Complex interplay between brain function and structure during cerebral amyloidosis in APP transgenic mouse strains revealed by multi-parametric MRI comparison

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Abstract

Alzheimer's disease is a fatal neurodegenerative disorder affecting the aging population. Neuroimaging methods, in particular magnetic resonance imaging (MRI), have helped reveal alterations in the brain structure, metabolism, and function of patients and in groups at risk of developing AD, yet the nature of these alterations is poorly understood. Neuroimaging in mice is attractive for investigating mechanisms underlying functional and structural changes associated with AD pathology. Several preclinical murine models of AD have been generated based on transgenic insertion of human mutated APP genes. Depending on the specific mutations, mouse strains express different aspects of amyloid pathology, e.g. intracellular amyloid- β (A β) aggregates, parenchymal plaques, or cerebral amyloid angiopathy. We have applied multi-parametric MRI in three transgenic mouse lines to compare changes in brain function with resting-state fMRI and structure with diffusion tensor imaging and high resolution anatomical imaging. E22 Δ A β developing intracellular A β aggregates did not present functional or structural alterations compared to their wild-type littermates. PSAPP mice displaying parenchymal amyloid plaques displayed mild functional changes within the supplementary and barrel field cortices, and increased isocortical volume relative to controls. Extensive reduction in functional connectivity in the sensory-motor cortices and within the default mode network, as well as local volume increase in the midbrain relative to wild-type have been observed in ArcA β mice bearing intracellular A β aggregates as well as parenchymal and vascular amyloid deposits.

39 Patterns of functional and structural changes appear to be strain-specific and not directly related to
40 amyloid deposition.

41 **Keywords**

42 Mouse, APP, Alzheimer's disease, MRI, resting-state, amyloid

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1. Introduction

Neuroimaging tools, in particular magnetic resonance imaging (MRI), offer a unique window for studying morphological and physiological changes associated with progression of Alzheimer's disease (AD) pathology (Buckner et al., 2005). For example, MRI-based volumetric analyses have revealed a marked volume reduction, especially in the temporal lobe, the rate of which correlated with the rate of cognitive decline of patients (Fox et al., 1999). Recently, the spotlight in MR-related AD research has turned towards functional MRI (fMRI) as characteristic functional alterations have been shown in patients with AD (Greicius et al., 2004), but also in groups at risk of developing the disease such as individuals with mild cognitive impairments (Rombouts et al., 2005; Sorg et al., 2007), cognitively healthy elderly positive for amyloid deposits (Sheline et al., 2010b), and healthy mid-life APO ϵ 4 risk allele carriers (Filippini et al., 2009; Sheline et al., 2010a). Though the results are promising in human studies, the origin of these fMRI/MRI changes in the disease remains poorly understood.

Murine models of AD have revealed many molecular and cellular mechanisms underlying AD pathology. Accumulation and aggregation of amyloid- β (A β) peptide, a cleavage product of the transmembrane amyloid precursor protein (APP), is a pathomorphological hallmark of AD. Not surprisingly, a large number of transgenic models of AD involving overexpression of APP have been developed, each having their specificities in terms of onset, severity and nature of A β accumulation (intracellular aggregates, extracellular parenchymal and perivascular deposits). Characterization of these murine models of AD, which mimic different aspects of the disease, should allow linking structural and functional alterations at a system level to aspects of the pathophysiological processes elicited by APP overexpression. For example, the analysis of functional connectivity (FC) in various APP overexpressing mouse lines showed that alterations in functional networks (Grandjean et al., 2014; Shah et al., 2013), similar to the results human resting-state fMRI studies, precede the intracerebral deposition of amyloid plaques (Grandjean et al., 2014). In addition to functional alterations, a large number of preclinical MRI studies have investigated structural phenotypes in models of AD assessing overall and region specific volume changes (Allemang-Grand et al., 2015; Badhwar et al., 2013; Grand'maison et al., 2013; Lau et al., 2008; Maheswaran et al., 2009; Redwine et al., 2003; Van Broeck et al., 2008) or changes in white matter structure (Grandjean et al., 2014; Shu et al., 2013; Song et al., 2004; Zerbi et al., 2013). Despite the growing body of information revealing characteristic structural and functional alterations in murine models of cerebral amyloidosis, linking these measures to the molecular and cellular aspects of the pathology in these mice remains an unresolved issue. This is due to lack of molecular and cellular specificity of MRI/fMRI readouts, and biological complexity, i.e. the actual phenotype being the result of multiple pathophysiological processes co-occurring in these mice, which are specific to each model. For instance, the ArcA β model (Grandjean et al., 2014) is characterized by intracellular amyloid deposits, extracellular plaques, as well as marked vascular pathology (Klohs et al., 2011; Knobloch et al., 2007a; Merlini et al., 2011), thus rendering it difficult to attribute the fMRI changes to a specific cause. Yet, comparing different mouse models that differ in the nature of cerebral amyloidosis, e.g. in the extent and cellular location of amyloid aggregates, might yield information on the relevance of these various deposits in determining the macroscopic phenotype.

