Early pathologic amyloid induces hypersynchrony of BOLD resting-state networks in transgenic mice and provides an early therapeutic window before amyloid plaque deposition

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Abstract

Introduction: In Alzheimer’s disease (AD), pathologic amyloid-beta (Aβ) is synaptotoxic and impairs neuronal function at the microscale, influencing brain networks at the macroscale before Aβ deposition. The latter can be detected noninvasively, in vivo, using resting-state functional MRI (rsfMRI), a technique used to assess brain functional connectivity (FC).

Methods: RsfMRI was performed longitudinally in TG2576 and PDAPP mice, starting before Aβ deposition to determine the earliest FC changes. Additionally, the role of pathologic Aβ on early FC alterations was investigated by treating TG2576 mice with the 3D6 anti-Aβ-antibody.

Results: Both transgenic models showed hypersynchronized FC before Aβ deposition and hypo-synchronized FC at later stages. Early anti-Aβ treatment in TG2576 mice prevented hypersynchronous FC and the associated synaptic impairments and excitatory/inhibitory disbalances.

Discussion: Hypersynchrony of FC may be used as a new noninvasive read out of early AD and can be recovered by anti-Aβ treatment, encouraging preventive treatment strategies in familial AD.

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Keywords: Amyloidosis; Alzheimer’s disease; Transgenic mouse models; BOLD functional connectivity; Resting-state fMRI; Hypersynchrony; TG2576; PDAPP

1. Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder, characterized by deficits in learning, memory, and cognitive function [1]. The pathologic hallmarks of AD include the progressive accumulation of extracellular amyloid plaques and intracellular tangles.

The amyloid cascade hypothesis postulates that abnormal accumulation of amyloid-beta (Aβ), one of the earliest events in AD pathology, triggers the formation of tau tangles and neurodegeneration, eventually resulting in the clinical expression of AD [2,3]. The nonlinear correlation between Aβ plaque deposition and dementia has led to a shift of
the amyloid cascade hypothesis toward the soluble form of Aβ, which exerts synaptotoxic and neurotoxic effects [4]. Indeed, several transgenic mouse models of amyloidosis show cognitive dysfunctions and impaired synaptic function before the manifestation of Aβ plaques [5–11] supporting a destructive role of soluble Aβ on neurologic processes that underlie learning and memory.

Synaptic dysfunction elicited by soluble Aβ leads to impaired communication between brain regions, resulting in deficits of neuronal networks which could be expressed as cognitive disturbances [4,12]. The noninvasive detection of such network alterations would allow elucidating the spatiotemporal relation between amyloid pathology and functional impairments and provide a tool for early diagnosis. Alterations at the neuronal network level can be detected noninvasively using resting-state functional magnetic resonance imaging (rsfMRI). In rsfMRI, low frequency (0.01–0.1 Hz) fluctuations of the blood-oxygenation-level-dependent (BOLD) signal are considered to reflect underlying fluctuations of neuronal activity. Functional connectivity (FC) is defined as the temporal correlation of BOLD fluctuations between spatially distinct brain regions [13].

RsfMRI has been applied to study functional disruptions in AD patients [14] and to a lesser extent in mouse models of amyloidosis [15]. These studies showed FC impairments between brain regions important for learning and memory after Aβ plaque deposition. However, the ability to noninvasively detect early-stage FC changes in mouse models of amyloidosis, thus when no plaques are present yet, would open doors in terms of early diagnosis. The present study longitudinally investigated BOLD FC networks in two transgenic mouse models of amyloidosis at a preplaque stage, that is, the TG2576 [16] and PDAPP mice [17].

2. Material and methods

2.1. Ethical statement

All procedures were performed in strict accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes. The protocols were approved by the Committee on Animal Care and Use at the University of Antwerp, Belgium (permit numbers 2013-62 and 2015-07), and all efforts were made to minimize animal suffering.

