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Makoto Higuchi, Jun Maeda, Bin Ji, Masahiro Maruyama, Takashi Okauchi, et al.. visualization of key molecular processes involved in Alzheimer's disease pathogenesis: Insights from neuroimaging research in humans and rodent models. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 2010, 1802 (4), pp.373. 10.1016/j.bbadis.2010.01.003 . hal-00566734

HAL Id: hal-00566734

<https://hal.science/hal-00566734>

Submitted on 17 Feb 2011

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Accepted Manuscript

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PII: S0925-4439(10)00010-4
DOI: doi: [10.1016/j.bbadis.2010.01.003](https://doi.org/10.1016/j.bbadis.2010.01.003)
Reference: BBADIS 63050

To appear in: *BBA - Molecular Basis of Disease*

Received date: 4 August 2009
Revised date: 17 December 2009
Accepted date: 5 January 2010



Please cite this article as: Makoto Higuchi, Jun Maeda, Bin Ji, Masahiro Maruyama, Takashi Okauchi, Masaki Tokunaga, Maiko Ono, Tetsuya Suhara, *In-vivo* visualization of key molecular processes involved in Alzheimer's disease pathogenesis: Insights from neuroimaging research in humans and rodent models, *BBA - Molecular Basis of Disease* (2010), doi: [10.1016/j.bbadis.2010.01.003](https://doi.org/10.1016/j.bbadis.2010.01.003)

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***In-vivo* visualization of key molecular processes involved in Alzheimer's disease pathogenesis: insights from neuroimaging research in humans and rodent models**

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Running title: Imaging of neurodegenerative dementias

Abstract

Diverse age-associated neurodegenerative disorders are featured at a molecular level by depositions of self-aggregating molecules, as represented by amyloid β peptides ($A\beta$) and tau proteins in Alzheimer's disease, and cascade-type chain reactions are supposedly commenced with biochemical aberrancies of these amyloidogenic components. Mutagenesis and multiplication of the genes encoding $A\beta$, tau and other pathogenic initiators may accelerate the incipient process at the cascade top, rationalizing generations of transgenic and knock-in animal models of these illnesses. Meanwhile, these genetic manipulations do not necessarily compress the timelines of crucial intermediate events linking amyloidogenesis and neuronal lethality, resulting in an incomplete recapitulation of the diseases. Requirements for modeling the entire cascade can be illustrated by a side-by-side comparison of humans and animal models with the aid of imaging-based biomarkers commonly applicable to different species. Notably, key components in a highly reactive state are assayable by probe-assisted neuroimaging techniques exemplified by positron emission tomography (PET), providing critical information on the *in-vivo* accessibility of these target molecules. In fact, multispecies PET studies in conjunction with biochemical, electrophysiological and neuropathological tests have revealed putative neurotoxic subspecies of $A\beta$ assemblies, translocator proteins accumulating in aggressive but not neuroprotective microglia, and functionally active neuroreceptors available to endogenous neurotransmitters and exogenous agonistic ligands. Bidirectional translational studies between human cases and model strains based on this experimental paradigm are presently aimed at clarifying the tau pathogenesis, and would be expanded to analyses of disrupted calcium homeostasis and mitochondrial impairments. Since reciprocal

causalities among the key processes have indicated an architectural interchangeability between cascade and network connections as an etiological representation, longitudinal imaging assays with manifold probes covering the cascade from top to bottom virtually delineate the network dynamics continuously altering in the course of the disease and its treatment, and therefore expedite the evaluation and optimization of therapeutic strategies intended for suppressing the neurodegenerative pathway over its full length.

Imaging pathogenic cascade: a multistep link between the gain of neurotoxicity and loss of neuronal integrity

Aging is a prime risk factor in the etiology of Alzheimer's disease (AD) and diverse allied dementing illnesses. The fact that few nonhuman species exhibit histopathological and phenotypic features of neurodegenerative dementias [1-3] implies that the pathogenetic mechanisms in these disorders unfold in a slow, gradual process beyond the lifespan of such organisms. While rodents do not fully model a chronological compression of human aging, genetic engineering techniques allow them to start their lives at a prodromal stage of the disease, skipping a nonpathological preincubation period. A simple methodology in this gene manipulation is to transgenically overexpress pathogenic candidates known to accumulate in diseased brains [4]. In AD, two major hallmark lesions, senile plaques and neurofibrillary tangles, are extracellular and intraneuronal aggregates of amyloid β peptide ($A\beta$) and tau protein, respectively, and thus it is feasible to conceive of them as molecular culprits responsible for the initiation of the disease processes [5]. This view has been supported by the discoveries of familial AD mutations in the genes coding amyloid precursor protein, presenilin-1 (PS-1) and presenilin-2 [6-10], as all of these genetic defects result in an overproduction of either total $A\beta$ or relatively fibrillogenic subspecies of $A\beta$ termed $A\beta_{42}$. Likewise, mutations in the tau gene have been causally implicated in a class of hereditary non-AD neurodegenerative disorders referred to as frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) [11-14]. Important insights from these revelations are that the $A\beta$ deposition is sufficient to induce fibrillar tau pathologies, and that the tau abnormalities alone are capable of provoking neurotoxicities. A sequential pathway consisting of the $A\beta$ -tau-neurotoxicity continuum

can accordingly be illustrated as the central scheme in the “amyloid cascade” hypothesis on the AD pathogenesis [15]. Meanwhile, mice transgenic for the mutant APP or doubly transgenic for the mutant APP and PS-1 have been shown to develop behavioral alterations and senile plaques but not display any explicit evidence for tau inclusions and neuronal loss [16-19]. Although the generation of transgenics overexpressing the wild-type or FTDP-17 mutant tau has led to the demonstration of tau-induced massive neuronal death in mice [14, 19-25], the mechanistic intermediates between tau aggregation and neurotoxic injuries are yet to be elucidated.

Biomolecular interactions putatively involved in neurological conditions are currently assessable with multiple analytical modalities exemplified by biochemistry and immunohistochemistry. Since most of these technologies examine homogenized or thin-sliced tissue samples, the amount and structural conformation of truly “bioactive” molecules accessible to the interactants in living brains may not be accurately monitored. In addition, numbers of molecular dialogues and signaling take place at a low concentration far below 1 μM , precluding precise nonlabeling detections of these events. To date, neuroradiological technologies with a high spatial resolution are applicable to small laboratory animals, and offer a characterization of *in-vivo* statuses of pathophysiological elements existing at nM and pM levels. Positron emission tomography (PET) in particular is suitable for near-simultaneous targeting of different nervous system molecules, as a wide variety of low-molecular-weight organic compounds decorated with positron-emitting nuclides (^{11}C , ^{13}N , ^{15}O , ^{18}F , etc.) can be synthesized without modifying their original chemical structures, providing decent flexibility in designing imaging agents [26]. Rapid radioactive decays in these isotopes (e.g. $T_{1/2} \sim 20$ min for ^{11}C) are also advantageous in serially capturing distinct targets

with a short interval. Moreover, the capability of PET scans in evaluating biologically accessible modes of brain components has been indicated in recent studies showing that cell-surface, neurotransmitter-attachable neuroreceptors can be visualized together with or separately from internalized forms by using two specific radioligands with different cell membrane permeabilities [27, 28].

Despite the lack of definite evidence, several neuromolecular and neurocellular alterations downstream of the amyloid fibrillogenesis have been highlighted in the neurodegenerative cascade as critical steps linking the gain of neurotoxicities by these fibrillar assemblies to the loss of neuronal functionalities (left panel in Fig. 1). PET in conjunction with an array of radiochemical probes enables an exhaustive tracking of these key processes and their interrelations. In rodents, this monitoring is performable parallel to genetic and pharmacological interventions in amyloid formation and other pathogenic changes, in order to clarify whether such therapeutic regulations are practically effective for suppressing the entire cascade, and what processes might remain unmanageable, leading to a poor outcome, in the course of the treatments. These nonclinical data are immediately translatable to clinical studies, owing to the compatibility of the PET tracers between small animals and humans. Conversely, PET observations in human subjects can be used for a side-by-side comparison with rodent models, and this bidirectionality reinforces translational research and development as a dynamic cycle.