The objective of this study was therefore to investigate phenotypic structural and functional alterations in three mouse models of cerebral amyloidosis comprising APP mutations described in familial onset AD. We compared the ArcA β , the E22 Δ A β , and the PSAPP model, each strain expressing different aspects of amyloid pathology. ArcA β mice, which over-expresses the human gene encoding for APP (hAPP) with the Swedish mutation (K670N+M671L) and the Arctic mutation (E693G) develop cognitive deficits by 6 months of age, intracellular A β aggregates at 3 months, followed by the formation of parenchymal and vascular amyloid deposits (Knobloch et al., 2007b). The E22 Δ A β model carries the Swedish and the Osaka mutation (E693 Δ). In this model, cognitive deficits were observed as early as at 3 months of age along with the formation of intracellular plaques. However, no extracellular plaques were observed in this model except in very old (24 month-old) animals (Kulic et al., 2012). Finally, the PSAPP mice overexpresses both the APP encoding gene with Swedish mutation and the PS1 gene with the Δ E9 mutation leading to cognitive decline, and mainly extracellular parenchymal amyloid plaque deposition occurring by 6 months (Borchelt et al., 1997; Jankowsky et al., 2004). We opted for a multi-parametric strategy comparing several fMRI/MRI read-outs which, in their combination should yield a more comprehensive and discriminative characterization of the models as compared to each readout on its own (Klohs et al., 2013). Parameters assessed were FC based on resting-state fMRI, volumetric measurements derived from voxel-based morphometric analysis of structural MRI data, and analysis of anisotropic diffusion properties (diffusivity, fractional anisotropy) derived diffusion tensor imaging. Comparing results obtained from mice with only intracellular deposits (E22 Δ A β), mainly extracellular deposits (PSAPP) and a line displaying both intra- and extracellular in combination with vascular deposits (ArcA β) should indicate the contributions of the various amyloid forms to the functional and structural phenotype.

109 2. Material and methods

110 2.1 Animal preparation

111 All experiments were performed in accordance to the federal guidelines for the use of animals in
112 research, and under a license from the Zürich Cantonal veterinary office. ArcA β and E22 Δ A β strains
113 were developed in-house, PSAPP breeders were obtained from Jackson laboratory (Maine, USA).
114 Both ArcA β and E22 Δ A β strains were generated on hybrid C57Bl/6 and DBA/2 background, and
115 crossed to C57Bl/6 background for at least 5 generations. PSAPP mice were generated on hybrid
116 C57Bl/6 and C3H background and crossed to C57Bl/6 background for at least 10 generations. Group
117 size for each of the lines tested was between 9 and 14, as listed in table 1. Efforts were made to
118 equilibrate gender; however equal distribution was not feasible in all cases due to limitations in animal
119 breeding and maintenance. Animals were housed in standard-house, with food and water *ad libido*,
120 and 12h day and night cycle. In preparation for the MRI scan, anesthesia was induced with 3.5%
121 isoflurane. The animals were endotracheally intubated, positioned on a MRI-compatible cradle, and
122 artificially ventilated at 80 breath per minute, 1:4 O₂ to air ratio, and 1.8 ml/h flow (CWE, Ardmore,
123 USA). During preparation, isoflurane was reduced to 2%. The tail vein of the animal was cannulated to
124 administer medetomidine and muscle relaxant. A bolus injection of medetomidine 0.05 mg/kg and
125 pancuronium bromide 0.2 mg/kg was administered, and isoflurane was reduced to 1.5%. After 5
126 minutes, an infusion of medetomidine 0.1 mg/kg/h and pancuronium bromide 0.4 mg/kg/h was
127 administered, and isoflurane was further reduced to 0.5%. The animal temperature was monitored
128 using a rectal thermometer probe, and maintained at 36.5°C $\pm$ 0.5 during the measurements. Animal
129 preparation and data acquisition took 45 minutes. Following MRI measurements, the animals were
130 deeply anesthetized with Ketamine (100 mg/kg) / Xylazine (10 mg/kg) / Acepromazine maleate (3
131 mg/kg), and transcardiacally perfused with Phosphate-buffered saline (PBS).

132 2.2 Immunohistology

133 Histological stainings were performed on 5 μ m sagittal paraffin sections according to standard
134 procedures. For the detection of amyloid deposits, antigen retrieval treatments were carried out by
135 placing the sections in 95% formic acid for 5 min, followed by boiling the sections in 10mM sodium
136 citrate buffer. After washing steps in PBS the sections were incubated in 3% H₂O₂ solution to eliminate
137 endogenous peroxidase activity, blocked in 10% horse serum and incubated with monoclonal 6E10
138 antibody (1:200 dilution; Convance) overnight at 4°C. After additional washing steps in PBS, sections
139 were incubated with secondary antibody obtained from Vector laboratories (Vectastain Elite ABC Kit)
140 for 3,3'-diaminobenzidine peroxidase stainings. For the immunostainings for glial fibrillary acidic
141 protein (GFAP), ionized calcium binding adaptor molecule (Iba1), synaptophysin, and myelin basic
142 protein (MBP), the sections were pretreated by boiling them in 10mM sodium citrate buffer, followed by
143 washing steps in Tris-buffered saline containing 0.2% Triton X-100 (TBS-T). The sections were
144 blocked for 1 h at room temperature in TBS-T containing 10% donkey serum and incubated with
145 primary antibodies (GFAP 1:200; Acris, Iba1 1:200; Wako Chemicals, Synaptophysin 1:200; Abcam,
146 MBP 1:200; Abcam) at 4°C overnight. Finally the sections were washed in TBS-T and incubated in
147 fluorophor-conjugated secondary antibodies (1:400) for 2 h at room temperature.