2.2. Animals

2.2.1. Characterization studies

The TG2576 mouse model of amyloidosis (kindly provided by Karen Hsiao Ashe, University of Minnesota) overexpresses the mutant form of amyloid precursor protein (APP) carrying the Swedish mutation (KM670/671NL) controlled by the hamster prion protein promoter [16]. Aβ plaque development starts at the age of 9–11 months [16]. Female mice were assessed with rsfMRI at the age of 3 months (TG2576N = 10, wild type N = 13), 5 months (TG2576N = 10, wild type N = 12), 8 months (TG2576N = 9, wild type N = 12), 14 months (TG2576N = 9, wild type N = 9), and 18 months (TG2576N = 11, wild type N = 12). At each time point, TG2576 and wild-type mice were sacrificed for immunohistochemistry to assess Aβ plaques (thioflavin-S), astrocytosis (GFAP), and microgliosis (Iba1; TG2576N = 4/age group, wild type N = 2/age group). Immunofluorescence analyses were performed according to a previously described protocol [18]. Additional analyses were performed in 5-month-old TG2576 and wild-type mice: Synaptic plasticity was assessed using electrophysiology (N = 6/group) as described previously [19], real-time reverse transcription (RT)-qPCR was performed to assess microglial markers (Iba1, interleukin-1β, macrophage inflammatory protein, interleukin-10, argininase-1, and chitinase-like-3, N = 5/group), and ELISA was used to determine the level of soluble Aβ (N = 4/group). For a detailed description of immunohistochemistry, electrophysiology, RT-qPCR and ELISA procedures, see Supplementary Methods.

The PDAPP mouse model of amyloidosis (kindly provided by Janssen Alzheimer Immunotherapy, South San Francisco, CA) overexpresses the mutant form of APP carrying the Indiana mutation (V717 F) controlled by the platelet-derived growth factor (PDGF) promotor [17]. These mice start to develop Aβ plaques around the age of 8–9 months. In this study, female mice were evaluated with rsfMRI at 3 months (PDAPP N = 20, wild type N = 20), after which a subgroup of mice was sacrificed for immunohistochemistry and ELISA (N = 4/group), to assess Aβ plaques (thioflavin-S) and soluble Aβ respectively. The remaining mice were scanned with rsfMRI again at 5 months (PDAPP N = 16, wild type N = 15) and at the age of 7 months (PDAPP N = 16, wild type N = 15). For a detailed description of immunohistochemistry and ELISA procedures, see Supplementary Methods.

2.2.2. Treatment study

TG2576 and wild-type mice (N = 40/group) were scanned with rsfMRI at the age of 3 months, after which they were divided into 4 treatment groups (N = 20/group), that is, wild-type and TG2576 shams and wild type and TG2576 treated. The sham groups received a weekly intraperitoneal (i.p.) injection of 0.02-mL/kg body weight (BW) phosphate buffered saline (PBS, injected volume 0.5 mL) and the treated groups received 10-mg/kg BW (injected volume 0.5 mL) 3D6 antibody (kindly provided by Janssen Alzheimer Immunotherapy, South San Francisco, CA) to clear Aβ. The treatment regimen started when all mice were 3 months of age and lasted 10 weeks, after which all mice were scanned again with rsfMRI. After the rsfMRI scans a subgroup of mice was sacrificed for immunohistochemistry to assess glutamatergic (VGLUT-1) synapses, GABAergic (GAD65-67) synapses, and presynaptic (synaptobrevin) and postsynaptic (synaptobrevin) markers (N = 4/group), electrophysiological analyses to investigate synaptic
plasticity (N = 6/group), and ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS, N = 4/group) to determine the ratio of glutamate/GABA neurotransmitters. The remaining mice (N = 10/group) were scanned again with rsfMRI at the age of 7, 8, and 10 months. For a detailed description of immunohistochemistry, electrophysiology, and UPLC-MS/MS procedures, see Supplementary Methods.