Multispecies PET imaging of amyloidosis: lighting up the A β chronicle

The self-assembly of amyloidogenic molecules occurs on a common biochemical basis of β -pleated sheet formation, which is a secondary structure of proteins constituted

by intramolecular and intermolecular hydrogen bonds between particular β -strand sequences. The stack of β -sheets displays a quite uniform geometric dimension irrespective of constituent molecules, and therefore yields an X-ray diffraction pattern with a highly preserved 0.47-nm meridional spacing, corresponding to the periodic interstrand distance within a β -sheet [29, 30]. A group of chemicals identified as β -sheet ligands are fluorescent compounds with a long, flat π -electron-conjugated backbone, which is thought to allow them to enter the canal-like architecture of amyloid fibrils and cling to the β -sheet surface [31]. Consistent with this context, the vast majority of these chemicals exhibit a high affinity for a wide range of *in-vitro* amyloid assemblies, including A β , tau and α -synuclein filaments, with a dissociation constant (K_D) and/or inhibition constant (K_i) below 50 nM [32-38]. One might thus infer their applicability to brain imaging as an all-purpose amyloid probe capturing senile plaques, neurofibrillary tangles and Lewy bodies (i.e. intraneuronal α -synuclein aggregates characteristic of Parkinson's disease and dementia with Lewy bodies). Dozens of ^{11}C - and ^{18}F -labeled chemicals have thereby been generated for visualizing brain amyloidoses [33, 35, 39], by trying to determine the chemostructural requirements in designing neuroimaging agents, such as: (1) reasonably high lipophilicity as indicated by the log ratio of differential solubilities in octanol and water (logP) ranging from 2.5 to 3.5, enabling an efficient plasma-brain transfer of the compound through the blood-brain barrier (BBB); (2) rapid clearance of target-unbound compounds from the brain tissue preferably with a half-life around 15-30 min for yielding a high amyloid contrast; and (3) high selectivity of the binding to pathological fibrils with minimal nonspecific attachments to the myelin and other lipid-rich nervous system elements. Several classes of compounds with a compact core structure have been proven to readily penetrate the BBB, and have

consequently been used for clinical PET studies for demented patients [40-44]. It is noteworthy that most of these relatively small-sized radiotracers preferentially bind to senile plaques without high-affinity associations with tau and α -synuclein aggregates [38, 45, 46]. Such “A β -selective” binding properties have been well demonstrated in [^{11}C]Pittsburgh Compound-B ([^{11}C]PIB), which is the most intensively investigated amyloid radioligand due to its superior sensitivity and specificity in the detection of A β deposits besides the qualification of the aforementioned requirements [39, 41, 45, 47]. Radiochemical binding assays as well as autoradiographic and PET imaging in APP transgenic and APP/PS-1 double transgenic mice have also revealed that [^{11}C]PIB may primarily bind to a selected subset of A β aggregates, since there was only a tiny amount of high-affinity binding components for this probe in model mouse brains as compared with human AD cases [48, 49]. Notwithstanding the fact that these animals generate abundant insoluble A β peptides exceeding AD levels, [^{11}C]PIB signals were barely detectable by high-resolution PET scans of their brains.

In consideration of these observations, Maeda and coworkers applied [^{11}C]PIB synthesized with a high specific radioactivity (~300 GBq/ μmol ; 5-10 fold of previous achievements) to capture a small number of specific binding sites, and reported the first successful demonstration of the age-dependent brain amyloidosis imaged in APP transgenic mice [50]. The rationale for the high-specific-radioactivity imaging is that the use of hotter radiotracers reduces blockage of the binding sites by nonradioactive competitors, leading to an improved contrast of target signals against nonspecific backgrounds. There was also considerable diversity in the amount of specifically bound [^{11}C]PIB among transgenic strains (Fig. 2A). Indeed, amyloid-associated radiosignals were noticeable even with a regular specific radioactivity approximating 37 GBq/ μmol

in very old (> 25 months) transgenic mice expressing the Swedish mutant APP under the control of murine Thy1.2 promoter (APP23 mice), but the binding potential for [¹¹C]PIB in this transgenic line reached only half of the value in human AD cortices even at 30 months of age. These data then raised a crucial issue, namely, the particular composition or conformation of A β fibrils responsible for the constitution of [¹¹C]PIB binding elements. As documented by numerous earlier studies [18, 48, 51-53], C-terminal A β heterogeneities primarily comprised of A β subtypes ending at residues 40 and 42, dubbed A β 40 and A β 42, respectively, accumulated in an insoluble protein fraction extracted from brains of these mice. Their levels exceeded those in postmortem AD brains, and accordingly had no correlation with the radioligand binding. Moreover, the ratio of A β 42 to A β 40, which is markedly low in transgenics relative to humans [52], did not account for the PET observations, because transgenic productions of the mutant APP and PS-1 resulted in an elevated A β 42/40 ratio [18, 51-53] but no overt enhancement of amyloid PET signals [50]. Hence, there has been no evidence for the interference between A β 40 and A β 42 in the formation of “AD-like” plaques preferred by [¹¹C]PIB. These indications have been confirmed by an independently conducted radiochemical comparison of PIB binding capacities in aged human and monkey brains [54], demonstrating that no overt emergence of binding components for this amyloid probe arises from the deposition of simian endogenous A β 40 and A β 42 identical to human peptides in an A β 42-dominant manner. By contrast, heterogeneities in the A β N-terminus were intimately associated with the affinity of PIB, according to the good spatial agreement of autoradiographic [¹¹C]PIB signals with the immunoreactivity for an N-terminally truncated and modified A β termed A β N3pE (Fig. 2B) [50]. A great abundance of A β N3pE, which begins with pyroglutamated residue 3, was deposited in

AD brains, consistent with earlier findings [55, 56], while the accumulation of this A β subspecies in transgenics was observed at a relatively low level, and was restricted to the dense cores of amyloid plaques (Fig. 2B). Unlike A β N3pE, the N-terminal-intact A β subtype, A β N1D, in all transgenic strains examined was accumulated at a level comparable to AD brains, showing disagreement with the interspecies and interstrain variabilities in PIB labeling (Fig. 2B). This profile is in line with the PIB-reactivity in the same brain slices, and implies a pivotal role of A β N3pE in the construction of radioligand binding sites. Further experiments indicated that the binding capacity for [¹¹C]PIB in an *in-vitro* assembly of synthetic A β N3pE peptides was approximately 3-fold that in full-length A β N1D filaments [50], but these test-tube results might not fully explain the preferential binding of PIB to A β N3pE-rich plaques in living brains. The significance of the N-terminal A β processing is relevant to the facts that N-terminal A β fragments as well as antibodies raised against N-terminal A β sequences are particularly effective in the immunotherapeutic removal of senile plaques [57-60], and that vaccination with full-length A β 42 generates antibodies against the A β N-terminus [61]. Based on these observations, we propose a structural concept that the N-terminus of A β peptides forming the outer surface of amyloid fibrils [62] confronts contacts by endogenous and exogenous materials, and therefore determines the accessibility of the β -sheet-layered interior by these visitor molecules. Since the conversion from A β N1D to A β N3pE involves a loss of two negative and one positive electric charges on residues 1-3, A β N3pE fibrils are conceived to possess a more lipophilic exterior than unprocessed A β aggregates [55], allowing the entrance of brain-penetrable, lipophilic agents exemplified by PIB. Our postulation also brings a view that many endogenous lipophilic components can dock at the electrostatically neutral outpost of A β N3pE

assemblies, yielding a heteromolecular shell capable of regulating the passage of β -sheet ligands on an electrochemical basis. This may also amplify the gain of *in-vivo* accessibility to β -sheets through N-terminal A β processing beyond the *in-vitro* dependency of the PIB binding on A β subtypes.

The paucity of A β N3pE in model mice has been biochemically documented in several reports [52, 63], and may be linked to the relative dominance of two distinct pathways in the A β catabolism. The majority of A β peptides undergo proteolytic cleavages by neprilysin and other endopeptidases [64-66], while a small proportion could be N-terminally truncated by aminopeptidases or dipeptidyl peptidases, followed by the cyclation of the third glutamate residue presumably with the aid of glutaminy cyclase (QC) [67-69]. This enzymatic manipulation of the A β N-terminus is usually overwhelmed by endopeptidase-mediated degradation, and the uncyclized intermediates lacking the first 1 or 2 residues are putatively labile for further cleavages by endopeptidases. We therefore hypothesize that the deposition of A β N3pE progresses at a very slow rate, not reaching a threshold for the massive fibrillization of this A β subspecies within 2-3 years in mice, and within 30-40 years in nonhuman primates. Once its level passes the critical point, A β N3pE may rapidly aggregate to pathological plaques, because it is more resistant to peptidases, has more propensity to self-polymerize, and exerts more cytotoxicity than A β N1D [70-72]. In light of the time-consuming steps toward its generation, we consider A β N3pE as an aged A β , and accumulations of total and pyroglutamated A β peptides in human brains emerge in a parallel fashion. Since this “aging of A β ” indicated by the A β N3pE level is not recapitulated in rodents and monkeys, they may be models of disproportionate depositions of total A β and A β N3pE.

