Multimodal imaging in rat model recapitulates Alzheimer’s Disease biomarkers abnormalities

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Abbreviated title: Alzheimer’s Biomarkers Progression in a Rat Model

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ABSTRACT

Imaging biomarkers are frequently proposed as endpoints for clinical trials targeting brain amyloidosis in Alzheimer’s Disease (AD); however, the specific impact of amyloid-β (Aβ) aggregation on biomarker abnormalities remains elusive in AD. Using the McGill-R-Thy1-APP transgenic rat as a model of selective Aβ pathology, we characterized the longitudinal progression of abnormalities in biomarkers commonly used in AD research. Middle-aged (9-11 months) transgenic animals (both male and female) displayed mild spatial memory impairments and disrupted cingulate network connectivity measured by resting-state fMRI, even in the absence of hypometabolism (measured with PET [18F]FDG) or detectable fibrillary amyloidosis (measured with PET [18F]NAV4694). At more advanced ages (16-19 months), cognitive deficits progressed in conjunction with resting connectivity abnormalities; furthermore, hypometabolism, Aβ plaques accumulation, reduction of CSF Aβ1-42 concentrations and hippocampal atrophy (structural MRI) were detectable at this stage. The present results emphasises the early impact of Aβ on brain connectivity and support a framework in which persistent Aβ aggregation itself is sufficient to impose memory circuits dysfunction, which propagates to adjacent brain networks at later stages.

SIGNIFICANCE STATEMENT

The present study proposes a “back-translation” of the Alzheimer pathological cascade concept, from human to animals. To do so, we used the same set of Alzheimer imaging biomarkers typically used in large human cohorts and assessed their progression over time in a transgenic rat model, which allows for a finer spatial resolution not attainable with mice. Using this translational platform, we demonstrated that Aβ pathology recapitulates an Alzheimer-like profile of biomarker abnormalities, even in the absence of other hallmarks of the disease such as neurofibrillary tangles and widespread neuronal losses.
INTRODUCTION

Alzheimer’s Disease (AD) is characterized by the accumulation of amyloid-β (Aβ) aggregates in various conformations (Glenner and Wong, 1984; Masters et al., 1985; Dickson, 1997; Selkoe, 2001), the occurrence of neurofibrillary tangles (NFTs) comprised of hyperphosphorylated tau proteins (Grundke-Iqbal et al., 1986; Kosik et al., 1988; Goedert et al., 1992) and synaptic dysfunction (Masliaih et al., 1989; Mufson et al., 2000).

Considering that all of these pathological manifestations are measurable at least several years before the onset of AD clinical symptoms (Jack et al., 2011; Sperling et al., 2011; Bateman et al., 2012; Buchhave et al., 2012; Jansen et al., 2015), increasing attention has been turned toward biomarkers for AD as measurable proxies of pathophysiological progression, particularly in presymptomatic stages, now referred to as preclinical AD (Dubois et al., 2016). Most notable among those biomarkers is amyloid deposition in the brain as evidenced by Positron Emission Tomography (PET) with radiopharmaceuticals specific to fibrillar amyloid (Klunk et al., 2004) such as \([^{18}\text{F}]\text{NAV4694}\) (Cselenyi et al., 2012) and by the reduction of Aβ concentration in the cerebrospinal fluid (CSF) (Shibata et al., 2000; Strozyk et al., 2003; Deane et al., 2008).

In addition, glucose metabolism as measured by \([^{18}\text{F}]\text{FDG}\) PET and regional brain volumetry from structural magnetic resonance imaging (MRI) both reveal AD-specific regional patterns of synaptic dysfunction and neurodegeneration (Lehericy et al., 1994; Minoshima et al., 1997; Silverman et al., 2001; Killiany et al., 2002). Finally, resting state (task-free) functional MRI (rs-fMRI) has been proposed as an indicator of brain connectivity abnormalities likely due to synaptic dysfunctions (Celone et al., 2006; Sperling et al., 2009). There exists an important co-localization of these disruptions with areas of early preferential deposition of amyloid plaques; both tend to be...
localized to the Default Mode Network (Klunk et al., 2004; Buckner et al., 2008), primarily involving the precuneus as well as the posterior cingulate, lateral parietal and medial prefrontal cortices.

The temporal sequence of biomarker abnormality in AD has been extensively modeled using cross-sectional data (Jack et al., 2010; Jack and Holtzman, 2013). What remains unclear, however, is the extent to which this progression can be explained by the vulnerability to Aβ toxicity (Alonso et al., 1994; Jagust, 2016). Biomarker studies in animal models expressing a mutated human amyloid precursor protein (hAPP) gene constitute a powerful platform for addressing such questions. To date however, the literature studying AD imaging biomarkers in transgenic animals is comprised almost exclusively of research on mice models, and has yielded varying and often contradictory results (Kawarabayashi et al., 2001; Bondolfi et al., 2002; Toyama et al., 2005; Maeda et al., 2007; Kuntner et al., 2009; Zimmer et al., 2014). We previously highlighted that this discrepancy is likely due to the diversity of pathological phenotypes across models, the cross-sectional nature of most of these studies, and limited imaging resolution of PET cameras for the mice brain size (Zimmer et al., 2014).