Data acquisition was performed on a Bruker 94/30 Biospec spectrometer (Bruker BioSpin MRI, Ettlingen, Germany) equipped BGA-S gradient system, a linear volume resonator coil for transmission, and a 2x2 phased-array cryogenic surface receiver coil. Images were acquired using Paravision 6 software. Triplot images were acquired to ensure proper positioning of the animals with respect to the coil and the magnet isocenter. Shim gradients were adjusted using mapshim protocol, with an ellipsoid reference volume covering the whole cerebrum. Functional imaging was performed with gradient-echo echo planar images that were acquired with repetition time TR=1000ms, echo time TE=9.2ms, flip angle FA=90°, matrix size MS=90x70, field of view FOV=20x17.5 mm², in-plane resolution PR = 0.22 x 0.25 mm², number of slice NS=12, slice thickness ST=0.5mm, slice gap SG=0.2mm, and bandwidth BW=250000.Hz, 360 volumes. 3D volumetric scans were acquired with a spin echo RARE sequence (Hennig et al., 1986) , TR=2000ms, TE=48.6ms, MS 150x200x80, FOV=15x20x8mm³, PR = 0.1 x 0.1 x 0.1 mm³, FOV saturation slice positioned on the inferior portion of the head. Diffusion tensor imaging (DTI) was acquired with multi-shot DTI-EPI sequence, 4 segments, TR=2000ms, TE=22ms, FA=90°, MS=128x128, FOV=20x17.5 mm², PR = 0.15 x 0.13 mm², NS=20, ST=0.3mm, SG=0.15m, 5 volumes acquired with b=0s/mm², b-value=1000 s/mm², 36 direction encoding.

2.4 Data processing

The data used in this study is made freely available in NIFTI format on XNAT data repository (project_ID: fMRI_AD_mouse2)

FMRI images were first temporally realigned using 3dvolreg (AFNI 2011_12_21_1014, <http://afni.nimh.nih.gov>), non-brain regions were removed using Bet (FSL 5.1, <http://fsl.fmrib.ox.ac.uk>), and then directly co-registered to the Australian Mouse Brain Mapping Consortium (AMBMC) MRI template using ANTS (ANTS 2.1.0, <http://picsl.upenn.edu/software/ants>). Temporal artifacts in the fMRI signal were removed using FMRIB's ICA-based Xnoiseifier (FIX) as in Zerbi et al. (2015). A set of 17 components extracted from a previous group-independent component analysis (Zerbi et al., 2015) was used as a spatial reference for FSL Dual Regression analysis (DR) for within-component and FSLNets for between-component analysis. To account for the poor sensitivity of the DR approach at the voxel-wise level, the component maps were used as ROIs to extract individual network strength from the individual network maps estimated during DR. Between-component interaction strength was computed with normalized full correlations.

Structural images were converted to MINC format, were iteratively registered, firstly to a reference image using 6 degrees of freedom transform, secondly by 12 degrees affine transformation between each individual images pairs, thirdly with 3 sequential iterations of non-linear transformation between each image pairs (Lerch et al., 2008), using ANTS for the non-linear transformation. Region of interest analysis was performed using a set of ROI derived from the Allen Mouse Brain atlas (Allen Institute for Brain Science, <http://atlas.brain-map.org>). Voxel-wise analysis from voxel-based morphometry (VBM) analysis was performed on the scaled-Jacobians, blurred at 0.2 mm full widths at half maximum.

Diffusion tensor images were corrected for eddy current and parameters reconstructed in FSL. The b0 images were co-registered to an *in-house* reference template using ANTS. The computed transformations were applied to the fractional anisotropy (FA) maps and the three eigenvalues. Axial diffusivity (D_{ax}) was derived from the first eigenvalue, while radial diffusivity (D_{rad}) maps were generated by averaging the last two eigenvalues. FA, D_{ax} , and D_{rad} values were extracted using a mask with ROI placed on selected white matter fibers.

2.5 Statistical analysis

We use 'strain' to refer to the 3 lines (ArcA β , PSAPP, and E22 Δ A β) and 'genotype' to discriminate between transgenic (TG) and wild-type (WT) littermate control animals within a strain. For the statistical analysis we combined the genotype and strain factors to obtain a single factor with 6 levels, ArcA β WT, ArcA β TG, PSAPP WT, etc. We created a design matrix modeling the genotype and strain effect, as well as gender effect. We applied 3 sets of contrast to test for the following hypothesis: ArcA β TG different from ArcA β WT, PSAPP TG different from PSAPP WT, E22 Δ A β TG different from E22 Δ A β WT. Voxel-wise statistical analysis for the DR, FSLNets, VBM, and FA analysis were established with permutation testing using FSL Randomize and 5000 permutations. Statistical analysis on the parameters extracted from the ROIs analysis from DR, VBM and DTI was performed in R (3.0.1, The R Foundation for Statistical Computing, Vienna, Austria) using a linear model and contrast analysis using multcomp package. The FA values were corrected with Fisher's z transform for bounded values prior to statistical analysis. During the first establishment of the model, a selection of parameters from the DR, VBM, and DTI analysis were used. Models with either genotype/strain and gender factors or genotype/strain factor alone were compared using likelihood test ratio. The assumption of normal distribution of the residuals was tested with QQ-plots to inspect normal distribution, Tukey–Anscombe plots for the homogeneity of the variance and skewness, and scale location plots for homoscedasticity (i.e., the homogeneity of residual variance). The assumption of a normal distribution was considered plausible for all 3 modalities. The presence of a gender effect could not be excluded in many of the variable tested and was therefore included in the final model. In order to account for a potential gender effect on the pathology, a gender x genotype interaction effect was modeled, but no significance of this effect could be established. Because there were notable differences in the parameters between the difference strains, the analysis was restricted to TG and WT littermates within each strain. Voxel-wise analyses were corrected with threshold-free cluster enhancement (TFCE) correction (Smith and Nichols, 2009). Statistical maps from the DR analysis were further corrected between resting-state networks with Bonferroni correction ($p \leq 0.05/17$). FSLNets and ROI analysis for local brain volumes were corrected for multiple comparisons with false discovery rate (FDR). The null hypothesis, i.e., the absence of a difference between TG and WT animals for a given strain, was rejected at the threshold set by the correction method and is denoted in the text as "genotype effect" for simplicity.