It subsequently becomes a matter of concern whether [^{11}C]PIB captures neurotoxic $\text{A}\beta$ lesions, provided that $\text{A}\beta\text{N3pE}$ fibrils serve β -sheets accessible by exogenous radioligands. One notable feature of the [^{11}C]PIB-PET scan in humans is the presence of PIB-positive cases in the nondemented elderly population, who account for 20-40% of total subjects aged over 65 years, and develop PIB-positivities with a prototypical configuration involving the frontal cortex and several other cortical and subcortical areas [73-75]. A combined histochemical and immunohistochemical examination of autopsied materials illustrated that non-cored senile plaques without overt dystrophies of surrounding neurites, referred to as diffuse plaques, are intensely labeled with PIB, and are enriched with $\text{A}\beta\text{N3pE}$ in the frontal region of human brains (Fig. 2B) [50, 56]. This was in sharp contrast with the concentration of $\text{A}\beta\text{N3pE}$ in the dense plaque cores of transgenic mouse models (Fig. 2B) [50]. We presume that the aggregation of $\text{A}\beta\text{N3pE}$ into diffuse plaques can induce functional deficits in neurons, according to a recent neuropathological investigation of normal elderly subjects whose cognitive performances were antemortemly assessed by neuropsychological test batteries [76]. Interestingly, the counts of diffuse plaques and neurofibrillary tangles were correlated with degraded scores for seemingly distinct cognitive subdomains. In this sense, [^{11}C]PIB-PET images in the nondemented elderly may signal the progress of brain aging to an initial pathological stage, which we reductionistically interpret at a molecular level as packing of aged $\text{A}\beta$ species represented by $\text{A}\beta\text{N3pE}$ in diffuse plaques toward a compromise of neuronal integrities. A recent PET analysis has presented evidence for a correlation between increased cortical [^{11}C]PIB binding and impaired episodic memory performance [74], although it is still to be confirmed by further longitudinal neuroimaging assessments whether this stage is consecutively

connected to the onset of AD or is merely a mechanistic basis of age-associated memory impairments virtually unrelated to AD pathogenesis.

Our second insight from the A β metabolism governed by the dual proteolytic pathways is that a decline in neprilysin activity may induce increased levels of substrates for N-terminal processing, eventually resulting in an overproduction of A β N3pE. Hence, decreased neprilysin levels in the brain as a function of age [77-80] could underlie pathological brain aging and AD via the neurotoxicity of A β N3pE. The causal link between deficits of neprilysin and selective augmentation of A β N3pE deposition has begun to be supported by our preliminary experiment on APP transgenic mice deficient in the neprilysin gene, and these bigenic animals have also exhibited enhanced [11 C]PIB signals in the neocortex and hippocampus. As neprilysin activity is thought to be upregulated by the stimulation of somatostatin receptors [81], somatostatinergic agents may offer a pharmacological suppression of A β N3pE, which can be specifically monitored by PET with [11 C]PIB and other similar radioprobes. It is also feasible to block the conversion of degradable N-terminally truncated A β intermediates to A β N3pE mediated by the enzymatic activity of QC, and efficacies of QC inhibitors on the protection of neurons against A β N3pE toxicity have been demonstrated in APP transgenic mice [69]. Significantly, the amount and activity of QC were reported to increase in AD brains possibly for responding to the overflow of its substrate [69], rationalizing the therapeutic targeting of QC to reverse its activity to a normal level as an anti-amyloid strategy without pronounced adverse effects. In this way, a series of basic and clinical studies interconnected through the identification and characterization of *in-vivo* imaging signals in humans and animal models have contributed to the mutual facilitation among the revelation of molecular etiologies and

diagnostic and therapeutic developments.

Targeting key holes on tau fibrils with a molecular lockpick

The above-mentioned clinicopathological study of dementia-free elderly people [76] indicates at least two modes of neurotoxicities reflected by diffuse plaques and neurofibrillary tangles, drawing attention to the role of fibrillary tau lesions as a winder of the aging clock in human brains. Additionally, intraneuronal tau inclusions have been found to more tightly correlate with dementia severity in AD brains than senile plaques [82, 83]. The pathologically enhanced incorporation of tau proteins into self-aggregates is also inevitably accompanied by a loss of normally functioning tau species, eventually disrupting the dynamics and statics of axonal cytoskeletons, because tau proteins are microtubule-associated components in charge of promoting the assembly of axonal tubulins into microtubules and stabilizing tubulins in their polymerized form [14]. These items of evidence give rise to the idea that either distinct or synergistic involvements of A β (presumably A β N3pE) and tau deposits in neuronal dysfunction can be mapped by using a radioligand for tau fibrils in combination with a plaque tracer. This technique may also aid the diagnostic differentiation between AD and non-AD tauopathies based on the presence or absence of amyloid plaques and the spatial distribution of tau aggregates. In the pursuit of a mechanistic pathway that connects tau fibrillization to neurodegenerative changes, clarification of the chronological order of the generation of primitive and mature tau filaments and neuronal deficits in living humans by means of tau scans in conjunction with functional and morphometric neuroimaging tests is an issue of particular interest. This time course could also be captured in transgenic mouse models of tauopathies, and neuronal death and behavioral phenotypes in different

transgenic strains have indeed been proven to result from the emergence of early-phase tau assemblies rather than complete neurofibrillary tangles [22-24]. However, it should be noted that the excessive expression of transgenic products might artificially provoke neurotoxicities at a premature stage of tau pathogenesis without any loss of functions of endogenous murine tau molecules, which need to be compared with putative mechanisms underlying tau-triggered neurodegenerative injuries in humans identifiable by imaging-based biomarkers.

The development of imaging agents preferentially binding to tau fibrils could be initiated by screening known and potential β -sheet ligands, but structural determinants of the selective affinity for tau aggregates remain elusive. We could conceive that tau filaments, the outer layer of which is electromicroscopically characterized as a diffuse coat and is formed by an alignment of densely charged N- and C-terminal portions of tau molecules [84, 85], are accessible by less lipophilic agents than are A β N3pE amyloids, bearing a chargeless surface and allowing contacts by lipophilic probes. It should also be considered that the equatorial spacing of amyloid fibrils reflecting the intersheet distance in X-ray diffraction assays is somewhat divergent and is largely affected by the component side chains, unlike the rigorous conservation of the 0.47-nm meridional spacing [30]. Interestingly, an FTDP-17 mutant tau protein was reported to aggregate in a test tube at an equatorial spacing of 1.3 nm, which was considerably longer than the usual value ranging from 1.0 to 1.1 nm [85]. Hence, our current theoretical view is that chemicals with a longer backbone might gain a high affinity for tau fibrils by acquiring the ability to cross-bridge two adjacent β -sheets. This possibility is supported by the fact that β -sheet ligands with a relatively long frame (> 1.3 nm), such as thioflavin-S and X-34 analogs, tend to prefer neurofibrillary tangles and

neuritic/cored plaques to diffuse plaques, although the determination of structure-selectivity relationships in an extensive group of tau and A β probes is an ongoing issue. Notably, all established β -sheet tracers bind tightly to the epicenter of dense-cored plaques, impeding the designing of pure ligands for tau lesions. A joint use of imaging agents leaning to A β and tau lesions would accordingly be required for extracting spatial maps of fibrillar tau pathologies.

Compelling proof of a direct interaction between intracellular tau inclusions and exogenous ligands might be revealed by comparative *in-vivo* and *ex-vivo* imaging assays with the use of animal models developing tangle-like tau aggregates. *Ex-vivo* labeling of tau fibrils with an intravenously injected X-34 analog was actually demonstrated in a transgenic mouse strain dubbed PS19 (Fig. 3) and similar mice expressing the P301S FTDP-17 mutant tau gene [86, 87], validating the notion that BBB-permeable β -sheet ligands can penetrate neuronal cell membranes as well. The X-34 family may not fulfill the kinetic criteria for optimal PET tracers [89], while agents with faster tissue uptake/clearance rates are presently under development for PET imaging purposes. Putative and potential PET probes for AD neurofibrillary tangles with more compact chemical structures have also been reported, as exemplified by [^{18}F]FDDNP [89, 90] and [^{11}C]BF-158 [35]. However, they do not firmly bind to non-AD tau inclusions, such as Pick's bodies in Pick's disease, neuronal and glial fibrillary tangles in progressive supranuclear palsy and corticobasal degeneration, and fibrillar lesions in tau transgenic mice, and therefore *ex-vivo* and *in-vivo* evidence for the binding of these compounds to tau deposits may not be provided by animal models. This is in contrast with labeling of both AD and non-AD tau pathologies with thioflavin-S and X-34 derivatives [91], and could justify "the law of size effect" for

designing a versatile tau tracer with affinities for diverse tau aggregates. The difference in acceptable ligands between AD-type tangles and other tau inclusions also suggests conformational distinctions stemming from incorporations of all six tau isoforms in the former versus less than three isoforms in the latter. In terms of tau isoform compositions, most tau transgenic animals, which have been generated with a transgene construct coding a cDNA sequence of a single tau isoform, might be incomplete models of *in-vivo* tau fibrillogenesis and tau-induced neurodegeneration, although it is unclear whether the conformational heterogeneity of tau assemblies affects their neurotoxicities.

