB-positive plaque formation nearly plateaus prior to the progression of brain atrophy in AD (Engler et al., 2006), but tau abnormalities may bridge the chasm between A βfibrillogenesis and neuronal death. Consistent with this notion, our PET/MRI data indicate that the deposition of tau inclusions as visualized by the intense [^{11}C]PBB3 labeling but lacking overt [^{11}C]PIB binding is closely associated with a local volume reduction in the hippocampal formation. Indeed, our pilot clinical PET study

demonstrated that localized accumulation of [^{11}C]PBB3 in the medial temporal region of AD patients was accompanied by marked hippocampal atrophy (Fig. 7B). It is noteworthy that [^{11}C]PBB3-PET signals were substantially increased, notwithstanding the atrophy-related partial volume effects on PET images, and this observation may support the contribution of tau fibrils to toxic neuronal death in AD. However, these data do not immediately imply neurotoxicities of [^{11}C]PBB3-reactive tau fibrils, in light of MRI-detectable neurodegeneration uncoupled with [^{11}C]PBB3 retention in the hippocampus of PS19 mice. In the hippocampal formation of AD patients, neurons bearing NFTs resembling those in the PS19 hippocampus may drive neurodegeneration similar to that observed in either of the PS19 hippocampus or brain stem, and this issue could be addressed in future studies using [^{11}C]PBB3-PET and MRI in diverse mouse models, including PS19 and rTg4510 mice, and human subjects.

Our analyses of multiple β-sheet ligands illustrated electrochemical and/or conformational diversities of β-pleated sheets among amyloid aggregates, producing a selectivity of these compounds for a certain spectrum of fibrillar pathologies (Figs. 1 and S1). Lipophilicities of the β-sheet ligands could determine their reactivity with non-cored plaques, as noted among the PBBs studied here (Fig. 1), although the molecular properties underlying this variation are yet to be elucidated. Meanwhile, it should also be noted that all β-sheet ligands tested in the present study were reactive with dense core plaques irrespective of their lipophilicities. This may affect *in vivo* PET signals particularly in AD brain areas with abundant cored plaques, such as the precuneus. However, our combined autoradiographic and histochemical assessments indicated that [^{11}C]PBB3 bound to dense core plaques accounts for less than 10% of total specific radioligand binding in these areas, and this percentage in fact includes binding to tau fibrils in plaque neurites in addition to A β-amyloid core. A second possibility to account for the diversity of ligand reactivity to tau lesions may arise from the packing distance between two juxtaposed β-sheets in tau filaments, and is discussed in the supplement (Supplemental Discussion).

It is also noteworthy that selectivity of [^{11}C]PBB3 for tau versus aggregates may depends on free radioligand concentration in the brain. Our autoradiographic binding assays suggested that affinity of [^{11}C]PBB3 for NFTs is 40–50 fold higher than senile plaques, but binding components on tau fibrils may be more readily saturated by this radioligand than those on A β-fibrils. [^{11}C]PBB3-PET data in humans indicated that uptake of this radioligand into the brain is less than 1/3 of [^{11}C]PIB uptake, and that free radioligand concentration in the brain at a pseudo-equilibrium state is approximately 0.2 nM or lower. In this range of concentration, [^{11}C]PBB3 could preferentially interact with high-affinity binding components formed by tau assemblies. Excessive amount of radioligand in the brain would result in saturation of its binding to tau lesions and increased binding to low-affinity, high-capacity binding components in A β-plaques, and such overload of free radioligand is more likely in regions with less abundant tau pathologies. This could be even more critical in capturing early tau pathologies originating in the hippocampal formation, and may require technical improvements and methodological refinements, including high-resolution imaging, correction for motions of subjects during scans and robust definition of VOIs on the atrophic hippocampus.

Although nonspecific [^{11}C]PBB3-PET signals in control human subjects were generally low, radioligand retention in dural venous sinuses was noticeable in all scanned individuals. Possible mechanisms underlying this property are discussed in the supplement (Supplemental Discussion).

The present work has also implied the potential utility of multimodal imaging systems for translational development of therapeutic agents counteracting tau fibrillogenesis. Optical

imaging with a near-infrared fluorescent probe such as PBB5 could provide the least invasive technique to assess tau accumulation in living mouse models. As demonstrated by our *in vitro* and *ex vivo* fluorescence labeling, all PBBs share a similarity in terms of their reactivity with tau aggregates. Hence, PBB5-optics may be applicable to early screening of therapeutic agents suppressing tau deposition, and the data on abundance of tau lesions obtained by this approach may be translatable to advanced stages of assessments using [^{11}C]PBB3-PET in animal models and humans. By contrast, pharmacokinetic properties of PBB5 (Fig. S5) were found to be distinct from those of electrically neutral PBBs, including PBB2 and PBB3. These considerations would be of importance in developing and using fluorescent ligands applicable to optical and PET imaging.