To test the hypothesis that Aβ aggregation can itself lead to declines in large-scale brain connectivity, metabolism and cognitive function, we conducted a longitudinal, multimodal biomarker study using the McGill-R-Thy1-APP transgenic (Tg) rat model of AD-like Aβ pathology, which expresses human APP with Swedish and Indiana mutations. This model displays progressive Aβ aggregation and cognitive deficits but no NFTs inclusions or widespread cell death (Leon et al., 2010; Galeano et al., 2014; Wilson et al., 2017). Considering that rat models have a larger and more complex CNS as well as higher cognitive abilities compared to mice (Do Carmo and Cuello, 2013), they are particularly advantageous for longitudinal pre-clinical studies involving imaging modalities (Zimmer et al., 2014). Using the McGill-R-Thy1-APP rat model, we designed a longitudinal and
multimodal study for quantifying age-dependent brain Aβ deposition (measured with PET [^{18}F]NAV4694 and CSF Aβ1-42), progressive neuronal dysfunction (measured with hippocampal volumetry sMRI, rs-fMRI connectivity and PET [^{18}F]FDG) and cognitive impairment (measured with a spatial memory task) as compared to wild-type animals (WT).

METHODS

All procedures described here were performed in accordance with the Canadian Council on Animal Care guidelines and were approved by a McGill Animal Care Ethics Committee.

Experimental design and statistical analysis

A sample of 26 rats (13 wild-type Wistar, 13 homozygous McGill-R-Thy1-APP; 7 males and 6 females in each group) was used for this project. Transgenic McGill-R-Thy1-APP rats (generated and bred by the McGill Cuello Laboratory at the Department of Pharmacology and Therapeutics) express human APP751 with the Swedish and Indiana mutations under the control of the murine Thy1.2 promoter, resulting in accumulation of Aβ peptides starting 1 week postnatal which progress to extracellular plaques at 6-10 months of age in homozygous animals. By 12 months, mature plaques are thioflavin S positive (Leon et al., 2010). Homozygous McGill-R-Thy1-APP also exhibit detectable CSF Aβ38, 39, 40 and 42 species (Iulita et al., 2014), as well as progressive cognitive deficits (Galeano et al., 2014; Qi et al., 2014; Wilson et al., 2017).

All rats were housed at the Douglas Mental Health University Institute animal facility on a 12/12h light/darkness cycle and ad libitum access to food and water. All animals underwent the procedures described below twice: once at a baseline time-point (aged 9-11 months old) and one
follow-up (16-19 months); imaging modalities of each animal for each time-point were acquired within 2 to 6 weeks.

**PET acquisition and processing**

PET acquisition was performed using a CTI Concorde R4 microPET for small animals (Siemens Medical Solutions) and two radiotracers: $[^{18}\text{F}]$NAV4694 for imaging Aβ and $[^{18}\text{F}]$FDG for imaging glucose metabolism. For $[^{18}\text{F}]$NAV4694 scans, anesthesia was first induced using 5% isoflurane in 0.5l/min oxygen and then maintained throughout the procedure with 2% isoflurane. A 9 minutes transmission scan using a rotating $[^{57}\text{Co}]$ point source was followed by a bolus injection of the radiotracer in the tail vein (13.3 ± 0.9 MBq in 200μl, with a specific activity of 85.97 ± 46.47 GBq/μmol), concomitant with the beginning of the emission scan, which lasted for 60 minutes in list mode. The data was then reframed into 27 sequential time frames of increasing durations (8x 30sec, 6x 1min, 5x 2mins, 8x 5mins). For $[^{18}\text{F}]$FDG, tracer injection was done in the tail vein of awake animals (12.7 ± 1.1 MBq in 200μl), who were anesthetized (5% isoflurane in 0.5l/min oxygen for induction, reduced to 2% during the scan) 50 minutes later to perform a 20 minutes emission scan (in a single static time frame) and a 9 minutes transmission scan. Breathing rate was monitored throughout both scanning procedures; temperature was monitored using a rectal thermometer and maintained at 37 ± 1°C using an electric blanket. Images for both tracers were reconstructed using a Maximum a posteriori (MAP) algorithm (voxel size: 0.6 · 0.6 · 1.2mm) and corrected for scatter, dead time and decay.

MINC tools (www.bic.mni.mcgill.ca/ServicesSoftware) were used for image processing and analysis. Image processing steps are summarized in Figure 1; briefly, parametric maps were generated: for $[^{18}\text{F}]$NAV4694, the Binding Potential ($\text{BP}_{ND}$) was calculated for each voxel using the
Simplified Reference Tissue Method at the voxel-level (Gunn et al., 1997) with cerebellar gray matter as a reference region. For $[^{18}\text{F}]$FDG, Standardized Uptake Value ratio (SUVr) images were generated by normalizing the tissue-radioactivity image using the pons as a reference tissue. Each resulting parametric image was first co-registered to the individual animal’s structural MRI (see below) using six degrees of freedom (rigid body transformation), then non-linearly transformed to a standardized rat brain space created from the wild-type Wistar rats used in the present study, in order to account for differences brain morphology.

**MRI acquisition and processing**

MRI acquisition was performed in a 7T BioSpec 70/30 USR dedicated animal MRI (*Bruker*) equipped with Avance III electronics and the 500V/300A B-GA12S2 gradient upgrade with a standard 40mm quadrature volumetric transceiver. Animals were anesthetized with a 1% isoflurane/medical air mix. A constant 37°C air flow was used to maintain the animals warm.