Emerging tau imaging techniques would also be allied with anti-tau treatments in order to allow translational and clinical proof-of-mechanism studies on the therapeutants. Since hyperphosphorylation of tau proteins is characteristic of tauopathies [92-94], inhibitors of kinases responsible for tau phosphorylation have been developed and aimed at halting tau pathogenesis [95]. The phosphorylation of tau proteins diminishes their ability to bind to microtubules [96-98], disrupting the axonal cytoskeletal stability and presumably promoting the concentration of free tau molecules prone to self-aggregate. The efficacy of drugs counteracting tau phosphorylation could thereby be examined by monitoring tau depositions with the assistance of radioligands for filamentous tau lesions [95]. In view of the redundancy of tau-phosphorylating enzymes (e.g. glycogen synthase kinase 3 β [99], cyclin-dependent kinases 2 and 5 [100, 101] and mitogen-activated protein kinase [96, 102, 103]), we infer that the pathologies of phosphorylated tau proteins might not be highly responsive to the pharmacological suppression of a single kinase, while a full blockage of this enzyme may impair physiological functions of its more specific substrates.

Therapeutic interventions in tau pathogenesis would also be enabled by inhibiting

the self-polymerization of tau molecules into fibrils, as indicated by previous *in-vitro* and *in-vivo* experiments using organic chemicals [104-110]. A significant subset of these anti-aggregation agents are the β -sheet ligands capable of binding to most amyloid filaments, but they are slightly larger than known imaging probes for A β and tau aggregates. It can be expected that a compound with sufficient steric bulk may exert effective interference with the β -sheet formation [111], and appropriate molecular mass and total surface area of such chemicals could be determined by trade-offs between their BBB-permeability and anti-aggregation potency. Congo red was one of the ancestor compounds shown to attenuate the *in-vitro* fibrillization of either A β or tau [112], and its derivatives, the X-34 family, retain the ability to suppress the growth of amyloid filaments [108, 113]. In addition, these bis-styrylbenzene group members are hitherto the only chemicals proven to bind to intraneuronal tau inclusions in living brains after being systemically administered to tau transgenics (Fig. 3) [86, 87]. It therefore seems feasible to design a tau amyloidogenesis inhibitor by structurally modifying these chemicals, and decorations of them with adequate side chains may augment their interference with tau aggregation. The chemical “bodybuilding” using bulky substructures, however, should be done to such an extent that their transferability through BBB remains acceptable as a therapeutic drug. Since the X-34 family exhibits high affinities for A β and tau lesions in AD and non-AD tau inclusions, modifications of its members would also become versatile amyloid filament blockers targeting a wide range of neurodegenerative disorders. Although anti-fibrillogenic activities of these inhibitors could be quantitatively monitored with the aid of β -sheet-binding PET radiotracers, a pitfall in the neuroimaging-based therapeutic evaluation is that a reduction of *in-vivo* radiolabeling may occur as a consequence of either effective

removal of tau fibrils or occupancy of the radioligand binding sites by the test drugs. This competition might be circumvented by choosing an imaging agent constituted of a core structure distinct from those of the anti-fibrillogenics. For instance, thioflavin-type benzothiazoles do not compete with X-34-like bis-styrylbenzenes for *in-vitro* A β assemblies [88, 114], and similar features could be examined for tau filaments.

Even though certain classes of small molecules display efficacies in suppressing the formation of high-order tau polymers, there may be a possibility that less mature species of tau aggregates play essential roles in the induction of neurodegenerative alterations. This is relevant to the implication of oligomeric A β peptides indicated by multiple lines of cell culture and animal studies [115-118], and to the observation of a neuronal loss antecedent to thioflavin-S-positive tau inclusion in murine transgenic models [22-24]. From this viewpoint, nonclinical and clinical validations by visualization of amyloid depositions parallel to volumetric and/or functional imaging of neuronal damages are required regarding the beneficial effects of anti-A β and anti-tau aggregation therapies on nervous system integrities. These research efforts would also shed light on the neuropathological resemblance of model mice and humans in relation to the neurotoxicity of oligomeric A β and tau, and would open an avenue to better replicate human pathogenesis in experimental animals.

Surveillance of neuroimmunity - trooping minesweepers or minelayers?

Accumulating evidence has unraveled an essential part played by dialogs between neurotoxic amyloidosis and neuroimmune responses in the evolution of neurodegenerative disorders [119-124]. A remarkable nature of immunocompetent cells in the brain, represented by activated microglia, is their strong potency in manipulating

depositions of proteinaceous aggregates. A β immunotherapies stimulating neuroprotective microglia have been shown to effectively eliminate amyloid plaques [58-60, 121, 123, 125-129], and are currently in a clinical trial stage. Meanwhile, a recent study on a tauopathy mouse model, PS19, has revealed an enhancement of tau abnormalities and neuronal death by toxic microgliosis, which could be therapeutically diminished by oral administration of an immunosuppressant, FK-506 [24]. A substantial amelioration of fibrillar tau pathologies by systemic treatment with minocycline has also been observed in ‘humanized’ tau transgenic mice, which overexpress all six human tau isoforms but lack endogenous murine tau proteins [130]. Although both FK-506 and minocycline act on neuronal kinases, phosphatases and multiple other targets apparently unrelated to immune reactions, it is plausible to attribute the beneficial outcomes of these drugs primarily to the arrest of the pro-inflammatory activities mediated by a microglial subpopulation. Hence, these interventions in A β and tau pathogeneses sharpen two incompatible functions of glial cells, leading to either restoration or deterioration of neurons [131, 132]. They also support the assertion that the amyloid cascade is not a unidirectional pathway but could be partially self-regulated through positive or negative feedback loops between upstream (e.g. amyloidosis) and downstream (e.g. microgliosis) processes.

Substantive research attempts have also enabled the visualization of microglial activities in living individuals by means of PET and radiolabeled ligands for translocator proteins (TSPO; also known as peripheral benzodiazepine receptors). TSPO is an outer component of mitochondrial permeability transition pores (mPTPs) [133-136], and its upregulation is known to accompany microglial activations in response to ischemic, traumatic and chemical brain damages (Fig. 4A) [137-145]. An

initial ^{11}C -labeled TSPO probe, [^{11}C]PK-11195, has been applied to the assessment of neuroinflammatory changes in patients with neurodegenerative disorders, such as AD and Parkinson's disease [146-151], as well as experimental models of these illnesses [152, 153]. Despite the observation of elevated TSPO levels in a large subset of AD patients, several properties of TSPO imaging do not necessarily represent its utility as a diagnostic marker for AD and associated diseases. Indeed, a notable variability of TSPO levels in elderly controls and patients diminishes the discrimination power of TSPO-PET assays [149-151]. Furthermore, spatiotemporal relationships between TSPO and neurodegenerative amyloidosis seem elusive, because TSPO signals become intensified in neocortices, thalamus and other extensive areas of AD brains without any correlation with the clinical stage of cognitive impairments [146, 149-151]. These data, however, have led us to a new insight into the AD etiology, namely, that an age-associated neuroinflammatory change attended with a widespread TSPO surge emerges antecedent to or independent of $\text{A}\beta$ and tau depositions, but accelerates these amyloidotic processes once they are initiated.

The use of laboratory animals has facilitated the development of second-generation TSPO radioprobes with improved pharmacokinetic properties. Studies of rodents given neuronal injuries and normal monkeys proved efficient BBB passage and high-affinity binding to target sites achieved by groups of chemicals, including DAA-1106 ([^{11}C]DAA1106, [^{18}F]fluoroethyl-DAA1106, etc.) [24, 50, 154-158] and Ac5216 ([^{11}C]Ac5216, [^{18}F]fluoroethyl-Ac5216, [^{18}F]fluoroethyl-desmethyl-Ac5216, etc.) [159, 160] families. Three of these ligands have so far been applied to human subjects [161-163], and a comparative PET analysis of nondemented elderly and AD cases was conducted with [^{11}C]DAA1106 [164]. The

high-contrast TSPO images in this [^{11}C]DAA1106-PET assay [164] brought an explicit indication that the subglobal TSPO upregulation observed in [^{11}C]PK-11195-PET studies is reproducible with different tracers and analytical methods. Researchers then revisited small animals to assess whether TSPO-positive glia are diffused or tightly associated with $\text{A}\beta$ and tau aggregates in the presence of AD-like pathologies. Autoradiographic and PET mapping of APP23 and PS19 mice with the use of tritiated and ^{18}F -fluorinated DAA1106 illustrated the localization of TSPO signals confined to the proximity of $\text{A}\beta$ plaques and intraneuronal phospho-tau deposits, respectively, in contrast to the outcome of clinical PET studies (Fig. 4B) [24, 158]. Thus, multiregional activations of TSPO-expressing glial cells in humans, which we assume to be in concurrence with pathological brain aging and to worsen the tissue vulnerability to amyloid-induced toxicities, are not recapitulated in the existing mouse models of AD.