2.3. MRI procedures

For MRI handling procedures, mice were anesthetized with 2% isoflurane. During the rsfMRI measurements, a combination of isoflurane (0.4%) and medetomidine (0.3 mg/kg) was used to sedate the animals as described previously [20]. Breathing rate, heart rate, blood oxygenation, and body temperature were monitored throughout the MRI procedure. MRI scans were performed on a 9.4-T Biospec MRI system, and images were acquired using a standard Bruker crosscoil setup using a quadrature volume coil and a quadrature surface coil designed for mice. Three orthogonal multislice Turbo Rapid Imaging with Refocused Echoes (RARE) T2-weighted images were acquired to render slice-positioning uniform (repetition time, 2000 ms; echo time, 15 ms; 16 slices of 0.4 mm). Field maps were acquired for each animal to assess field homogeneity, followed by local shimming, which corrects for the measured inhomogeneity in a rectangular volume within the brain. RsfMRI signals were measured by a $T_2^*$-weighted single shot echo-planar-imaging sequence (repetition time, 2000 ms; echo time, 15 ms; 16 slices of 0.4 mm; 150 repetitions). The field-of-view was (20 × 20) mm² and the matrix size (128 × 64), resulting in voxel dimensions of (0.156 × 0.312 x 0.4) mm³. RsfMRI preprocessing was performed as described previously [15]. Independent component analysis (ICA) was performed in 3-month-old wild type animals to determine which brain networks can be discerned. Next, the regions that were part of the neurologically relevant ICA components (Supplementary Table 1) were used for region-of-interest (ROI) correlation analyses, which resulted in FC matrices. Additionally, individual z-transformed FC maps were obtained for all animals with the right hippocampus as seed region. For a detailed description of MRI procedures and processing, see Supplementary Methods.

2.4. Statistical analyses

Statistical analyses of FC strength per brain network and electrophysiological recordings included a two way ANOVA, with the Holm–Sidak correction for multiple comparisons (P < .05). Statistical analyses of the zFC maps include a one sample T-test for within group analyses, a two sample T-test for comparison between two groups and a one way ANOVA for comparison between multiple groups, with the false discovery rate correction for multiple comparisons (P < .05) and a threshold of minimum 10 voxels. Statistical analyses of the immunohistochemical stainings were performed using the Kruskal–Wallis test with the Dunn’s correction for multiple comparisons (P < .05). Reported values are mean ± standard error.

3. Results

3.1. BOLD rsfMRI shows pre-plaque stage hypersynchrony in TG2576 mice

BOLD FC was assessed in TG2576 mice before (3, 5 and 8 months) and after Aβ plaque deposition (14 and 18 months). Fig. 1 shows BOLD FC strength matrices of TG2576 and age-matched wild types at each time point. The networks that were assessed are presented in Supplementary Fig. 1, which shows the neurologic ICA components observed in 3-month-old wild-type animals, that is, the hippocampus, default-mode-like (DMN-like), cingulate, frontal, caudate putamen, sensorimotor, frontal-thalamus, and piriform networks. At 5 months, TG2576 mice showed hypersynchronous BOLD FC compared to age-matched wild-type littermates. Starting from 8 until 18 months of age, BOLD FC decreased in TG2576 mice compared to wild-type mice. Fig. 2A shows that the hippocampus network demonstrated a trend toward hypersynchrony of BOLD FC as early as 3 months (P = .07). At the age of 5 months, hyper-synchrony of BOLD FC was more extensive and most significant in the hippocampus (P = .0004). Additionally, hypersynchronous BOLD FC was observed in the DMN-like network (P = .029), and a trend was observed in the cingulate network and frontal-thalamus network. Fig. 2B shows that hippocampus BOLD FC strength was decreased in TG2576 mice compared to wild types at 8 months (P = .044) and 18 months (P < .0001). The evolution of BOLD FC over time is shown for each network in Supplementary Fig. 2. Fig. 2C shows a seed-based analysis of the right hippocampus, which confirmed hypersynchronous BOLD FC in the hippocampus of 5-month-old TG2576 mice (P = .031).

Immunohistochemistry in the hippocampus of 5-month-old TG2576 and wild-type mice demonstrated no detectable increase in astrocytosis (GFAP) or microgliosis (Iba-1; Fig. 3A). Using qPCR, mRNA expression of the following inflammatory markers was assessed, and no difference was observed between TG2576 and wild-type mice (Fig. 3B): Iba-1, arginase-1 (Arg-1), interleukin 1b (IL-1b), macrophage inflammatory protein 2 (MIP-2), interleukin-10 (IL-10), and chitinase 3-like-3 (Chi-3l3). Aβ plaques (thioflavin-S) were not yet detectable (Fig. 3C), but ELISA (Fig. 3C) showed an increase in soluble Aβ load in the TG2576 mice (1520 ± 80 pg/mL) compared to wild-type mice (129 ± 13 pg/mL). Immunohistochemistry of astrocytes (GFAP), microglia (Iba-1), and amyloid plaques (thioflavin-S) is shown for each time point in Supplementary Fig. 3.