Structural imaging was obtained using the Bruker standard 3D-True Fast Imaging with Steady State Precession pulse sequence, (3D-TrueFISP, a balanced Steady State Free Precession type sequence). To remove banding artifacts, a root-mean-square image of eight phase advance (angles of 0 to 315 degrees in increments of 45) acquisitions was obtained. Each TrueFISP phase angle acquisition was acquired as follows: slices oriented in the rostrocaudal axis, FOV of 36x36x36mm with a matrix of 180x180x180, TE/TR of 2.5/5.0ms, NEX of 2, flip angle of 30°, bandwidth of 50kHz, and no accelerations were employed. The resulting image is an average of 16 acquisitions, with an isotropic 200μm resolution, and was acquired in a total scanning time of 46m30s. Hippocampal volumes were measured using manual segmentation performed by an experimenter blinded to the group conditions, and normalized by the intracranial volume.
The rs-fMRI acquisitions were completed immediately after the anatomical scans using the standard Bruker 2D-Spin Echo, Echo Planar pulse sequence (2D-SE-EPI) and the following parameters: slices oriented in the rostrocaudal axis, FOV of 25.6x25.6mm with a matrix of 64x64 and 32 slices of 1.0mm for a final resolution of 400x400x1000μm, interslice distance of 1.0mm, TE/TR of 15/2000ms, flip angle of 70°, bandwidth of 300kHz, 4 dummy scans to establish steady state, and 450 repetitions for a total scan time of 15min. A partial-FT acceleration factor of 1.34 (16 overscans) was employed, with standard fat suppression and 5 standard saturation slices to isolate the brain volume; the 5th saturation band was used over highly fatty throat areas. Finally, the standard EPI Navigator was employed along with Automatic Ghost Correction, and Automatic Trajectory Adjustment.

The first 4 volumes (8 seconds) were discarded to account for transient drift. Using AFNI (https://afni.nimh.nih.gov), the dynamic functional images were corrected for slice-time and motion and then bandpass-filtered between 0.01 and 0.15 Hz. Connectivity maps were generated by correlating with a seed point in the cingulate cortex, a component of the rat’s default mode network (Lu et al., 2012), which has been shown in this model to be vulnerable to fibrillary Aβ accumulation (Parent et al., 2013). Resulting images were non-linearly transformed to the standardized rat brain space.

Spatial Memory

The spatial memory of each rat was assessed using the Morris Water Maze (Morris, 1984) over four consecutive days, with four trials per day, a maximum trial length of 90 seconds (rats were placed on the platform after unsuccessful 90-seconds trials) and one hour between each trial. Each trial was started in a different quadrant, and external cues were placed outside the pool for
navigation. The time to find the platform was measured automatically using overhead camera
tracking with ANY-maze video tracking software (Stoelting Co.) and used as an outcome measure.
One hour after the last trial on the fourth day, one probe (no platform) trial to assess reference
memory and one visible platform trial was also conducted to account for swim speed and gross
visual deficits.

CSF sampling

Under 5% isoflurane anesthesia, 100-150 μl of cerebrospinal fluid was collected from each
rat through direct puncture of the cisterna magna. Concentrations of Aβ1-42 in the CSF samples
were measured using a multiplex xMAP Luminex platform (Luminex Corp.) using the ELISA kit
INNOTEST β-AMYLOID1-42 (Fujirebio Europe NV), with calibrators from 62.5 to 4000 pg/mL.

Immunohistochemistry

After completion of the 2nd time-point experiments, rats were anesthetized using equithesin
(pento-barbitol based, 2.5 ml/kg, intraperitoneal injection) before trans-cardiac perfusion with cold
0.1 M phosphate buffer (PB; pH 7.4). The brains were removed, divided into hemispheres, post-
fixed in 4% paraformaldehyde in 0.1 M PB for 24 hours at 4°C and then equilibrated in a solution of
30% sucrose in 0.1 M PB. Coronal sections of 40 μm-thickness were obtained using a freezing
sledge microtome (SM 2000R, Leica, Wetzlar, Germany). Free-floating sections (3 sections per
animal) were collected in phosphate-buffered saline (PBS; 10 mM Na2HPO4, 150 mM NaCl, 2.7
mM KCl) and processed for immunohistochemistry. Brain sections were incubated first in McSA1
(MediMabs, QC, Canada) a mouse monoclonal antibody detecting human Aβ (Grant et al., 2000),
and then in goat anti-mouse antibody (MP Biochemicals), followed by a mouse anti-peroxidase
monoclonal antibody complex (MAP/HRP complex, MediMabs) and developed using 3,3′-
diaminobenzidine as the chromogen (Vector Laboratories, Inc.). Images of Aβ-immunoreactivity were acquired using a Zeiss microscope equipped with an AxioCam HRe digital camera (Carl Zeiss, Toronto, Canada) and the Axiovision 4.8 Software.

Statistical analyses

For imaging outcome measures ([18F]NAV4694, [18F]FDG and rs-fMRI), group effects were estimated using a voxel-level general linear model. Resulting t-statistical maps were corrected for multiple comparisons using a Random Field Theory based approach (Worsley et al., 1996) for an adjusted threshold of \( p < 0.05 \) in clusters of at least 30 mm\(^3\). Longitudinal changes in [18F]NAV4694 binding and [18F]FDG uptake were measured with the voxel-level differences between baseline and follow-up parametric images, normalized by the baseline and expressed as maps of average percentage changes.

RESULTS

No significant effect of sex was found for any measurement; male and female were thus grouped together for all subsequent analyses.