The lack of diffusely increased TSPO signals, however, renders these mice useful for highlighting the local crosstalk between glia and AD amyloidosis. Interestingly, immunohistochemical data have shown that plaque lesions in APP23 mice are encompassed by TSPO-positive astrocytes and TSPO-negative microglia, presenting a prominent contrast to microglia-dominant TSPO expressions induced by the phospho-tau accumulation in PS19 mice [158]. Our pilot investigation of autopsied brain samples has supported a microglial but not astrocytic induction of TSPO immunoreactivities in AD brains, and implies that the TSPO-positive microglia observed may be actively involved in neurotoxic insults, giving rise to a massive neuronal loss in PS19 mice (Fig. 4B) and AD patients. Unlike these neurodegenerative devastations, no or minimal neuronal death takes places in APP23 and many other APP transgenics [165-167], and we accordingly hypothesize that activations of

TSPO-positive astrocytes and TSPO-negative microglia could be linked to the neuritic and synaptic regeneration in the vicinity of plaques as noted in these animals [168]. These views were consistent with indications in other models of neurodegenerative insults, represented by TSPO-expressing microglia contacting unrecoverably damaged neurons in kainate-triggered excitotoxicity and ethanol-induced necrotic injuries, versus clustering of TSPO-positive astrocytes and TSPO-negative microglia in the spontaneous recovery from demyelinating conditions following treatments with cuprizone (Fig. 4C) [158]. Demonstrations of neurotrophic supports by TSPO-positive astrocytes were further supplemented by correlations of TSPO and glial cell line-derived neurotrophic factor expressions in activated astrocytes responding to these model pathologies [158].

There appears to us a possibility that an elevated TSPO level beyond a certain threshold is interconnected to the conversion of microglia to a neurotoxic form. Hence, amyloid-provoked TSPO increase may easily top this threshold in human cases, if their microglia in advance express a decent amount of TSPO in extensive brain areas. It is similarly likely in our view that A β immunization in AD patients drives microglia to a hyperactive state featured by a TSPO rise, making a detrimental impact on tau pathologies and neuronal survival. As increased TSPO signals in the treatment of APP23 mice with anti-A β antibodies have been demonstrated by PET and [18 F]fluoroethyl-DAA1106 [50], *in-vivo* TSPO imaging techniques would allow the maintenance of microglial activities in an appropriate range during immunotherapeutic approaches to AD.

The potential limitation of TSPO assays in living subjects may stem from their incapability of dissociating between microglia and astrocytes overpresenting TSPO. A high-throughput exploration of molecules specifically existing in TSPO-positive and

TSPO-negative microglia could be targets in differential visualizations of aggressive and neuroprotective microglia. Toward this aim, experimental transplantation of cultured microglia producing TSPO at low and high levels into brains of model mice could be performed, and correlations of their *in-vivo* functions with microglial components are assessable by a combination of antemortem neuroimaging and postmortem biochemical examinations.

In addition to its significance as a visible biomarker, TSPO may pivotally contribute to the switching of glial characters, and can be targeted by therapeutic ligands. As aforementioned, TSPO is a major constituent of mPTPs (Fig. 4A), but its roles beyond the trafficking of intracellular cholesterol related to the neurosteroid synthesis are generally unknown. The opening of mPTPs promotes abolishment of the mitochondrial membrane potential, leading to either mitochondrial autophagy or necrotic and apoptotic cell deaths [169-171]. Moreover, exposure to excessive oxidative stresses gives rise to the opening of mPTPs and consequent collapse of mitochondrial membrane potential. This in turn reinforces the generation of reactive oxygen species (ROS) and its release into the cytosolic compartment, causing an intermitochondrial transmission of ROS waves [172, 173]. Thus, TSPO is presumed to eventually participate in the regulation of glial vitality and ROS-mediated glial toxicity. It has been documented that the conventional knock-out of TSPO in mice is lethal [174], implying the need for the generation of animals conditionally deficient in microglial and/or astrocytic TSPO for the pursuit of its pathophysiological roles. Parent chemicals of some TSPO imaging probes are known to act as anti-anxiety and anti-depressant drugs [175-178], putatively by modulating mitochondrial functions in the nervous system cells, and would be structurally optimized for the purpose of “glial rehabilitation”, which

mends glial profiles from deleterious to beneficial toward the protection of neurons against amyloid-initiated injuries.

Unskippable intermediates, functional deficits, cellular lethality, and bi-causality - grasping the recurrent cascade correlations

Besides neuroinflammatory responses accompanied by glial TSPO expressions, a number of other molecular and cellular events mediating between amyloidogenesis and neuronal death in the neurotoxic cascade are potentially targetable by imaging and disease-modifying agents, which are often structural derivatives of each other. Quite a few researchers support the view that oligomeric or polymeric assemblies of A β disrupt the intraneuronal calcium homeostasis via several proposed mechanisms such as induction of membrane lipid peroxidations inhibitory for ion-motive ATPases [179, 180]. Aberrantly elevated levels of cytoplasmic calcium ions activate calcium-dependent cysteine-proteases, calpains, which catalyze conversion of p35 to p25 [181, 182]. As p25 fortifies activities of cyclin-dependent kinase 5 in a deregulated manner, phosphorylation of tau proteins by this kinase may be markedly accelerated. Calcium mistrafficking and calpain upregulation thus link A β and tau abnormalities, and immunohistochemical assays have revealed activation of calpains by a self-cleavage in AD brains [183, 184]. *In-vivo* models represented by mice transgenic for and deficient in an endogenous calpain-inhibiting protein, calpastatin, have permitted investigation of calpain-mediated neuropathologies [185, 186]. An injection of kainic acid into brains of these animals demonstrated roles of calpains in both cytoskeletal disorganization involving tau phosphorylation and overdrive of pro-apoptotic signals [185, 186]. Participation of calpains in synaptic dysfunctions has also been proven in APP/PS-1

double transgenic mice, as treatments with low-molecular-weight calpain inhibitors reversed perturbation of long-term potentiation (LTP) and behavioral deficits in these mice [187]. This study has also raised the possibility of generating imaging probes for calpains derived from inhibitory compounds. The present mainstream strategy for the development of calpain inhibitors is based on the incorporation of P1 and P2 residues of the substrates [188], but these peptidyl derivatives may not undergo trans-*BBB* delivery sufficient for visualization of calpains in living brains. To date, the crystal structure of calcium-bound calpains interacting with calpastatin has been identified [189], and would bring insights into new classes of synthetic chemicals reactive with active calpains bound to calcium.

Pathological consequences of A β and tau aggregation may also converge on mitochondrial disturbances. The integrity of this organelle is maintained by its axonal transport supported by normally functioning tau proteins, and retarded movements of fast (e.g. organelles) and slow (e.g. neurofilaments) transport components along the axon have been observed in multiple tau transgenic strains [14, 20, 190]. In addition, an entrapment of swollen mitochondria in cytoplasmic tau fibrils is ultrastructurally illustrated in PS19 mice [24]. On the other hand, access of A β peptides to an inner component of mPTP, cyclophilin D (Fig. 4A), has been implicated in AD, and APP transgenic mice lacking cyclophilin D have exhibited alleviation of ROS overproduction and mitochondrial dysfunctions, leading to improvements of LTP and behavioral phenotypes [191]. As described earlier, the opening of mPTP diminishes mitochondrial membrane potentials triggering a cell death signaling pathway [169, 170]. This process is also augmented by a release of cytochrome c through mPTPs, which is subject to coupling with apoptotic protease-activating factor 1 and resultant activation

of caspases [192]. Interventions in mPTP-mediated cellular insults could accordingly be a central focus in therapeutic controls of the neurodegenerative cascade, and monitoring of mPTPs with a specific probe is an urgent requirement. Radioligands of TSPO may help meet this demand, if enhanced recruitment of TSPO into the pore complex is concurrent with the mPTP activation. TSPO has been believed to be exclusively localized to glial lineages in the brain, yet its expression in neuronal cells is under investigation by our research group. Moreover, imaging agents for cyclophilin D would enable assessments of mitochondrial deteriorations in a diseased condition, because levels of this molecule are reportedly elevated in AD brains [191]. Although several organic compounds, including cyclosporin A, exert antagonism to cyclophilin D [193], these ring-shaped polypeptides may not enter brain tissue with high efficiencies, impeding the application of their radiolabeled analogs to *in-vivo* mapping of cyclophilin D and mPTPs.