To characterize changes in synaptic functioning in 5-month-old TG2576 mice, field recordings of paired-pulse
Fig. 1. BOLD FC matrices in the TG2576 mouse model at pre-plaque and post-plaque stages. This figure shows mean FC strength matrices of wild type (WT) (lower half) versus TG2576 (upper half) mice. The BOLD FC matrices show functional correlation strengths between pairs of brain regions at 3, 5, and 8 (pre-plaque stages) and 14 and 18 (post-plaque stages) months. The color scale represents the strength of the functional correlation. The different brain ROIs and their abbreviations are listed in Supplementary Table 1.
responses and long-term potentiation were performed in the hippocampus CA1 region. No significant differences were observed in basal synaptic transmission by stimulation of the Schaffer collateral-CA1 pathway when comparing TG2576 and wild-type mice (Fig. 3D). Thereafter, paired-pulse facilitation (PPF) was inspected, a phenomenon that reflects presynaptically mediated short-term plasticity. While at most inter-stimulus intervals, no genotype difference was detectable, PPF was significantly decreased in TG2576 mice at 200 ms ($P < .03$) compared to wild types (Fig. 3E). Finally, long-term potentiation was measured, an established correlate of learning and memory at the cellular level, and no significant difference was observed between TG2576 and wild-type mice (Fig. 3F).

3.2. BOLD rsfMRI shows pre-plaque stage hypersynchrony in PDAPP mice

To demonstrate that the observation of hypersynchronous BOLD FC is independent of the hAPP transgenic mouse model, PDAPP mice were subjected to rsfMRI at the age of 3 months, that is, 3–5 months before the appearance of amyloid plaques. The FC matrices in Fig. 4A reveal that PDAPP mice show increased FC compared to their wild-type littermates. Hypersynchronized BOLD FC was most significant in the frontal network ($P = .04$; Fig. 4B). At 7 months, the frontal network showed decreased BOLD FC in PDAPP versus wild-type mice ($P = .037$; Fig. 4C). At the age of 3 months, no Aβ plaques were detected (Fig. 4D), but ELISA showed an increase in soluble Aβ load in PDAPP mice ($721 \pm 44.7$ pg/ml) compared to wild-type mice ($82 \pm 6.3$ pg/ml) (Fig. 4D).

3.3. Neutralizing Aβ prevents BOLD hypersynchrony and synaptic deficits in TG2576 mice

Next, TG2576 mice were treated with the 3D6 anti-Aβ–antibody to further investigate the role of pathologic
Aβ in the observed hypersynchrony of FC. The FC matrices in Fig. 5A show that treatment with 3D6 recovered FC hypersynchrony and FC patterns were rendered comparable to wild-type mice. This was confirmed by seed-based analyses of the right hippocampus (Fig. 5B), which demonstrates hypersynchronous BOLD FC in PBS-treated TG2576 animals compared to wild-type animals (P = .016), whereas the 3D6-treated TG2576 mice showed BOLD FC patterns comparable those in wild types.

The data shown in Fig. 6A confirm hypersynchronous BOLD FC in the hippocampus of PBS-treated TG2576 mice compared to wild types (P < .0001), whereas the TG2576 group that received the 3D6 antibody showed BOLD FC that was comparable to wild-type mice. At 8 months, that is, 3 months after the treatment was concluded,
PBS-treated TG2576 mice showed decreased BOLD FC compared to wild types, whereas 3D6-treated TG2576 mice still showed BOLD FC comparable to wild types. Interestingly, at 10 months, that is, 5 months after the treatment was concluded, PBS-treated TG2576 mice still showed lower levels of BOLD FC compared to wild types, whereas 3D6-treated TG2576 mice demonstrated hypersynchrony of BOLD FC compared to wild types \((P = .003)\).

Additionally, 3D6-treated TG2576 mice displayed a rescue of impaired PPF at 200 ms, whereas the same antibody treatment had no effect in wild-type mice at 5 months (Fig. 6B). Consistent with the findings reported above, PPF of PBS-treated TG2576 mice was significantly reduced at 200 ms \((P = .004)\).