Aβ accumulation induces time-dependent changes in glucose metabolism and connectivity

We first examined the consequences of incremental aggregation of Aβ in the McGill-R-Thy1-APP Tg rat model of AD-like Aβ pathology by PET and rs-fMRI. While some fibrillary Aβ is visible at the baseline time-point (9-11 months) using immunohistochemistry, especially in the dorsal hippocampus (see Fig. 6b), no significant group difference in [18F]NAV4694 binding was found at that age. Similarly, glucose metabolism as measured by [18F]FDG uptake was not significantly altered in younger Tg animals. Group contrast for rs-fMRI showed clusters of significantly lower
cingulate connectivity in the Tg group, centered on the orbital cortex and the thalamus ($k = 34.74$ mm$^3$, peak $t(24) = 6.528, p < 0.0001$) as well as higher connectivity with the dorsal hippocampus and sensorimotor cortical areas (see Fig. 2).

At the follow-up time point (16-19 months of age), all three imaging outcomes showed significant group differences (see Fig. 3). For PET Aβ load, the Tg group had significantly higher binding in a cluster covering the olfactory bulb and the infralimbic cortex and spreading laterally to the insular, perirhinal and entorhinal cortices ($k = 113.34$ mm$^3$, peak $t(17) = 6.659, p < 0.0001$), where the ratio of BP$_{ND}$ in the Tg group compared to the non-specific binding observed in WT animals was $1.734 \pm 0.428$. A second cluster covering the dorsal hippocampi, the caudal piriform cortex and amygdala ($k = 70.24$ mm$^3$, peak $t(17) = 7.854, p < 0.0001$) showed an average ratio of $1.767 \pm 0.467$ (Fig. 3a-d).

Group contrast of $[^{18}F]$FDG PET hypometabolism revealed two symmetrical clusters of significant differences where the WT group had higher uptake than Tg animals ($t > 3.58$), located in the ventral orbital, secondary motor, cingulate, prelimbic, barrel and entorhinal cortices (left hemisphere: $k = 133.09$, peak $t(17) = 6.46, p < 0.0001$; average SUVr of $1.449 \pm 0.157$ for WTs and $1.343 \pm 0.14$ for Tg; right hemisphere: $k = 117.48$ mm$^3$, peak $t(17) = 6.403, p < 0.0001$; SUVr of $1.484 \pm 0.123$ for WTs and $1.369 \pm 0.099$ for Tg). A third, median cluster covered the ventral thalamus and medial geniculate as well as the hippocampal genus and the inferior colliculi ($k = 82.36$ mm$^3$, peak $t(17) = 4.417, p = 0.0004$) where the WT group had an average SUVr of $1.448 \pm 0.121$ compared to $1.343 \pm 0.112$ for the Tg group (Fig. 3e-h).

Connectivity with the cingulate seed point, as measured by rs-fMRI, was lower in Tg animals, including the pre- and infralimbic cortices, the basal forebrain, the ventral caudate putamen...
as well as the dorsal hippocampi, parietal association cortex and endopiriform nucleus ($k = 242.442$ mm$^3$, peak $t(15) = 7.392, p < 0.0001$) (Fig. 3i-k).

In terms of age effect, the Tg but not the WT group showed a progressive increase of fibrillar Aβ, with $^{[18]}$FNAV4694 BP$_{ND}$ reaching differences of up to 47.44% in the parietal association and retrosplenial cortices, 91.22% in the caudal entorhinal cortex and 94.08% in the basal forebrain and olfactory bulb (see Fig. 4a). Conversely, $^{[18]}$FDG uptake decreases (see Fig. 4b) were observed throughout the brain in Tg animals, with the highest reductions located in frontal (38%) and parietal (37%) cortices as well as the cerebellum (36%). Together these findings suggest that Aβ deposition per se is sufficient to cause abnormalities in glucose metabolism and connectivity.

$Aβ$-induced functional deficits are reflected by Spatial Memory Impairments

Spatial memory, as tested using the Morris water maze (MWM) task, showed a learning effect for both groups at both time points, depicted as the latency to locate the platform in the learning phase of the task from days one to four (see Fig. 5). For the baseline time-point, there was a significant genotype effect only on the fourth day of testing, with the WT taking significantly less time to locate the platform than the Tg animals ($F = 8.996, p = 0.007$). At follow-up, the WT group performed significantly better than Tg for both the third ($F = 5.903, p = 0.028$) and fourth days ($F = 5.352, p = 0.038$) of learning. Additionally, both groups performed significantly better on the first day of testing at follow-up than they did at baseline ($F = 8.243, p = 0.001$). Despite the differences during the learning phase, both groups reached comparable performance during the probe trial as measured by proportion of time spent in the target quadrant (WT: $38.9 \pm 14.9\%$; Tg: $39.9 \pm 9.5\%$). Latency to reach the platform during a trial where the platform was visible did not differ
significantly between Tg (17 ± 10.4s) and WT (14.4 ± 5.2s). These findings illustrate observable
cognitive impairments as a consequence of Aβ-induced metabolic and synaptic dysfunctions.