At the bottom of the hierarchy in the amyloid cascade exist disordered neurotransmissions and neuronal death, yet they are of prime importance in the mechanisms of symptomatic manifestations. It is also noteworthy that dysregulation of the neurotransmitter systems may in turn aggravate the intermediate processes, as exemplified by a compromised calcium homeostasis attributable to activations of ionotropic glutamate receptors [194, 195]. The predicted significance notwithstanding, there have been no radioligands suitable for PET imaging of these receptors. The majority of antagonists for glutamate and glycine binding sites on N-methyl-D-aspartic acid (NMDA) receptors possess more than one charged moiety [196], hampering their entries into the brain. Electrically neutral quinolone derivatives have shown affinities for glycine binding sites, and their ^{11}C -labeled versions have been applied to animal and

human assays [197-199]. Contrary to expectations, the brain uptake of these probes was not satisfactory for detecting subtle changes in pathological conditions, putatively due to their tight binding to plasma albumin and their property as substrates for P-glycoprotein (Pgp) efflux pump expressed on the luminal surface of cerebral endothelial cells [199]. The functional BBB against glutamatergic agents formed by Pgp well indicates the elaborate protection of the brain from exogenous components likely to jeopardize neuronal functionality and survival. Taking a lesson from these results, designing and characterization of PET imaging ligands for NMDA and other types of ionotropic glutamate receptors are still ongoing.

As a matter of fact, clinically approved and in-development drugs for symptomatic treatments of AD are chemicals acting on specific neurotransmitter systems [200], which may also display abnormalities in transgenic models of brain amyloidoses. A volume of literature has documented cholinergic deficits in APP23 and other APP transgenic and APP/PS-1 bigenic mice [201-204]. Importantly, a recent biochemical study has indicated that acetylcholinesterase (AChE) and choline acetyltransferase activities are significantly reduced in the basal forebrain nuclei of APP23 mice, prior to the emergence of plaque lesions in the neocortical and hippocampal regions [203]. This observation suggests the possibility of capturing cholinergic impairments in prodromal AD cases by a radioprobe before they become positive for amyloid PET tests. Several PET tracers, such as radiolabeled substrates [205, 206] and inhibitors [207] for AChE, have revealed a profound reduction of this enzyme in AD patients, but clinical data have seemingly supported the superiority of [^{11}C]PIB-PET to AChE imaging in detecting early pathological alterations, judging from the presence of PIB-positive subjects even in nondemented elderly populations [73-75]

versus no overt age-related changes in AChE levels [206, 208]. Of course, this might be due to difficulties in spotting slight irregularities of the cholinergic system initiated in a small anatomical structure by means of PET scans. To assess the *in-vivo* detectability of cholinergic deficiencies, comparative PET, biochemical and immunohistochemical examinations of the cholinergic elements in animal models would be required.

While cholinergic neurotransmission appears particularly vulnerable to the A β -triggered neuronal insults, other modalities, including monoaminergic and glycinergic systems, may also be affected by an incipient toxicity, as proven by the above-referenced study [203]. Furthermore, the formation of fibrillary tau inclusions is likely to promote synaptic disruptions independently of or synergistically with pathological A β species. Our pilot PET imaging has shown a severe loss of serotonin 1A receptors in the hippocampus of PS19 mice with occurrence of atrophies in this region (Fig. 5A), and younger mice are currently being tested to verify the possibility that serotonergic damages precede neuronal death. This finding raises the possibility that the serotonin neurotransmission is susceptible to tau abnormalities, and could offer a mechanistic explanation for depressed levels of hippocampal serotonin 1A receptors in AD brains documented in recent clinical PET studies [209, 210]. However, implications of these alterations in serotonergic components in cognitive and psychiatric manifestations remain to be determined. The identification of susceptible neurotransmissions would offer a sensitive determination of whether therapeutic regulations of the cascade top with anti-amyloid agents can ameliorate the bottom events featured by such synaptic dysfunctions, and eventually clarify whether the unidirectionality or bidirectionality of causalities governs the amyloid cascade. As pathogenic molecules in transgenics are often expressed under the control of

heterologous promoters, neurotransmitter systems predisposed to transgene-provoked impairments might not be identical to those selectively vulnerable to endogenous A β and tau accumulations in humans. A comparison of neurotransmission PET data across different transgenic strains and humans would address this issue, and would define the validity of these animals as models of AD-characteristic synaptic injuries.

It should also be noted that the intensity of radioligand signals, convertible to kinetic parameters such as binding potential and metabolic rate, may not necessarily reflect the amount of target neurotransmission components, but can be influenced by their (subcellular) translocation, posttranslational modifications (phosphorylation, etc.) and coupling with their endogenous orthosteric and allosteric ligands [211]. The occurrence of endocytotic internalization of receptors and transporters can be indirectly probed by using two radioligands with different cell-membrane permeabilities [27, 28]. Altered levels of synaptic neurotransmitters are also measurable as changes in the concentration of specifically bound radioligands due to competitive inhibitions, provided that the affinity of the radiolabeled probes is in an adequate range so that their binding can be displaced by the neurotransmitters with relative ease (Fig. 5B) [211]. As demonstrated by visualization of dopaminergic overflows by amphetamines, agonistic rather than antagonistic agents may suffice the purpose of sensitively detecting the neurotransmitter release and depletion, because of their selective binding to agonistic binding sites displaceable by endogenous ligands [212-215]. These studies have shown that a 10-fold increase in extracellular dopamine by psychostimulant yielded a 20% reduction in agonistic radioligand binding (Fig. 5C, 5D), raising the concern that pathophysiological fluctuations of synaptic neurotransmitter concentrations, being putatively milder than pharmacological modulations, might not be detectable by PET

scans with a test-retest variability of 5-15%. It is thus indispensable to verify the *in-vivo* measurability of such alterations using combined PET and microdialysis assays in animals (Fig. 5C, 5D) [215]. Importantly, the trans-BBB delivery, clearance and specific binding of radioligands for neurotransmission elements are sensitive to the use of anesthetics, and scanning of unanesthetized animals (Fig. 5D) may markedly contribute to an improvement of intra- and inter-subject reproducibility [215]. As this experimental procedure is now applicable to mice, functional viabilities of the neurotransmitter systems in the course of amyloid-induced pathologies and their therapeutic reversals would be noninvasively investigated in transgenic disease models by neurotransmission PET scans following pharmacological and non-pharmacological stimulations.

The total magnitude of neuronal damages irrespective of neurotransmitter types is measurable by glucose metabolic PET imaging and volumetric magnetic resonance imaging (MRI) [216]. Metabolic scans using PET and [¹⁸F]fluoro-deoxy-glucose ([¹⁸F]FDG) in principle reflect both loss of synaptic energy consumptions and death of neurons [217], and is conceivably advantageous over MRI in probing brains for synaptic deteriorations antecedent to the neuronal decline. However, loss of inhibitory synapses leads to a disinhibition of excitatory neurons, which might partially rebound the metabolic drop. In addition, increased glucose utilization by growing astrocytic and microglial populations in response to tissue damages could incompletely cancel the hypometabolic state. These factors possibly provide an accountable reason for the fact that well-calibrated MRI volumetry is equivalent or somewhat superior to [¹⁸F]FDG-PET in terms of the precision of a two-point longitudinal test for separation between AD and controls [218]. In rodent experiments, high-field MRI devices permit a topographic analysis of living brains with a spatial resolution below 50 μm [219]. Such

detailed mapping provides a remarkable benefit in detecting atrophies of tiny structures as compared with small animal PET with a spatial resolution approximating 1.3 mm [220], particularly in case pathological changes take place unevenly among brain subregions. The metabolic rate for glucose is variable as a function of the anesthetic depth [221], and this effect may display regionality. Similar to selective neurotransmission PET mapping, [^{18}F]FDG-PET and MRI are of potential utility in determining whether the therapeutic effects of anti-amyloid agents can penetrate the pathological cascade pathway toward a suppression of neuronal lethality, or are faded by inverse causalities from downstream to upstream events.

Concluding remarks

The concept of the amyloid cascade has lately been updated by the nonclinical evidence that a topical administration of $\text{A}\beta$ oligomers has adverse impacts on LTPs and behavioral test performances in rats [116-118], supporting the presence of a direct pipeline between $\text{A}\beta$ aggregation and disordered neurotransmissions. However, little is known as to whether this shortcut leads to neuronal death and disease onset, and we conceive that tangled network of mutual mechanistic links among diverse key processes is still paramount in the neurodegenerative etiology of AD and allied illnesses (right panel in Fig. 1). $\text{A}\beta$ and tau abnormalities thus have synergistic connections directly and by way of neuroinflammation and other network nodes, all of which could be stimulated as a function of age. This might provide a logical explanation for the pathogenesis of sporadic plaque-negative tauopathies and the lack of intense tau inclusions in APP transgenic models. Therapeutic monitoring backing onto unraveling of this webbed cascade should accordingly track these multiple steps in order to identify

treatment-resistant, self-amplifying cycles formed by two interpromoting processes. The commencement and reinforcement of the cascade may be efficiently arrested by simultaneously overturning more than two processes with co-administered agents counteracting individual pathological events. This approach would permit a synergistic use of drug candidates, each of which alone may not be potent enough to suppress the entire cascade, and would give a second chance to dropouts in the research and development stages. A critical notion highlighted by these insights is that neurodegenerative disorders in a mechanistic sense are not disorders but “pathological orders” of autonomic signaling networks architected in the cascade. This inevitably claims a systemic order of diagnostic and therapeutic strategies targeting manifold key steps, and time-course imaging studies of multigenic mice generated by crossbreeding of elemental models should crucially contribute to the elucidation of the network dynamics.