3 Results

3.1 Group description

Animals were aged 373 to 487 days when undergoing the MRI/fMRI procedure (Table 1). None of the animals presented observable comorbidities or obvious abnormalities in the MR anatomical images. There was no significant weight difference between the strains or between the genotypes, however there was a significant gender effect in the weight distribution of the animals denoting an increased weight in males compared to females ($p\text{-value}=9.1\times 10^{-10}$). Histological analysis confirmed the presence of amyloid-related pathology in each strain. TG ArcA β mice presented intracellular deposits, parenchymal and vascular amyloid plaques (Figure 1 A, D), while PSAPP mice only presented parenchymal amyloid plaques in the cortex and hippocampus (Figure 1 B, E). PSAPP mice also presented vascular amyloid deposits around pial vasculature (Figure S1). E22 Δ A β presented only intracellular deposits (Figure 1 C, F). Neuroinflammation was assessed with GFAP histological staining for astrocytes and Iba1 for microglia (Figure S2). PSAPP TG mice presented highest level of both cortical and hippocampal inflammation, followed by ArcA β mice. E22 Δ A β mice displayed limited number of cells positive for either marker. Histological staining for synaptic density (Figure S3 A) and myelin (Figure S3 A) indicated no noticeable differences in the hippocampus and cortex between the transgenic animals from the three strains. There was also no noticeable differences in myelin basic protein marker in minor forceps between strains (Figure S3 C).

3.2 Functional network analysis

Within-component FC was estimated by extracting the individual temporal signal from a given group-level component reference map, which was then regressed into the time series from the scans to obtain individual-level functional network maps. Within-component ROI analysis highlighted significant decrease network strength in motor ($p\text{-value}=0.0022$), supplementary ($p\text{-value}=6.2\times 10^{-6}$), barrel field 1 ($p\text{-value}=0.0043$) and 2 ($p\text{-value}=0.022$), limb ($p\text{-value}=0.01$), auditory ($p\text{-value}=1.8\times 10^{-7}$), prefrontal ($p\text{-value}=6.4\times 10^{-8}$), and cingulate ($p\text{-value}=1.2\times 10^{-7}$) cortical components, ventral hippocampus ($p\text{-value}=0.014$), dorsal ($p\text{-value}=0.011$) and ventral ($p\text{-value}=0.01$) striatum in the ArcA β TG compared to WT (Table S2). In PSAPP mice, a significant decrease compare to WT littermates was only observed in the supplementary ($p\text{-value}=0.036$) and barrel field 1 ($p\text{-value}=0.038$) cortices, while there was no significant difference between E22 Δ A β and WT mice in the ROI analysis. Network strength for all three strains are illustrated for supplementary, barrel field 1, and cingulate cortices, as well as ventral hippocampus (Figure 2 A). In order to test for the localized functional alterations within the components, voxel-wise statistical comparison of the individual network maps was conducted. Significant voxel clusters denoting decrease in FC have been identified within the motor, supplementary, limb, visual, auditory, prefrontal, and cingulate cortical components, ventral hippocampus, and dorsal striatum of ArcA β TG compared to WT (Figure 3 A), while there were no voxel clusters surviving Bonferroni correction when comparing PSAPP or E22 Δ A β TG mice to their respective WT littermates.

Network analysis was used to explore the functional alterations between-components. The group-level network maps between the WT of the 3 strains showed qualitative similarities (Figure 4 A, B, and C). Frontal sensory-motor cortical components such as motor, supplementary, barrel field 1 and 2 cortices, revealed the strongest correlations among each other. Also, areas typical of the human DMN default-mode network (DMN) such as prefrontal and cingulate cortices, dorsal and ventral hippocampus, also displayed strong inter-regional correlations. Similarly, sub-cortical components including dorsal, lateral and ventral striatum, and amygdala were found clustered together. Statistical analysis revealed a significant genotype effect in ArcA β mice between the supplementary cortex toward the barrel field 1 (p-value=0.043), prefrontal (p-value=0.037), and cingulate cortices (p-value=0.025), and between the barrel field 1 cortex towards prefrontal cortex (p-value=0.0001) and lateral striatum (p-value=0.027), and finally between the prefrontal and cingulate cortices (p-value=0.014) (Figure 4 D). There was no interaction surviving FDR correction in PSAPP and E22 Δ A β mice (Figure 4 E, F). Interaction strength for interactions between the barrel field 1 cortex towards the prefrontal cortex, and between the prefrontal cortex toward the cingulate cortex are shown in Figure 2B, revealing a significant increase in correlation between the barrel field and the prefrontal cortex in ArcA β TG mice compared to WT and a decrease between the prefrontal and cingulate cortex. For the latter interaction, a non-significant trend towards a decrease has been observed in PSAPP and E22 Δ A β TG mice as compared to the corresponding WT littermates.