To conclude, our new class of multimodal imaging agents offer the possibility of visual investigations of fibrillary tau pathologies at subcellular, cellular and regional levels. These assay systems are potentially powerful tools for the longitudinal evaluation of anti-tau treatments (Marx, 2007), as a single probe may facilitate a seamless, bidirectional translation between preclinical and clinical insights. PET tracers would also serve a more immediate therapeutic purpose by enabling the assessment of the effects of anti-A βas well as anti-tau therapies on tau pathologies in living AD patients.

EXPERIMENTAL PROCEDURES

Compounds and reagents

PBB1 (Wako Pure Chemical Industries), PBB2 (ABX), PBB3 (Nard Institute), PBB4 (ABX), mPBB5 (Nard Institute), desmethyl precursor of [^{11}C]PBB2 (2-[4-(4-aminophenyl)buta-1,3-dienyl]benzothiazol-6-ol; Nard Institute), desmethyl precursor of [^{11}C]PBB3 protected with a silyl group (5-[4-(6-tert-butyldimethylsilyloxy-benzothiazol-2-yl)buta-1,3-dienyl]pyridine-2-amine; Nard Institute), desmethyl precursor of [^{11}C]mPBB5 (2-[4-(4-dimethylaminophenyl)buta-1,3-dienyl]-3-ethyl-6-hydroxybenzothiazol-3-ium; Nard Institute) and 2-[8-(4-dimethylaminophenyl)octa-1,3,5,7-tetraenyl]-3-ethylbenzothiazol-3-ium (DM-POTEB; Nard Institute) were custom-synthesized. Information on other chemicals is provided in the supplement (Supplemental Experimental Procedures). ClogP for each compound was calculated using ACD Chemsketch logP software (Advanced Chemistry Development, Toronto, Canada).

Animal models

Tg mice heterozygous for human T34 (4-repeat tau isoform with 1 N-terminal insert) with FTDP-17 P301S mutation driven by mouse prion protein promoter, also referred to as PS19 mice (Yoshiyama et al., 2007), were bred and kept on a C57BL/6 background. All mice studied here were maintained and handled in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals and our institutional guidelines. Protocols for the present animal experiments were approved by the Animal Ethics Committees of the National Institute of Radiological Sciences.

Postmortem brain tissues

Procedures for preparation of human and mouse brain sections are given as Supplemental Experimental Procedures online.

In vitro and *ex vivo* fluorescence microscopy

Six- μm paraffin sections generated from patient brains and 20- μm frozen sections of mouse brains were stained with 10^{-3}% βsheet ligands dissolved in 50% ethanol for 1 hr at room temp. Images of the fluorescence signals from these compounds were captured by non-laser (BZ-9000; Keyence Japan) and confocal laser scanning (FV-1000; Olympus) microscopes.

In the confocal imaging, excitation/emission wave lengths (nm) were optimized for each compound as follows: 405/420–520 (PBB3, FSB, PIB, BF-227, BF-158, FDDNP, thioflavin-S), 488/520–580 (PBB2, PBB4), 515/530–630 (PBB1, curcumin) and 635/645–720 (PBB5, BF-189, DM-POTEB). Subsequently, the tested samples and adjacent sections probed serially with each ligand were autoclaved for antigen retrieval, immunostained with the anti-tau monoclonal antibody AT8 that is specific for tau phosphorylated at Ser 202 and Thr 205 (Endogen), as well as a polyclonal antibody against A β N3(pE), and inspected using the microscopes noted above. For *ex vivo* imaging, PS19 and non-Tg WT at 10–12 months of age were anesthetized with 1.5% (v/v) isoflurane, and were given 1 mg/kg PBB1–4, 0.1 mg/kg PBB5 or 10 mg/kg FSB by syringe via tail vein. The animals were killed by decapitation at 60 min after tracer administration. Brain and spinal cord were harvested and cut into 10- μm-thick sections on a cryostat (HM560). The sections were imaged using microscopes as in the *in vitro* assays, and were labeled with either FSB or AT8, followed by microscopic re-examination.

Ex vivo and *in vivo* multiphoton imaging

Experimental procedures are given as Supplemental Experimental Procedures online.