Aβ deposition in the brain is reflected by decreased Aβ CSF levels and brain volume

Lastly, two other common AD biomarkers, hippocampal volumetry and CSF Aβ1-42, were studied
here using the McGill-R-Thy1-APP model. The normalized hippocampal volumes of Tg animals
decreased from 79.606 ± 3.093 mm³ at baseline to 73.283 ± 3.93 at follow-up (paired t(8) = 6.328, p
= 0.0002), while there was no significant age effect for WT rats (see Fig. 6a, 6c). Additionally, the
CSF Aβ1-42 concentrations in older Tg rats decreased by 28.44% compared with the first time-point
assay (see Fig. 6d); from 2002.507 ± 453.008 pg/mL at baseline to 1433.031 ± 273.349 at follow-up
(paired t(8) = 4.513, p = 0.002), with overall concentrations being comparable to those observed in
elderly human cohorts (see Fig. 6e).

DISCUSSION

In summary, we have shown in this longitudinal study that Aβ aggregates secondary to the
expression of mutated human APP in the rat brain (devoid of NFTs or widespread neuronal
depletion) is sufficient to cause specific brain injury quantifiable using the same biomarkers that are
used as outcome measures in AD clinical studies. In aged McGill-R-Thy1-APP rats, amyloidosis
was observed by increased [¹⁸F]NAV4694 binding in addition to decreased CSF Aβ₁₋₄₂
concentrations. Compared with WT controls, the Tg animals showed progressive functional decline,
reflected by reduced resting brain connectivity and glucose metabolism, as well as spatial memory
impairments measured by the Morris Water Maze task.
Interestingly, both rs-fMRI and behavioral measures showed abnormalities before mature fibrillary plaques or glucose hypometabolism are detectable by microPET imaging. These results support the notion that human Aβ oligomeric aggregates exert toxic effects before the formation of mature, thioflavin-positive Aβ plaques (Walsh et al., 2002; Forny-Germano et al., 2014). Indeed, previous electrophysiological studies conducted in the McGill-R-Thy1-APP and other models of human brain amyloidosis conducted at this disease stage suggest that early brain connectivity changes or memory declines observed in our cohort are conceivably functional consequences of synaptic alterations (Iulita et al., 2014; Qi et al., 2014; Wilson et al., 2017).

As previously suggested, adaptations of network architecture such as recruitment as well as strengthening or weakening of specific connections might occur as consequences of Aβ aggregates (Greicius et al., 2004; Buckner et al., 2009; Gardini et al., 2015). In fact, the brain network abnormalities reported here possibly represent a large-scale signature of Aβ-induced synaptic dysfunction rather than disruption of the underlying structural connections (Lacor et al., 2007; Bao et al., 2012). Remarkably, the fact that human Aβ aggregates enhances the connectivity between hippocampus and cingulate cortex in the animal model as well as in mild cognitive impaired patients indicates susceptibility of this specific memory network component to Aβ (Bai et al., 2009; Elman et al., 2014; Gardini et al., 2015). In fact, early functional deficits preceding the onset of fibrillary Aβ are possibly related to synaptic vulnerabilities, and supports the concept that localized Aβ deposition may be dependent on the default patterns of activity preceding disease onset (Buckner et al., 2005). It should be noted that while these early functional changes underscore deleterious effects of pre-plaques Aβ aggregates, we cannot discard the possibility that further damage is imposed by fibrillary Aβ deposits in later disease stages.
While the progression rates of biomarker abnormalities over time vary significantly throughout the brain, both amyloidosis and hypometabolism are contained within a range of 20 to 40% in the frontoparietal areas between the cingulate and retrosplenial cortices. Specifically, in the parietal association and retrosplenial cortices, fibrillary Aβ deposition increased by an average of 34% over the 6 months’ period separating baseline and follow-up scans, while glucose metabolism decreased by an average of 31%.

Colocalization between Aβ fibrillary deposition, hypometabolism, and connectivity decline occurred in the vicinity of the rhinal fissure, encompassing somatosensory and limbic cortices, while other brain regions showed partial biomarker abnormality overlapping. For instance, both fibrillary Aβ and connectivity impairments converge in cortical and hippocampal regions, which confirms that Aβ is sufficient to predict functional connectivity decreases in resting-state networks and in the hippocampal formation in humans (Hedden et al., 2009). Conversely, while progressive accumulation of fibrillary Aβ was most prominent in the olfactory bulb and basal forebrain, decreases in glucose uptake was largest in the cortex. This regional dissociation likely reflects a selective vulnerability of these cortical areas to Aβ toxicity, and alternatively, could be explained by a downregulation of the basalo-cortical projections. Indeed, regional declines in metabolism measurable with [18F]FDG PET can be induced by an injury in a remote brain region (Meguro et al., 1999).

Of note, the basal Aβ1-42 levels observed in these animals are comparable to those in human populations (see Fig. 6d), which underlines the translational value of the present observations. Mild brain atrophy measured with structural MRI indicated a modest but significant effect of Aβ on volumetry limited to the hippocampus, which is the only structure where cell death is observed in
McGill-R-Thy1-APP rats (Heggland et al., 2015). Resilience of the surrounding cortical areas to atrophy might be explained by the absence of NFTs, since native murine hyperphosphorylated tau is not prone to aggregation. Alternatively, the follow-up time point of this study may not have been late enough for the initiation of more pronounced brain atrophy. Future studies of this model will investigate CSF levels of hyperphosphorylated tau to gain a better understanding of the progression of pre-atrophy neurodegeneration. Finally, the early memory impairment observed here seems to contrast with the human sequence of biomarkers abnormalities modeled by Jack and colleagues (Jack and Holtzman, 2013), which could be explained by an absence of neural reserve in the less evolved rodent CNS. Learning deficits were also observed for a visual association task in this animal model (Wilson et al., 2017), while hemizygous transgenic animals (+/-) showed impairments in working memory as early as 6 months of age (Galeano et al., 2014).