Immunohistochemistry (Fig. 6C) revealed that the density \((\text{puncta} / \mu m^2)\) of VGLUT1 was significantly increased in PBS-treated TG2576 compared to wild types \((P = .02)\), whereas 3D6-treated TG2576 mice were comparable to wild types \((P = .67)\). On the other hand, the density \((\text{puncta} / \mu m^2)\) of GAD65/67 was significantly decreased in PBS-treated TG2576 compared to wild types \((P = .03)\) but not in 3D6-treated TG2576 mice \((P = .99)\). Thus, the ratio of excitatory (VGLUT1) to inhibitory (GAD-65/67) synapses was significantly increased in the hippocampus of PBS-treated TG2576 mice \((P = .01)\), whereas 3D6-treated TG2576 mice showed a VGLUT/GAD-65/67 ratio comparable to wild types \((P = .99)\). Similarly, the ratio of glutamate/GABA neurotransmitters was significantly increased in the hippocampus of PBS-treated TG2576 mice \((P = .02)\), but 3D6-treated TG2576 mice were comparable to wild types \((P = .44; \text{Fig. } 6D)\). Additionally, the density \((\text{puncta} / \mu m^2)\) of the pre-synaptic marker synapsin was significantly increased in PBS-treated TG2576 mice compared to wild types \((P = .04)\) but not in 3D6-treated TG2576 mice \((P = .99; \text{Fig. } 6E)\). The density \((\text{puncta} / \mu m^2)\) of the post-synaptic marker synaptobrevin was not altered.
4. Discussion

This study investigated BOLD FC patterns at early stages of AD progression in transgenic mice. Hypersynchronous BOLD FC was observed before Aβ plaque deposition in two mouse models of amyloidosis, that is, the TG2576 and PDAPP mice. Early treatment of TG2576 mice with an anti-Aβ-antibody prevented hypersynchronous BOLD FC, as well as the associated synaptic impairments and excitatory/inhibitory disbalances.

The TG2576 and PDAPP mouse models of amyloidosis develop Aβ plaques between 9–11 months and 8–9 months, respectively [16,17]. Both mouse models show cognitive disturbances and synaptic deficits before Aβ plaque deposition [6,7,11,21], suggesting that soluble Aβ, rather...
Fig. 6. Treatment with 3D6 prevents FC and synaptic abnormalities in the hippocampus network in 5 months old TG2576 mice. (A) Mean BOLD FC strength in the hippocampus network for PBS-treated wild-type and TG2576 mice, and 3D6-treated wild-type and TG2576 mice at 3, 5, 7, 8, and 10 months of age. Treatment started at 3 months of age and was concluded at 5 months of age. (B) Mean PPF ratio at different inter stimulus intervals (ISI) for PBS-treated wild type and TG2576 mice, and 3D6-treated wild type and TG2576 mice at 5 months. (C) Mean density VGLUT1 (left y-axis), mean density GAD65-67 (left y-axis), and synapsin/synaptobrevin (right y-axis) in the hippocampus network.
than Aβ plaques, are responsible for the observed functional deficits. In the present study, TG2576 mice were characterized in terms of BOLD FC using rsfMRI. The results showed hypersynchronous BOLD FC in the hippocampus of TG2576 mice compared to wild types at age of 5 months, when soluble Aβ was increased, but no Aβ plaques or increased inflammatory responses were detected. Starting from 8 months, BOLD FC decreased in TG2576 mice, which is consistent with the observed hyposynchronous BOLD FC in memory-related regions in AD patients [14] and our previous study in old APP/PS1 mice [15]. The observation of hypersynchronous BOLD FC is consistent with several reports of hyperactive and hypersynchronous neurons associated with Aβ. Busche et al. reported hippocampal hyperactivity and hypersynchrony in mouse models of amyloidosis using two-photon Ca²⁺ imaging and showed that neuronal hyperactivity was caused by increased levels of soluble Aβ [12,22,23]. This observation was confirmed in a recent study in the TG2576 model [24]. Our results suggest that pre-plaque stage pathologic amyloid, which could include an accumulation of soluble Aβ, the overexpression of human APP and/or the accumulation of different APP fragments, is associated with hypersynchrony of BOLD FC.