In vivo and *ex vivo* pulsed laser scanning imaging

Noninvasive scans of isoflurane-anesthetized non-Tg WT and tau Tg mice at 12 months of age were performed using a small animal-dedicated optical imager (eXplore Optix; ART). Scan protocols are given in the supplement (Supplemental Experimental Procedures).

Radiosynthesis of [^{11}C]PBB2

Experimental procedures are given as Supplemental Experimental Procedures online.

Radiosynthesis of [^{11}C]PBB3

[^{11}C]Methyl iodide was produced and transferred into 300 μl of dimethyl sulphoxide (DMSO) containing 1.5–2 mg of *tert*-butyldimethylsilyl desmethyl precursor and 10 mg of potassium hydroxide at room temperature. The reaction mixture was heated to 125°C and maintained for 5 min. After cooling the reaction vessel, 5 mg of *tetra-n*-butylammonium fluoride hydrate in 600 μl of water was added to the mixture to delete the protecting group, and then 500 μl of HPLC solvent was added to the reaction vessel. The radioactive mixture was transferred into a reservoir for HPLC purification (CAPCELL PAK C_{18} column, 10 mm × 250 mm; acetonitrile/50 mM ammonium formate=4/6, 6 ml/min). The fraction corresponding to [^{11}C]PBB3 was collected in a flask containing 100 μl of 25% ascorbic acid solution and 75 μl of Tween 80 in 300 μl of ethanol, and evaporated to dryness under a vacuum. The residue was dissolved in 10 ml of saline (pH 7.4) to obtain [^{11}C]PBB3 (970–1990 GBq at EOS) as an injectable solution. The final formulated product was radiochemically pure (≥95%) as detected byanalytic HPLC (CAPCELL PAK C_{18} column, 4.6 mm × 250 mm; acetonitrile/50 mM ammonium formate=4/6, 2 ml/min). The specific activity of [^{11}C]PBB3 at EOS was 37–121 GBq/μmol, and [^{11}C]PBB3 maintained its radioactive purity exceeding 90% over 3 hrafter formulation.

Radiosynthesis of [^{11}C]mPBB5

Experimental procedures are given as Supplemental Experimental Procedures online.

Radiosynthesis of [^{11}C]PIB

Radiolabeling of PIB was performed as described elsewhere (Maeda et al., 2011). The specific activity of [^{11}C]PIB at EOS was50–110 GBq/μmol.

In vitro and *ex vivo* autoradiography

Experimental procedures are given as Supplemental Experimental Procedures online.

In vivo PET imaging of mice

PET scans were performed using a microPET Focus 220 animal scanner (Siemens Medical Solutions) immediately after intravenous injection of [^{11}C]PBB2 (28.3 ± 10.3 MBq), [^{11}C]PBB3 (29.7 ± 9.3 MBq) or [^{11}C]mPBB5 (32.8 ± 5.9 MBq). Detailed procedures are provided in the supplement (Supplemental Experimental Procedures).

In vivo PET imaging of humans

Three cognitively normal control subjects (64, 72 and 75 years of age; mean age, 70.3 years) and 3 AD patients (64, 75 and 77 years of age; mean age, 72 years) were recruited to the present work (Fig. 8). Additional information on these subjects is given in the supplement (Supplemental Experimental Procedures). The current clinical study was approved by the Ethics and Radiation Safety Committees of the National Institute of Radiological Sciences, Chiba, Japan. Written informed consent was obtained from the subjects or their family members. PET assays were conducted with a Siemens ECAT EXACT HR+ scanner (CTI PET Systems). Detailed PET scan protocols are provided in the supplement (Supplemental Experimental Procedures). A fraction of radioactivity corresponding to unmetabolized [^{11}C]PBB3 in plasma at 3, 10, 20, 30 and 60 min was determined by HPLC (Waters mBondapak C_{18} column, 7.8 mm × 300 mm; acetonitrile/ammonium formate mobile phase with gradient elution = 40/60, 52/48, 80/20, 80/20, 40/60 and 40/60 at 0, 6, 7, 8, 9 and 15 min, respectively; flow rate, 6 ml/min) as described elsewhere (Suzuki et al., 1999). The radiotracer injection and following scans and plasma assays were conducted in a dimly lit condition to avoid photoracemization of the chemicals.

Individual MRI data were coregistered to the PET images using PMOD software (PMOD Technologies). Volumes of interest (VOIs) were drawn on coregistered MR images, and were transferred to the PET images. Procedures of image analyses are provided in the supplement (Supplemental Experimental Procedures).

We additionally carried out PET scans of a patient who was clinically diagnosed as having corticobasal syndrome, as described in Supplemental Experimental Procedures online.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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