As such, our findings indicate that longitudinal biomarker acquisitions in rodents recapitulate large-scale observational and interventional studies in humans, specifically in prodromal and early stages of the disease. This is the first longitudinal multiparametric study using a robust rat model of Aβ pathology illustrating progressive abnormalities in AD biomarkers. With only a single transgene insertion site per allele, this rat model has minimal genetic invasion compared to other animal models, yet was able to reproduce a biomarker profile closely analogous to that of the human disease. Based on the present observations, we propose that biomarker abnormalities as a function of Aβ pathology are more evident at the level of large-scale brain networks connectivity and regional brain metabolism measurements than at that of brain atrophy or memory impairment measurements.
Alonso AC, Zaidi T, Grundke-Iqbal I, Iqbal K (1994) Role of abnormally phosphorylated tau in the breakdown of microtubules in Alzheimer disease. Proceedings of the National Academy of Sciences of the United States of America 91:5562-5566.

Bai F, Watson DR, Yu H, Shi Y, Yuan Y, Zhang Z (2009) Abnormal resting-state functional connectivity of posterior cingulate cortex in amnestic type mild cognitive impairment. Brain Res 1302:167-174.

Bao F, Wicklund L, Lacor PN, Klein WL, Nordberg A, Marutle A (2012) Different beta-amyloid oligomer assemblies in Alzheimer brains correlate with age of disease onset and impaired cholinergic activity. Neurobiol Aging 33:825 e821-813.

Bateman RJ et al. (2012) Clinical and biomarker changes in dominantly inherited Alzheimer’s disease. The New England journal of medicine 367:795-804.

Bondolfi L, Calhoun M, Ermini F, Kuhn HG, Wiederhold KH, Walker L, Staufenbiel M, Jucker M (2002) Amyloid-associated neuron loss and gliogenesis in the neocortex of amyloid precursor protein transgenic mice. J Neurosci 22:515-522.

Buckner RL, Andrews-Hanna JR, Schacter DL (2008) The brain’s default network: anatomy, function, and relevance to disease. Annals of the New York Academy of Sciences 1124:1-38.

Buckner RL, Sepulcre J, Talukdar T, Krienen FM, Liu H, Hedden T, Andrews-Hanna JR, Sperling RA, Johnson KA (2009) Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability, and relation to Alzheimer’s disease. J Neurosci 29:1860-1873.

Buckner RL, Snyder AZ, Shannon BJ, LaRossa G, Sachs R, Pototsky AF, Sheline YI, Klunk WE, Mathis CA, Morris JC, Mintun MA (2005) Molecular, structural, and functional characterization of Alzheimer’s disease: evidence for a relationship between default activity, amyloid, and memory. J Neurosci 25:7709-7717.

Celone KA, Calhoun VD, Dickerson BC, Atri A, Chua EF, Miller SL, DePeau K, Rentz DM, Selkoe DJ, Blacker D, Albert MS, Sperling RA (2006) Alterations in memory networks in mild cognitive impairment and Alzheimer’s disease: an independent component analysis. J Neurosci 26:10222-10231.

Cselenyi Z, Jonhagen ME, Forsberg A, Halldin C, Julin P, Schou M, Johnstrom P, Varnas K, Svensson S, Farde L (2012) Clinical validation of 18F-AZD4694, an amyloid-beta-specific PET radioligand. Journal of nuclear medicine : official publication, Society of Nuclear Medicine 53:415-424.

Deane R, Sagare A, Zlokovic BV (2008) The role of the cell surface LR and soluble LR in blood-brain barrier Abeta clearance in Alzheimer’s disease. Curr Pharm Des 14:1601-1605.

Dickson DW (1997) The pathogenesis of senile plaques. J Neuropathol Exp Neurol 56:321-339.

Do Carmo S, Cuello AC (2013) Modeling Alzheimer’s disease in transgenic rats. Molecular neurodegeneration 8:37.

Dubois B et al. (2016) Preclinical Alzheimer’s disease: Definition, natural history, and diagnostic criteria. Alzheimers Dement 12:292-322.

Elman JA, Oh H, Madison CM, Baker SL, Vogel JW, Marks SM, Crowley S, O’Neil JP, Jagust WJ (2014) Neural compensation in older people with brain amyloid-beta deposition. Nature neuroscience 17:1316-1318.

Forny-Germaino L, Lyra e Silva NM, Batista AF, Brito-Moreira J, Gralle M, Boehnke SE, Coe BC, Lablans A, Marques SA, Martinez AM, Klein WL, Houzel JC, Ferreira ST, Munoz DP, De Felice FG (2014) Alzheimer’s disease-like pathology induced by amyloid-beta oligomers in nonhuman primates. J Neurosci 34:13629-13643.

Galeano P, Martino Adami PV, Do Carmo S, Blanco E, Rotondaro C, Capani F, Castano EM, Cuello AC, Morelli L (2014) Longitudinal analysis of the behavioral phenotype in a novel transgenic rat model of early stages of Alzheimer’s disease. Front Behav Neurosci 8:321.

Gardini S, Venneri A, Sambataro F, Cuetos F, Fasano F, Marchi M, Crisi G, Caffarra P (2015) Increased functional connectivity in the default mode network in mild cognitive impairment: a maladaptive compensatory mechanism associated with poor semantic memory performance. J Alzheimers Dis 45:457-470.