Additionally, hypersynchronous BOLD FC was confirmed in the frontal network of PDAPP mice at 3 months, that is, when there were no Aβ plaques, but soluble Aβ was increased. These results suggest that the observation of hyper-synchronous BOLD FC itself is not model specific but might be attributed to pre-plaque soluble Aβ, human APP, and/or different APP fragments. Additionally, the pattern of early hypersynchronous BOLD FC and subsequent hyposynchronous BOLD FC at later stages of disease progression, as observed for the TG2576 mice, was also reproduced in the PDAPP mice. The discrepancy in region-specific vulnerability to amyloid pathology between the TG2576 and PDAPP models might be explained by the fact that both mouse models carry a different mutation, that is, the APP KM670/671NL (Swedish) mutation controlled by the hamster prion protein promoter and APP V717 F (Indiana) mutation controlled by the PDGF promoter in TG2576 and PDAPP mice, respectively. Another important difference is that both mouse models are created on different background strains, that is, a mixed C57BL6/SJL and C57BL6/Swiss-Webster/DBA background for TG2576 and PDAPP mice, respectively. A previous study in our group showed that different strains of mice presented a different regional pattern of BOLD FC and glucose metabolism [20]. These differences might result in a different regional vulnerability to amyloid pathology and thus a different spatiotemporal relation between amyloidosis and BOLD FC. In summary, our results strongly suggest that early stages of AD progression are associated with hypersynchrony of BOLD FC networks, before the presence of plaques. Importantly, the present study demonstrates that hypersynchronous neuronal activity, which until now has been reported in several transgenic models of amyloidosis using relatively invasive techniques, can be detected noninvasively, in vivo, with rsfMRI.

It is important to note that although our data show hypersynchronous BOLD FC, Grandjean et al. [25] demonstrated hyposynchronous BOLD FC before Aβ plaque deposition in the arc-Aβ mouse model. This discrepancy could be explained by the fact that they used isoflurane to anesthetize the animals. Isoflurane is a known vasodilator and thus might influence the BOLD signal. Moreover, as isoflurane targets the GABAergic system, it might affect the excitatory/inhibitory disbalances known to occur in amyloidosis models [23]. The fact that we observed hypersynchronous BOLD FC in two different mouse models of amyloidosis is encouraging and suggests that the observation is not model specific. Moreover, it is important to mention that a recent study showed increased BOLD FC in children carrying the presenilin1 (PSEN1) mutation compared to noncarriers [26], which strongly point toward a potential translational value of our findings.

To further investigate the involvement of pathologic Aβ in the observed hypersynchronous BOLD FC, TG2576 mice were treated with the 3D6 anti-Aβ-antibody. 3D6, the murine form of bapineuzimab, recognizes amino acids 1–5 of Aβ. It binds to the N-terminal of Aβ and does not recognize other hAPP fragments [27]. Studies have shown that 3D6 enters the central nervous system on systemic injection [28–30] and binds soluble Aβ [31,32] and improved behavior and synaptic impairments in amyloidosis models [31]. Treatment with the 3D6 antibody rescued hypersynchrony of BOLD FC in TG2576 mice at 5 months. Follow-up of the treated mice until 8 months, so 3 months after the treatment was concluded, showed that early treatment does not only prevent the hypersynchrony of BOLD FC at 5 months but also subsequent hyposynchrony of BOLD FC in TG2576 mice at 8 months of age. Our results strongly suggest that an early treatment might rescue or delay the effect of pathologic Aβ on brain function. A recent study by Busche et al. used two-photon imaging to show that anti-amyloidogenic treatment in TG2576 and PDAPP mice did not recover neuronal hyperactivity but instead
expression of the pre-synaptic marker synapsin in TG2576 pre-synaptic alterations is the observation of increased pulse ratio comparable to wild types. Another indication for Treatment of TG2576 mice with 3D6 rendered the paired synapses [12,22]. Consistent with those findings, our data associated with remodeling of excitatory and inhibitory finally, hypersynchronous firing of neurons has been compensated response for a loss of synaptic plasticity. [40,41]. Another possible explanation is a form of enhance synaptic excitation at the pre-synaptic level [34]. This type of synaptic plasticity is thought to allow neurons to detect input coherence, to maintain stability, and to promote synchronization [35,36]. In addition, it has been shown to play a vital role in important brain functions such as decision making, working memory, and spatial place learning [37–39].}