3.3 Voxel-based morphometry analysis

Voxel-wise analysis from the VBM analysis revealed multiple alterations between WT and TG animals in ArcA β and PSAPP mice (Figure 5); however, there was no significant genotype effect in the E22 Δ A β strain. ArcA β mice presented increased lateral and third ventricles, local increase in volume in the hippocampi, basal forebrain, and in regions spanning over the periaqueductal grey and locus coeruleus (Figure 5 A). These local increases were accompanied by local volume decreases in the anterior cingulate cortex and the cerebellum. The TG PSAPP mice showed local volume increases covering large domains of the isocortex, and only few and confined areas displaying a volume decrease in midbrain and brainstem (Fig 5 B). ROI analysis indicated no significant genotype effect in the total brain volumes in any of the strains, however, there was a significant gender effect as tested with likelihood test ratio (p-value=0.0051), and a significant strain effect in a post-hoc test comparing only the WT animals of each strain (p-value=0.018) (Figure 5 C). Comparison for genotype effects in each of the ROI for all three strains yielded results comparable to the voxel-wise analysis. There were increased volume in the hippocampus (dentate gyrus, CA1, CA2, CA3), lateral and basolateral amygdalar nucleus, and in specific midbrain ROIs such as the periaqueductal grey in ArcA β TG compared to WT (Figure S4). PSAPP mice displayed a general increase in cortical volume, which was found to be statistically significant for insular, prelimbic, primary somatosensory, visual, orbital, and somatomotor areas as compared to WT littermates (Figure S5). No significant volume difference surviving FDR correction has been detected for any of the ROIs in E22 Δ A β TG mice (Figure S6). The volume extracted from ventricle (Figure 5 D), barrel field cortex (Figure 5 E), and periaqueductal grey (Figure 5 F) ROIs illustrate absolute changes for the different strains, e.g. increased volume in the

ventricles (p-value=0.001) and periaqueductal grey (p-value= 3.9×10^{-5}) in ArcA β TG mice, and increased volume in the barrel field cortex in PSAPP TG mice (p-value=0.003).

3.4 Diffusion tensor imaging analysis

Voxel-wise analysis of FA did not highlight any significant voxels surviving the TFCE threshold. ROI analysis on a selection of white matter regions (Figure 6 A) indicated a significant increase in FA in the minor forceps in ArcA β TG animals (Figure 6 B, p-value=0.02), and a significant decrease in the external capsule in PSAPP mice (Figure 6 C, p-value=0.0093). Analysis of axial and radial diffusivity analysis suggests that the increase in FA in the minor forceps may be related to an increase in D_{ax} rather than a decrease in D_{rad} (Table 2, D_{ax} : p-value=0.017; D_{rad} : p-value=0.99). In the external capsule in PSAPP, changes in FA could neither be attributed to D_{ad} or D_{rad} (Table 2, D_{ax} : p-value=0.83; D_{rad} : p-value=0.3). ROI analysis did not yield any significant genotypic effects for the E22 Δ A β strain. In order to test if the changes in FA were associated with alterations in FC, within-component network strengths in the supplementary and cingulate cortical components were compared to FA values extracted from the external capsule and cingulum, respectively, the fibers closely associated with each of the cortical regions. For both regions we found no or only relatively weak correlations between structural and functional connectivity measures ($r=0.02$, and $r=0.07$ respectively).

4 Discussion

Preclinical imaging studies in models of AD hold the promise to highlight potential mechanisms underlying clinically used phenotypic neuroimaging markers. Cerebral amyloidosis is a pathological hallmark of the disease and is therefore appealing to link the presence of amyloid peptides in different forms to the observed functional and structural abnormalities. Based on our results obtained in three mouse lines displaying different types of amyloid aggregates we conclude that intracellular amyloid pathology appears not to be sufficient to elicit a functional or structural phenotype at a whole-brain level. In contrast, mice with extracellular pathology present significant functional changes, which were further exacerbated by the occurrence of cerebral amyloid angiopathy. Nevertheless, associating phenotypic MRI changes just to the presence of extracellular parenchymal and vascular amyloid deposits would certainly constitute an oversimplification as additional factors have to be considered such as, level of transgene expression and of soluble A β and A β -oligomers, as well as cumulative exposure to the transgene products over time.

4.1 Intracellular amyloid aggregates do not alter function or brain structure at macroscopic level

A major result of this study is the identification of significant differences among these three mouse lines regarding functional networks and macroscopic brain morphology. In particular, we could not identify any significant alterations in any of the parameters examined in E22ΔAβ mice as compared to their WT littermates despite the presence of extensive intracellular amyloid deposits and reported behavioural deficits. The Osaka mutation is associated with AD-like symptoms in human carriers (Tomiyama et al., 2008). Similarly, cognitive deficits have been reported for the E22ΔAβ mouse model (Kulic et al., 2012). In addition, mice overexpressing APP with the Osaka but not the Swedish mutation showed activation of microglia and abnormal Tau protein phosphorylation (Tomiyama et al., 2010) and impaired synaptic activity in hippocampal slices in the presence of the mutated Aβ peptides (Takuma et al., 2008). Based on these reports significant functional and macroscopic structural changes should be expected. The absence of such abnormalities is surprising, and may indicate that the effects of Aβ oligomers and intracellular deposits on behaviour and memory may be below the detection threshold of the fMRI/MRI read-outs. Alternatively, there might not be a direct coupling between behavior and FC readouts, as well as between behavior and structure, which would explain the absence of marked changes in the parameters recorded despite the behavioural changes.