Acknowledgements

The nonclinical and clinical studies including PET assays conducted at the National Institute of Radiological Sciences were assisted by the staffs of the Departments of Molecular Neuroimaging and Molecular Probe, Molecular Imaging Center. The authors also thank Drs. J. Q. Trojanowski and V. M. -Y. Lee at the University of Pennsylvania, Drs. T. C. Saido and N. Iwata at the RIKEN Brain Science Institute, Dr. Yasumasa Yoshiyama at the National Hospital Organization Chiba-East Hospital, Dr. M. Staufenbiel at the Novartis Institutes for Biomedical Research – Basel, Dr. R. B. Innis at the National Institute of Mental Health - National Institutes of Health, Dr. C. Halldin at the Kalorinska Institute and Drs. H. Mori and T. Tomiyama at Osaka City University for their collaborative support of these works.

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Legends for figures

Figure 1. Schematic diagrams of the molecular mechanisms linking brain amyloidoses and symptomatic onset of neurodegenerative disorders. The pathogenic cascade initiated by amyloid depositions (left) is interconvertible to a network presentation (right), in light of reciprocal causalities between key participants. P_1 through P_6 denote the magnitude of each alteration (e.g. amyloid accumulation and reduced cholinesterase activity), and a summation of these changes and their interactions represents the network activity. C_i and C_{ij} are scaling coefficients. Neuronal death and disease manifestations occur by elevations of this activity beyond thresholds for cellular lethality (T_L) and symptoms (T_S).

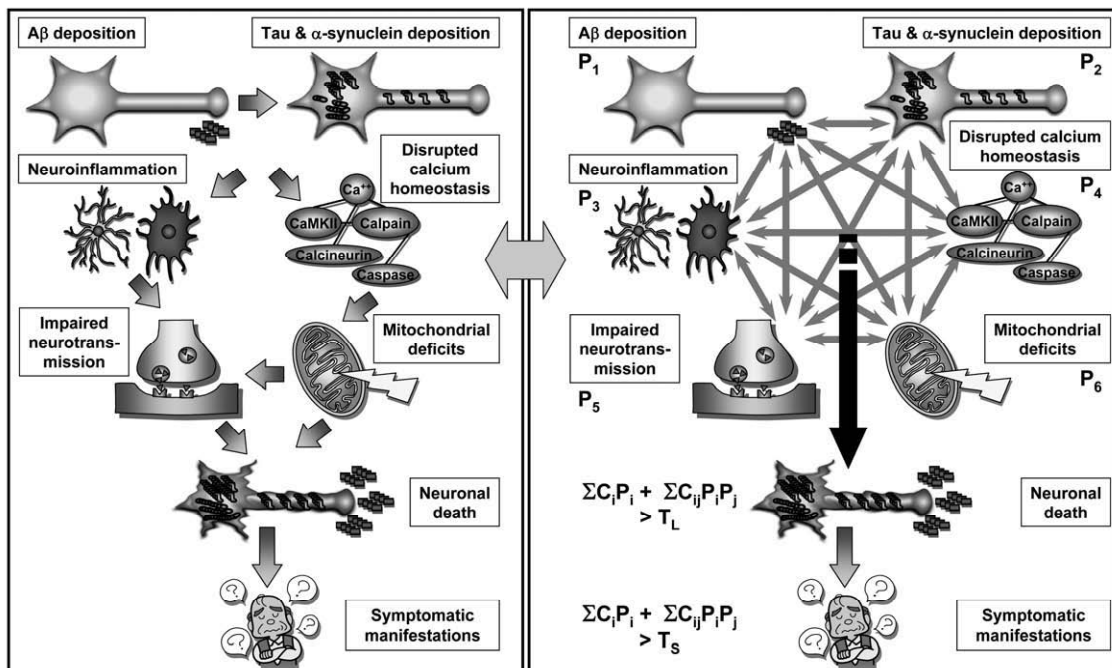


Figure 2. Roles of an A β subspecies as a major component of “AD-like” plaques preferentially detectable by PET radioprobes. (A) [¹¹C]PIB-PET images illustrating amyloid depositions in brains of an AD patient (axial view) and different strains of APP

transgenic or PS-1/APP double transgenic mice (coronal view) merged onto anatomical MRI maps. Note that the human and mouse images are differently scaled according to the maximal binding potential for the radiotracer in each species, as indicated by vertical bars. Tg2576 mice overexpress the Swedish mutant APP driven by the hamster prion protein promoter, and PS1/APP double transgenics were generated by crossbreeding Tg2576 mice with A260V mutant PS-1 transgenics. (B) Immunohistochemical assays for N-terminal heterogeneities of A β . In the frontal cortex of an AD patient (top panels), both A β _{N3pE} and A β _{N1D} are abundantly present in diffuse and cored plaques. A β _{N1D} immunoreactivity is also intensely observed in the neocortex of an APP23 mouse, while the localization of A β _{N3pE} in an adjacent section is confined to the plaque epicenters (middle panels). Despite its paucity, the A β _{N3pE} immunostaining in APP23 mice is well colocalized with [¹¹C]PIB signals (bottom panels; dual autoradiographic and immunohistochemical labeling).

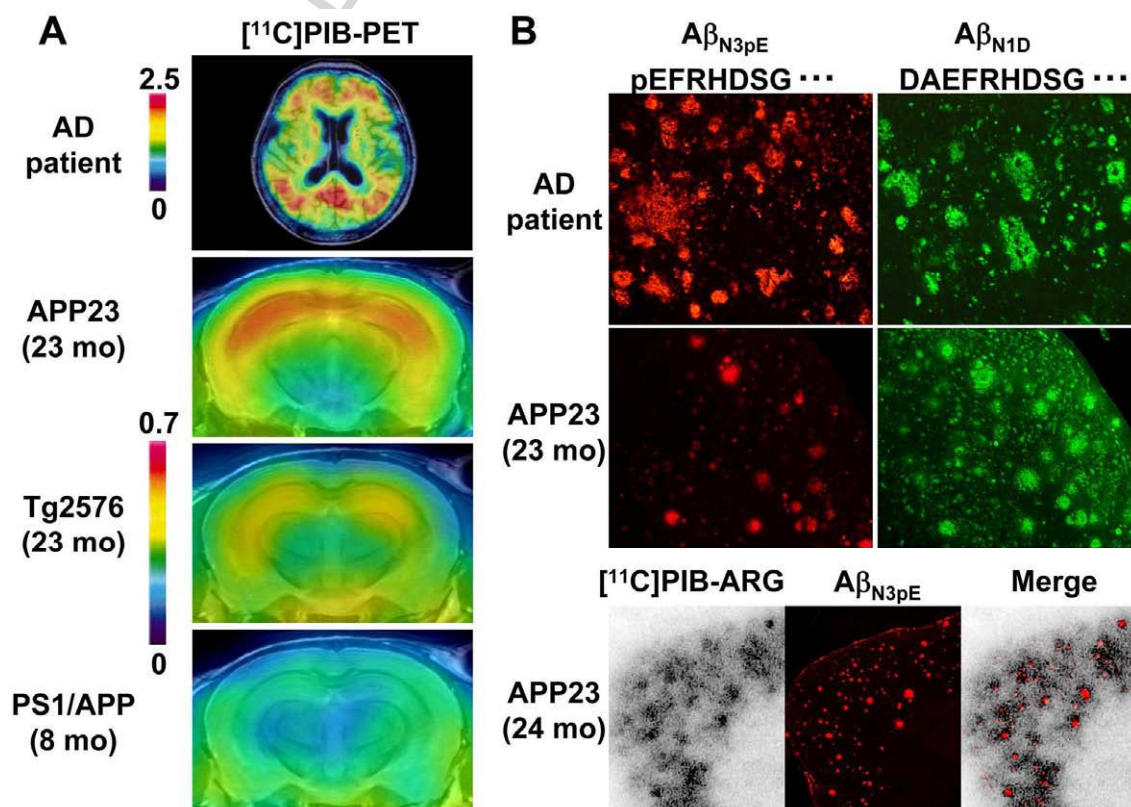


Figure 3. *Ex vivo* fluorescence labeling of intraneuronal tau inclusions with an analog of X-34 dubbed FSB. PS19 mouse was administered with 10 mg/kg of FSB, and its brain was removed at 3 hours after the termination of the tracer infusion. A coronal slice containing the brain stem displays numerous tau lesions doubly stained with administered FSB and postmortemly-applied antibody against phosphorylated tau (AT8). Areas indicated by white squares are presented at a higher magnification in the bottom panels.