Additionally, early treatment with the 3D6 antibody recovered synaptic impairments. At 5 months, TG2576 mice showed a reduced paired-pulse ratio, which is indicative of changed presynaptic short-term plasticity [34]. This type of synaptic plasticity is thought to allow neurons to detect input coherence, to maintain stability, and to promote synchronization [35,36]. In addition, it has been shown to play a vital role in important brain functions such as decision making, working memory, and spatial place learning [37–39].

Treatment of TG2576 mice with 3D6 rendered the paired pulse ratio comparable to wild types. Another indication for pre-synaptic alterations is the observation of increased expression of the pre-synaptic marker synapsin in TG2576 mice. Studies have demonstrated that low levels of Aβ enhance synaptic excitation at the pre-synaptic level [40,41]. Another possible explanation is a form of compensatory response for a loss of synaptic plasticity. Finally, hypersynchronous firing of neurons has been associated with remodeling of excitatory and inhibitory synapses [12,22]. Consistent with those findings, our data show an increased ratio of VGLUT1/GAD65-67 synapses and glutamate/GABA neurotransmitters in TG2576 mice, which were rescued in TG2576 mice treated with 3D6.

In conclusion, our data strongly suggest that early stages of AD progression are associated with hypersynchronous BOLD FC patterns before the presence of Aβ plaques. These results provide more insight into the earliest changes that occur in AD and might thus be of interest in terms of early biomarkers. As aberrant BOLD FC patterns were demonstrated using rsfMRI, a noninvasive in vivo imaging technique that is already being used extensively in clinical studies, our data potentially hold high translational value. Moreover, our data suggest that this hypersynchronous FC can be rescued at an early stage, as anti- amyloidogenic treatment prevented hypersynchrony of BOLD FC, suggesting that preventive strategies should be the focus of future treatment studies.

Acknowledgments

The authors wish to thank Elien Theuns (Laboratory of Cell Biology and Histology, University of Antwerp) and Tim Willems (Department of Biology, University of Antwerp) for their technical assistance. This research was supported by the European Union’s Seventh Framework Programme under grant agreement number 278850 (INMiND), by Molecular Imaging of Brain Pathophysiology (BRAINPATH) under grant agreement number 612360 within the Marie Curie Actions-Industry-Academia Partnerships and Pathways (IAPP) program, by the Institute for the Promotion of Innovation by Science and Technology (IWT) in Flanders, by Stichting Alzheimer Onderzoek (SAO-FRA, grant agreements 13026, 1402 and 14010) and by the Fund for Scientific Research Flanders (FWO) (grant agreement G.0D76.14, G.0587.14.). D.S. is holder of an IWT PhD grant (grant agreement 13160). J.P. is holder of an FWO postdoc grant (grand agreement 12G1416N). J.R.D. is holder of an IWT Baekeland grant (grand agreement 140775). Aspects of this work were supported by funding to S.R. from the German Research Council (DFG, grant # RO 2226/13-1) and from the JPND CrossSeeds consortium (BMBF, grant # 01ED1501B).

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jalz.2016.03.010.

RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the literature using traditional sources (e.g., Pubmed). There are several recent publications investigating the effect of pathologic amyloid on neuronal functioning using different techniques, such as electrophysiology and calcium imaging, and these are appropriately cited.

2. Interpretation: First, the article proposes that pathologic amyloid causes early-stage brain network hypersynchrony that can be detected noninvasively and thus might serve as a potential biomarker in AD pathology. These findings are consistent with other preclinical studies that use more invasive techniques. Second, our findings show that preplaque stage pathologic amyloid could be a potential therapeutic target.

3. Future directions: The article proposes a framework for the generation of new treatment hypotheses and the conduct of additional studies. Examples include (1) longitudinal examination of other amyloidosis models, focusing on early stages before amyloid deposition and (2) focus on preventive treatment regimens in amyloidosis models and humans.
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