4.2 Shared patterns of functional and white matter alterations between PSAPP and ArcAβ

Both the ArcAβ and PSAPP mice presented functional alterations in TG animals compared to WT, predominantly reduced in the sensory cortex and in elements of the DMN. Direct comparison between the two strains is rendered difficult by the potential difference in response to anesthetic and other strain-specific variables, which are likely to affect the FC read-out; however, it appears that the ArcAβ presents a more pronounced pattern of alteration with respect to WT littermates compared to the PSAPP mice. Both strains present aggressive amyloid pathology reflected by a high parenchymal plaque burden, but ArcAβ mice display in addition marked vascular pathology (Klohs et al., 2012; Klohs et al., 2011; Knobloch et al., 2007b; Merlini et al., 2011), which may further affect the hemodynamic response measured with fMRI (Princz-Kranz et al., 2010) and thus the FC metrics. Neuroinflammation has been proposed as a factor influencing FC, however, ArcAβ TG mice presented more sticking functional alterations than PSAPP, while presenting less evidence for neuroinflammation, suggesting the latter may not be directly linked to functional alterations. Further, there is evidence that the two strains also differ in the development of FC alteration from a young age. Bero et al. have used intrinsic optical imaging to study FC in the PSAPP model at 3 months and 11 months age and found increased FC in young TG animals in regions that later in life became dominant sites of amyloid plaque formation (Bero et al., 2012). In contrast, in the ArcAβ mice we found widespread functional alterations occurring over a time span from 2 to 21 months indicating that these mice fail to normally develop functional connectivity within the first months of adulthood and may not be directly related to amyloid deposition (Grandjean et al., 2014). The additional vascular pathology in ArcAβ mice might further compromise FC readouts based on the BOLD mechanism: perivascular Aβ deposition is likely to impair neurovascular coupling and thus the amplitude and temporal characteristic of the BOLD response. This notion is supported by previous observation of impaired vascular reactivity (Princz-Kranz et al., 2010) and reduced vascular density (Ielacqua et al., 2016) in these mice.

In addition to functional changes, studies in PSAPP and ArcA β mice indicate differential white matter changes that also depend on the age of the mice. Nevertheless there appear to be some general features regarding these changes: A general reduction of FA in the external capsule of PSAPP (Song et al., 2004) and ArcA β mice (Grandjean et al., 2014) has been reported, which is related to an increase D_{rad} indicative of demyelination processes (Song et al., 2004). Further, we found increased FA in the minor forceps in both the PSAPP and the ArcA β strain (Grandjean et al., 2014; Shu et al., 2013), which can be attributed to an increase in D_{ax} . While an decrease in D_{ax} is recognized as a marker for axonal injury (Song et al., 2004), it is unclear how to interpret an increase. Histological staining did not bring evidence for specific underlying causes for altered water diffusivity. Eventually, pruning of non-oriented fibers may explain such changes.

4.3 Strain-specific local volumetric alterations

The extent and rate of cerebral atrophy derived from brain volumetric analysis are considered useful clinical markers of disease progression (Fox et al., 1999). Not surprisingly, volumetric changes have also been extensively investigated in APP TG animals. The structural phenotype reveals large heterogeneity across the three mouse lines considered in this study. While we could not identify a genotype effect in the E22 Δ A β strain, ArcA β mice showed reduced cingulate/retrosplenial cortical volume, enlarged ventricles, and increased volume in nuclei located in the midbrain and pons, similar to observations in this strain at 5 months, prior to the onset of extracellular amyloid pathology (Grandjean, 2014). The PSAPP mice present mostly enlarged isocortex, and few foci of volume decrease. The phenotype of the PSAPP is comparable to that of the APP/J20 model (Swedish and Indiana mutations, V717F) with thicker cortex at 3.5 month (Grand'maison et al., 2013), overall larger isocortex and amygdala throughout life time, but smaller dentate gyri and external capsule by 6 months of age (Badhwar et al., 2013). Similarly, the TgCRND8 line (Swedish and Indiana mutations) was reported to have an enlarged cortex throughout its life span (Allemand-Grand et al., 2015). Apart from these similarities there are also major dissimilarities across the strains. For example, PDAPP mice (Indiana mutation, V717F) present an overall reduction in total brain volume, as well as in the hippocampi and corpus callosum, detectable from 3 months on (Redwine et al., 2003). In contrast, the TASTPM model harboring both mutations in APP (Swedish mutation) and PS1 (M146V) is characterized by enlarged whole brain and hippocampi observable already at 6 months of age (Maheswaran et al., 2009). The APP-Au (Austrian mutation, T714I) model, similar to E22 Δ A β mice, displays only intracellular amyloid deposits, but displays a smaller cortex and cerebellum in animals at 12 and 20 months compared to strain carrying the human WT APP (Van Broeck et al., 2008), while there was no effect on brain volume in E22 Δ A β .

The heterogeneity of the local volume changes among strains needs to be interpreted with caution. It has been speculated that elevated APP levels may have an effect on the rate of local region growth in adult mice. For instance, Maheswaran et al. (Maheswaran et al., 2009) reported a link between transgene expression, age, and volume increase over time in most of the ROIs considered and attributed this effect due to space occupancy by amyloidosis and astrogliosis. This however may not be generalized to other strains, for instance, we observed the same structural phenotype in ArcA β mice aged 5 months, prior to the onset of parenchymal amyloidosis onset (Grandjean, 2014), as those

reported in 12 months old animals, thus suggesting that volume changes may not be related to amyloidosis. Similarly, Grand'maison et al. and Allemang-Grand et al. reported a significant increase in cortical volume in APP/J20 and TgCRND8 compared to WT prior to the onset of amyloid deposition (Grand'maison et al., 2013), and as early as 1.5 week after birth (Allemang-Grand et al., 2015), respectively. This latter observation suggests rather a neurodevelopmental effect of elevated levels of APP, its cleavage products, or the indirect effects related to APP overexpression such as altered cellular homeostasis or effects on other signaling pathways, as a result of the transgene being constitutively expressed. This may lead to compensatory processes and APP mediated effects occurring at a very early stage in life affecting the developing brain, which may be particularly susceptible to the presence of potentially toxic molecular entities. These observations regarding brain volumes are consistent with our previous results of functional changes in the ArcA β model in the first months after birth suggesting impaired neuromaturation, i.e. the failure to develop strong FC. Interestingly, we could not detect any decrease in FC with increased amyloid burden in these animals, which might hint at soluble forms of amyloid peptides being responsible for functional deficits (Grandjean et al., 2014).