Glenner GG, Wong CW (1984) Alzheimer’s disease and Down’s syndrome: sharing of a unique cerebrovascular amyloid fibril protein. Biochemical and biophysical research communications 122:1131-1135.
Goedert M, Spillantini MG, Cairns NJ, Crowther RA (1992) Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. Neuron 8:159-168.

Grant SM, Ducatenzeiler A, Szfy M, Cuello AC (2000) Abeta immunoreactive material is present in several intracellular compartments in transfected, neurally differentiated, P19 cells expressing the human amyloid-beta-protein precursor. J Alzheimer's Dis 2:207-222.

Greicius MD, Srivastava G, Reiss AL, Menon V (2004) Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci U S A 101:4637-4642.

Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM (1986) Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. The Journal of biological chemistry 261:6084-6089.

Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ (1997) Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. Neuroimage 6:279-287.

Hedden T, Van Dijk KR, Becker JA, Mehta A, Sperling RA, Johnson KA, Buckner RL (2009) Disruption of functional connectivity in clinically normal older adults harboring amyloid burden. J Neurosci 29:12686-12694.

Heggland I, Storkaas IS, Soligard HT, Kobro-Flatmoen A, Witter MP (2015) Stereological estimation of neuron number and plaque load in the hippocampal region of a transgenic rat model of Alzheimer's disease. Eur J Neurosci 41:1245-1262.

Iulita MF, Allard S, Richter L, Munter LM, Ducatenzeiler A, Weise C, Do Carmo S, Klein WL, Multhaup G, Cuello AC (2014) Intracellular Abeta pathology and early cognitive impairments in a transgenic rat overexpressing human amyloid precursor protein: a multidimensional study. Acta neuropathologica communications 2:61.

Jack CR, Jr., Holtzman DM (2013) Biomarker modeling of Alzheimer's disease. Neuron 80:1347-1358.

Jack CR, Jr., Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, Petersen RC, Trojanowski JQ (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol 9:119-128.

Jack CR, Jr., Vemuri P, Wiste HJ, Weigand SD, Aisen PS, Trojanowski JQ, Shaw LM, Bernstein MA, Petersen RC, Weiner MW, Knopman DS, Alzheimer's Disease Neuroimaging I (2011) Evidence for ordering of Alzheimer disease biomarkers. Arch Neurol 68:S126-135.

Jagust W (2016) Is amyloid-beta harmful to the brain? Insights from human imaging studies. Brain 139:23-30.

Jansen WJ et al. (2015) Prevalence of cerebral amyloid pathology in persons without dementia: a meta-analysis. JAMA: the journal of the American Medical Association 313:1924-1938.

Kawarabayashi T, Younkin LH, Saido TC, Shoji M, Ashe KH, Younkin SG (2001) Age-dependent changes in brain, CSF, and plasma amyloid (beta) protein in the Tg2576 transgenic mouse model of Alzheimer's disease. J Neurosci 21:372-381.

Killiany RJ, Hyman BT, Gomez-Isla T, Moss MB, Kikinis R, Jolesz F, Tanzi R, Jones K, Albert MS (2002) MRI measures of entorhinal cortex vs hippocampus in preclinical AD. Neurology 58:1188-1196.

Kluck WE et al. (2004) Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Ann Neurol 55:306-319.

Kosik KS, Orecchio LD, Binder L, Trojanowski JQ, Lee VM, Lee G (1988) Epitopes that span the tau molecule are shared with paired helical filaments. Neuron 1:817-825.

Kuntner C, Kesner AL, Bauer M, Kremleshner R, Wanek T, Mandler M, Karch R, Stanek J, Wolf T, Muller M, Langer O (2009) Limitations of small animal PET imaging with [18F]FDDNP and FDG for quantitative studies in a transgenic mouse model of Alzheimer's disease. Molecular imaging and biology : MIB : the official publication of the Academy of Molecular Imaging 11:236-240.

Lacor PN, Buniel MC, Furlow PW, Clemente AS, Velasco PT, Wood M, Viola KL, Klein WL (2007) Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer’s disease. J Neurosci 27:796-807.

Lehericy S, Baulac M, Chiras J, Pierot L, Martin N, Pillon B, Deweer B, Dubois B, Marsault C (1994) Amygdalohippocampal MR volume measurements in the early stages of Alzheimer disease. AJNR American journal of neuroradiology 15:929-937.

Leon WC, Canneva F, Partridge V, Allard S, Ferretti MT, DeWilde A, Vercauteren F, Atifeh R, Ducatenzeiler A, Klein W, Szfy M, Alhonen L, Cuello AC (2010) A novel transgenic rat model with a full Alzheimer’s-like amyloid pathology displays pre-plaque intracellular amyloid-beta-associated cognitive impairment. J Alzheimers Dis 20:113-126.

Lu H, Zou Q, Gu H, Raichle ME, Stein EA, Yang Y (2012) Rat brains also have a default mode network. Proceedings of the National Academy of Sciences of the United States of America 109:3979-3984.
FIGURE LEGENDS

Figure 1. Processing and analytical pipeline for imaging data

PET data was acquired in list mode, then reconstructed with correction for dead-time, scatter and decay. Resulting tissue-activity images were filtered with a Gaussian kernel and parametric maps were generated for each radiotracer, then linearly registered to their respective structural (FISP) MRI. For rs-fMRI, dynamic resting-state images were corrected for slice timing and motion, then bandpass filtered. Resulting processed images were correlated at the voxel level to a seed point in the cingulate cortex to generate parametric maps of connectivity. Parametric maps of connectivity, SUVr and BPND in individual MRI space were then non-linearly co-registered to a standard space consisting of an averaged structural MRI. Group effects were assessed with a voxel-level linear model, corrected for multiple comparison using a random field theory approach. SRTM: Simplified Reference Tissue Method; SUVr: Standardized Uptake Value ratio; BPND: Binding Potential. BOLD: Blood-Oxygen-Level Dependent.