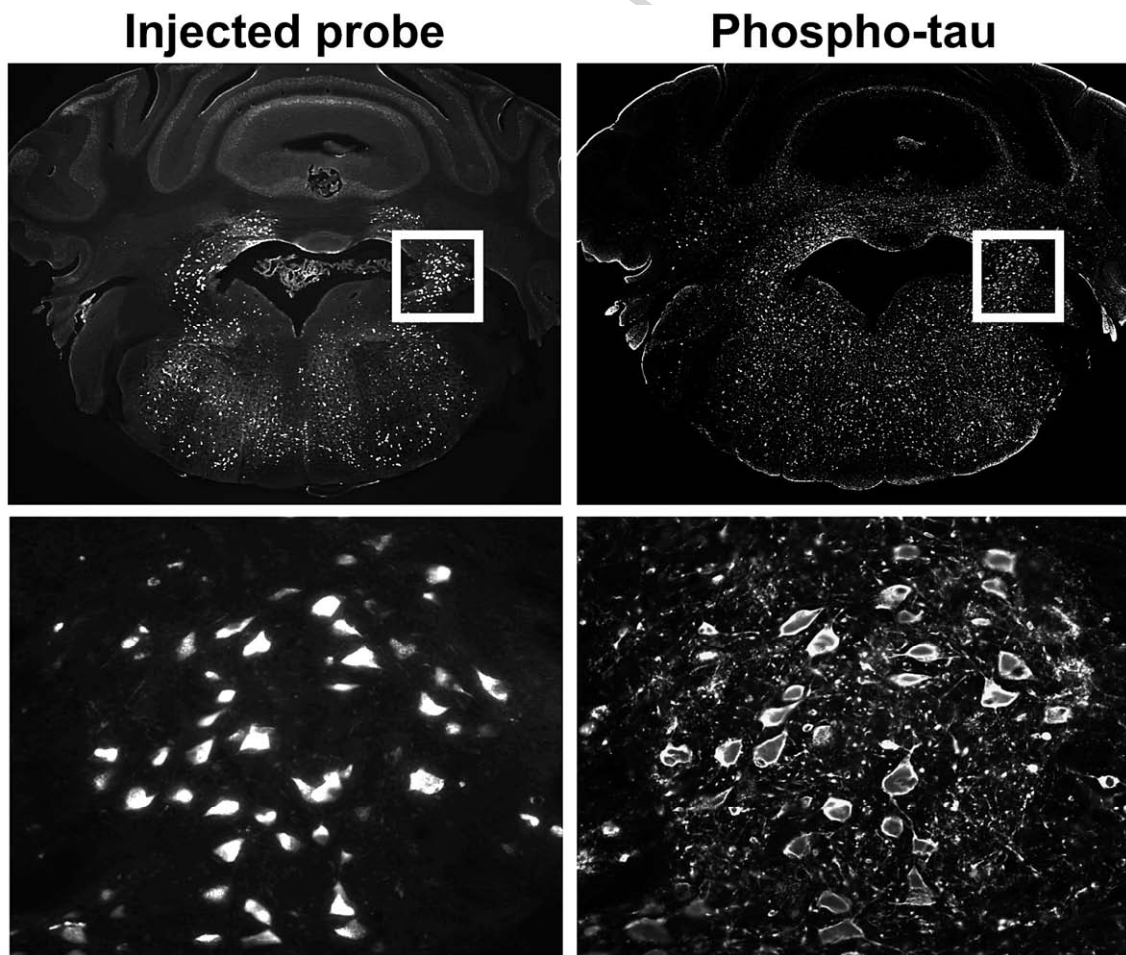


Figure 4. Detection of neuroinflammatory responses by imaging agents for TSPO. (A) Composition of mPTP in resting (top) and activated (bottom) glial cells. TSPO is located on the outer membrane (OM) of mitochondria, and is supposedly intensified in a

polymeric form upon glial activations. Another outer element, voltage-dependent anion channel (VDAC), and inner-membrane (IM) component, adenine nucleotide translocator (ANT), constitute a channel structure allowing transports of selected molecules, and receive excitatory and inhibitory controls by proapoptotic Bax and antiapoptotic Bcl-2, respectively. Hexokinase II (HK II) and cyclophilin D (Cyp D) are also structurally and functionally coupled with these translocators. (B) *In-vivo* T2-weighted MRI and [¹⁸F]fluoroethyl-DAA1106-PET scans of wild-type and PS19 mouse brains. The hippocampus, outlined by dots, exhibits pronounced atrophy and increased TSPO levels in PS19 mice. (C) Two distinct modes of the interplay between glia and injured neurons characterized by TSPO expressions. In case of recoverable damages, TSPO(-) microglia and TSPO(+) astrocytes act for neuronal repairs. Irreversible insults leading to neuronal death are featured by activations of TSPO(+) microglia and TSPO(-) astrocytes.

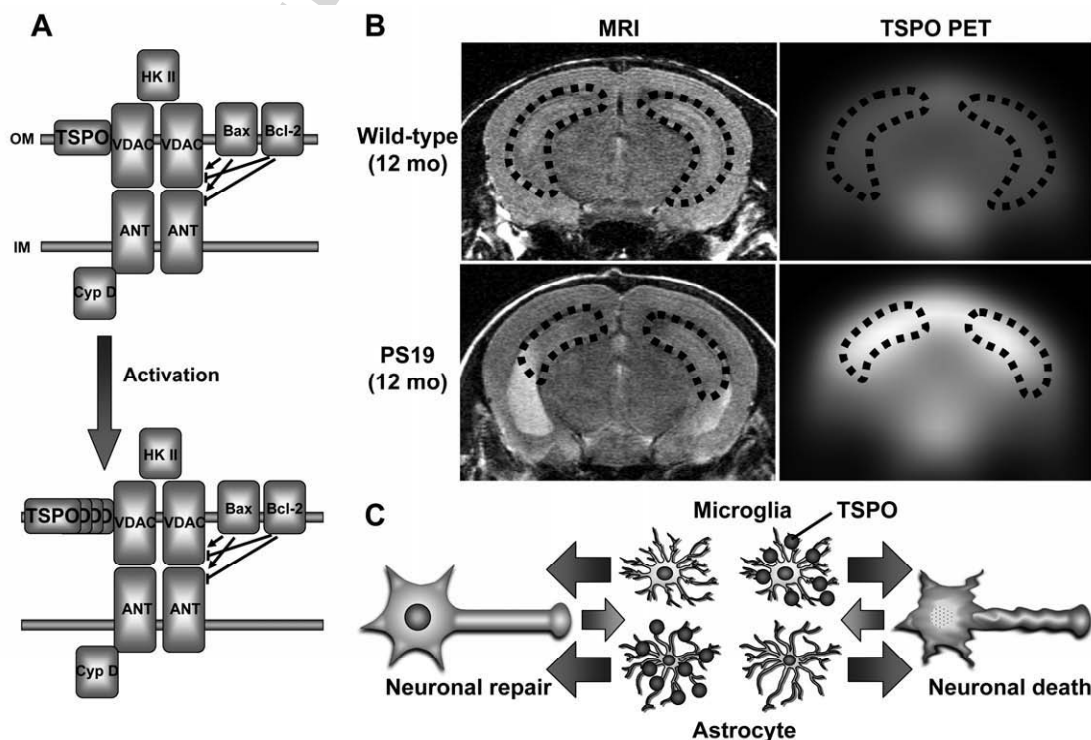


Figure 5. (A) Structural MRI and PET imaging of serotonin 1A receptors using a high-affinity radioligand, [^{11}C]WAY-100635, in wild-type and PS19 mice. Coronal views of the hippocampi (outlined by dots) indicate striking atrophy and serotonergic denervation in the transgenic. (B) Availability of neuroreceptors at baseline (left) and overflow by neurotransmitters (right). The radiotracer binding sites are competitively displaceable by these endogenous ligands. (C) Extracellular dopamine (DA) concentrations measured in microdialysis samples obtained from rat striata. A surge of DA by 10-fold of baseline is induced by intravenous injection of methamphetamine (MAP), and is strongly suppressed by pretreatment with an antagonist for metabotropic glutamate receptor 5, termed MPEP. (D) PET scans of a conscious rat in experimental conditions identical to the microdialysis assays. The binding of agonistic DA D2 receptor radioligand, [^{11}C]MNPA, is noticeably blocked by endogenous competitors following MAP injection. This effect is reversible by administration of MPEP prior to the MAP challenge.