4.4 Translational relevance for Alzheimer's disease

In sporadic AD there is a poor correlation between cerebral plaque burden and functional impairments, with amyloid deposition being preceded by the occurrence of functional alterations in groups at risk of developing the disease (Filippini et al., 2009). Similarly in studies with APP overexpressing mice, there is a poor correlation between FC changes and plaque load, with functional and structural changes occurring early in life long before solid amyloid aggregation (Allemang-Grand et al., 2015; Grandjean et al., 2014). This indicates a neuromaturation deficit in models involving constitutive APP overexpression which may not relate directly to late onset AD, but rather to familial early onset AD. In a recent study, teenagers aged 9 to 17 years and carriers of the PS1 E280A mutation showed increased FC in the cingulate cortex and increased cortical volume in temporal regions (Quiroz et al., 2015). These results present striking similarities with the FC results in young PSAPP mice from Bero et al. (Bero et al., 2012), and the volumetric changes in PSAPP mice from this study as well as from earlier reports in APP/J20 (Grand'maison et al., 2013) and TgCRND8 mice (Allemang-Grand et al., 2015). These observations become even more relevant as both a locally increased FC in PSAPP mice (Bero et al., 2012) and a local increase in cortical volume in APP/J20 (Grand'maison et al., 2013) were found predictive of local amyloid plaque burden later in life, suggesting a plausible causal relationship between functional impairments and cortical developments and amyloid pathology. It remains to be determined to what extent these alterations in these strains are shared by the human mutation carriers, as PS1 mutation may result in phenotypic changes independent of APP processing. The link between APP TG mice to sporadic late-onset AD remains harder to establish. While APP TG models have been of great scientific value to investigate the role of APP processing at molecular and cellular levels, and to assess therapeutic target engagements, many of the large-scale functional and structural observations discussed above do not seem to recapitulate the specific pathological landmarks of late-onset AD.

5 Conclusions

We have analyzed the phenotypic implication of APP overexpression in the brains of three genetically different mouse lines using multi-parametric MRI methods to link specific functional and structural signatures to pathological elements specific to the genetic variant. Our results indicate strain-specific alterations in local brain volumes, white matter structures, and FCs. Intracellular A β accumulation in the E22 Δ A β mice did not lead to any measurable alterations in any of the readouts, in contrast to mice bearing parenchymal and vascular A β deposits. However, the fact that functional changes occur prior to the onset of amyloid plaque formation indicates that the effects may not simply be due to the presence of extracellular amyloid deposits. Instead, the presence of soluble factors, including strain- and mutation-specific oligomeric A β species and/or overexpression of specific APP mutants, and their cumulative effect during development, might account for the striking differences between the transgenic strains studied. Conclusion derived from mouse models of cerebral amyloidosis need to be interpreted with caution and are rather of relevance with regard to the rare cases of familial AD than for the much more common sporadic form of the disease.

Acknowledgements

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477 **Table Legends**

478 **Table 1 | Group description**

479 The gender distribution, age and weight are indicated for the 3 strains evaluated in transgenic (TG)
480 and wild-type (WT) groups. Statistics are indicated as mean ± standard deviation.

481

482 **Table 2 | Water diffusion properties in white matter fibers**

483 Descriptive statistics for fractional anisotropy (FA), axial diffusivity (D_{ax}), and radial diffusivity (D_{rad})
484 parameters are shown for regions of interest positioned in the minor forceps and external capsule.
485 Significant genotype effects for a strain are highlighted in bold. Transgenic ArcA β mice present a
486 significant increase in the minor forceps for both FA ($p=0.02$) and D_{ax} ($p=0.017$). Transgenic PSAPP
487 present a decrease in FA in the external capsule ($p=0.009$), however no significant difference in either
488 D_{ax} or D_{rad} . D_{ax} and D_{rad} are indicated in [$10^{-3}\text{mm}^2/\text{s}$]. Values are given as as mean ± standard
489 deviation.

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491

492

493 **Figure Legends**

494 **Figure 1 | A β deposition**

495 Immunostainings with a monoclonal 6E10 antibody against A β showed punctate intracellular amyloid
496 deposits (arrow-head), extracellular plaques (asterisk) and vascular amyloid aggregates (arrow) in the
497 ArcA β model in the hippocampus (A), as well as in the cortex (D). In contrast, PSAPP mice showed
498 primarily extracellular plaques (B+E). The E22 Δ A β model demonstrated only intraneuronal amyloid
499 deposits in both, the hippocampus (C) and the cortex (F). Scale bars: 40 μm (A-C) and 125 μm (D-F).

500

501 **Figure 2 | Functional connectivity analysis**

502 Within-component analysis reveals decreased network strength in transgenic ArcA β mice relative to
503 littermate wild-type controls in the supplementary ($p\text{-value}=6.2\times10^{-6}$), barrel field ($p\text{-value}=0.004$), and
504 cingulate ($p\text{-value}=1.2\times10^{-7}$) cortical components and in the ventral hippocampus ($p\text{-value}=0.01$). In
505 PSAPP mice, a significant decrease was observed in the supplementary ($p\text{-value}=0.03$) and barrel
506 field ($p\text{-value}=0.03$) cortical component. No differences reach the threshold for significance in E22 Δ A β
507 for any of the components considered (A). Between-component analysis reveals increased interaction
508 strength between the barrel field and prefrontal cortical component in transgenic ArcA β mice relative
509 to wild-type littermates specifically ($p\text{-value}=0.0001$), and a decrease in interaction strength between

the prefrontal and cingulate component in ArcA β mice too (p-value=0.014). No significant differences surviving false discovery rate correction in transgenic relative to wild-type mice were observed for either PSAPP or E22 Δ A β (B). Group-level reference components are shown as overlay on the AMBMC MRI template. Bar plots show group mean $\pm$ 1 SD. * p $\leq$ 0.05, ** p $\leq$ 0.01, *** p $\leq$ 0.001.