Figure 2. Early functional connectivity disruption in transgenic animals

t-statistical rs-fMRI connectivity group contrasts (n = 13 animals per group) at baseline overlaid on a template structural image (sagittal slices 1 to 4 mm lateral to midline at 1mm intervals). (a) Connectivity with the cingulate seed point is reduced for transgenic rats in the orbital cortex, thalamus and amygdalohippocampal area, and (b) up-regulated in the dorsal hippocampus as well as primary sensory and motor cortical regions. Clusters of at least 30 mm³ are shown for values corresponding to p < 0.05 after multiple comparison correction.

Figure 3. Amyloid plaques, glucose hypometabolism and functional connectivity impairments in transgenic animals
t-statistical group contrasts at follow-up time-point, overlaid on a template structural image. Significant group contrasts after multiple comparison correction in clusters of at least 30mm³, are projected on sagittal slices (1 to 5 mm lateral from midline, at 1 mm intervals). Box-and-whisker plots show the distribution of the parametric measures for each cluster in WT (blue) and Tg (red) animals. (a) [¹⁸F]NAV4694 BPND (n = 10 Tg, 9 WT) is significantly higher for Tg rats in (b) the olfactory bulb and frontal cortex as well as (c) in the left and (d) right dorsal hippocampus and the entorhinal and insular cortices. (e) [¹⁸F]FDG uptake (n = 10 Tg, 9 WT) is lower in Tg rats across (f) the left and (g) right frontal cortex, and (h) in the genu of the hippocampus, ventral thalamic nuclei and colliculi. (i) rs-fMRI (n = 8 Tg, 8 WT) connectivity is weaker (j) in dorsal hippocampus and the retrosplenial cortex as well as in (k) prelimbic areas and the basal forebrain.

*** p < 0.0005; * p < 0.05

Figure 4. Regional progression of amyloid plaques and glucose hypometabolism over time
Percentage change values overlaid on a brain surface and mid-sagittal projections. Longitudinal progression of PET biomarkers in transgenic animals over a 6 month period (n = 10 animals with two time-point measurements per biomarker). (a) [¹⁸F]NAV4694 binding increase over time is most prominent in the parietal and entorhinal cortices as well as in the basal forebrain and olfactory bulb. (b) [¹⁸F]FDG uptake longitudinal decreases are present throughout the encephalon, with the highest changes across the cortical mantle and in the cerebellum.

Figure 5. Spatial memory deficits in transgenic animals
(a) Average time to find platform during the learning phase of the Morris Water Maze Task. Significant group effects were found at day 4 for baseline measurements (*), and for days 3 and 4 at follow-up (#). Additionally, a long-term learning effect is visible through improved day 1 performance at follow-up compared with baseline. Each bar represents mean ± S.E.M of each group (n = 13 per group at baseline; 11 transgenic and 9 wildtype animals at follow-up) with 4 repetitions per animal per day (b) Representative occupancy plots of one day 4 trial for each group and time-point.

Figure 6. Hippocampal volumetry and CSF Aβ1-42 concentrations
(a, c) Volumetric analysis of the hippocampus following manual segmentation showed average decrease of normalized volume from 79.606 to 73.283 mm³ (7.94% lower) in transgenic animals, with individual trajectories (n = 8 animals with two time-point measurements) of hippocampal volume between baseline and follow-up. (b) Aβ immunoreactivity
detection using McSA1 antibody in the brain tissue of representative wildtype (left) and transgenic animals at 11 (top right) and 20 months of age (bottom right). Slices are 3.3mm posterior from bregma, showing the dorsal hippocampus as well as the retrosplenial, somatosensory and auditory cortices. (d) Individual trajectories (n = 8 animals with two time-point measurements) of CSF concentrations of Aβ1-42 between baseline and follow-up for transgenic rats. Concentrations went from an average of 2002.507 to 1433.031 pg/mL, representing a 28.44% decrease. (e) Distribution of CSF concentrations of Aβ1-42 in elderly humans (n = 62, 75.5 ± 6.8 years old) compared to those of transgenic rats at the follow-up time point (n = 8).

*** p < 0.0005
**PET Native Space**
- List-mode acquisition
- Reconstruction with correction for dead-time, scatter & decay
- Dynamic tissue-activity images
  - Gaussian filtering; reference region modeling \([^{18}F]FDG\);
  - SRTM \([^{18}F]NAV4694\)
- Parametric maps: SUVr \([^{18}F]FDG\) or \(BP_{ND}\) \([^{18}F]NAV4694\)
- Linear registration (6 parameters)

**MRI Native Space**
- Dynamic BOLD images
- Slice timing & motion correction, bandpass filtering
- Processed BOLD images
- Connectivity with cingulate seed point
- Parametric maps: functional connectivity with cingulate cortex

**Standard Space**
- Non-linear registration
- Parametric maps in standard space
- Voxel-level general linear model
- t-value statistical maps
- Random field theory multiple comparison correction & thresholding
- Significant group effect maps
- Structural MRI