Figure 3 | Voxel-wise functional connectivity analysis

Voxel-wise analysis of within-component network strength reveal clusters of voxels presenting a significant genotype effect denoting a decrease in transgenic ArcA β compared to wild-type littermates in several components, namely the motor, supplementary, limb, visual, auditory, prefrontal, and cingulate cortices, ventral hippocampus, and dorsal striatum. Significant t-statistics are shown as color-coded overlays on the AMBMC MRI template.

Figure 4 | Between-component network analysis

Group average functional networks maps for ArcA β (A), PSAPP (B), and E22 Δ A β (C) mice, showing the normalized full correlation values for the transgenic group in the top right corner and the littermate wild-type controls in the bottom left corner. Network maps across the three strains are comparable, presenting clusters between frontal sensory-motor components (SSc, B1c, B2c), between elements of the default-mode network (Pfc, Cg, dHc, vHc), and between sub-cortical components (dStr, lStr, vStr, Amg). Statistical are shown as significant p-value following false discovery rate correction in the top right corner, and color-coded unthresholded t-statistics for the corresponding effect in the bottom left corner. FC alterations surviving false discovery rate correction were found in the ArcA β transgenic mice compared to wild-type between the SSc toward B1c, Pfc, and Cg; between B1c toward Pfc and lStr; and Pfc toward Cg (D), but not in PSAPP (E), or E22 Δ A β strains (F). Regions in network maps are: Motor (Mc), supplementary (SSc), barrel field 1 (B1c) and 2 (B2c), limb (Lc), visual (Vc), auditory (Ac), prefrontal (Pfc), and cingulate (Cg) cortices, dorsal (dHc) and ventral (vHc) hippocampus, piriform cortex (Pir), dorsal (dStr, lateral (lStr), and ventral (vStr) striatum, amygdala (Amg), and thalamus (Th).

Figure 5 | Brain volume changes

Voxel-wise analysis of the voxel-based morphometry (VBM) analysis indicate local volume increases in the basal forebrain, lateral and third ventricles, parts of the hippocampus, periaqueductal grey, and locus coeruleus in transgenic ArcA β mice compared to WT controls (A). In contrast, the volumes of olfactory bulb, cingulate cortex, and cerebellum volume are reduced in these mice. In the PSAPP mice, the volume of large parts of isocortex is increased in the transgenic mice compared to control, while there is some indication of local volume decreases in the midbrain/brainstem regions (B). Region of interest analysis did not indicate any genotype effect with respect to the total brain volumes (C),

however the analysis confirmed the results obtained with voxel-wise analysis in the ventricles (D), barrel field cortex (E), and periaqueductal grey (F). Transgenic ArcA β display a significant increase in the ventricular (p-value=0.001) and periaqueductal grey regions (p-value=3.9x10⁻⁵), while the PSAPP transgenic mice present increase volume in the barrel field cortex (p-value=0.003). Voxel-wise statistics are shown as t-statistics for the indicated effect as an overlay on the AMBMC MRI template. Individual values for the animals are represented as overlay on the boxplots and shape-coded for gender, male=triangle, female=circle.

Figure 6 | White matter alteration

Region of interest analysis of the fractional anisotropy maps was performed on selected white matter fibers (A). The minor forceps indicated significant increase in FA in the ArcA β transgenic animals compared to the WT (p-value=0.02), while there was no significant genotype effect in the PSAPP and E22 Δ A β mice. A significant decrease in FA in the external capsule was observed in the PSAPP mice (p-value=0.0093).

Regions of interest considered: minor forceps (mc), anterior commissure (ac), cingulum (cg), external capsule (ec), corpus callosum (cc), fimbria of the hippocampus (fb), internal capsule (ic), hippocampal commissure (hc).

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Strain	Genotype	Gender ratio [m/f]	Age [days]	Weight [g]
ArcA β	TG	4/8	402 $\pm$ 18	28.8 $\pm$ 3.8
ArcA β	WT	6/6	406 $\pm$ 25	35.8 $\pm$ 7.3
E22 Δ A β	TG	6/6	380 $\pm$ 8	35.3 $\pm$ 6.4
E22 Δ A β	WT	5/7	382 $\pm$ 6	38.2 $\pm$ 5
PSAPP	TG	5/4	473 $\pm$ 19	36.7 $\pm$ 6.5
PSAPP	WT	4/7	480 $\pm$ 13	37.6 $\pm$ 4.8

711

712 Table 1 | Group description

713 The gender distribution, age and weight are indicated for the 3 strains evaluated in transgenic (TG) and wild-type (WT) groups.

714 Statistics are indicated as mean $\pm$ standard deviation.

715

716

Strain	Minor forceps			External capsule		
	FA	D _{ax}	D _{rad}	FA	D _{ax}	D _{rad}
ArcAβ TG	0.38±0.03	0.94±0.05	0.51±0.02	0.39±0.02	0.98±0.04	0.54±0.04
ArcAβ WT	0.35±0.03	0.90±0.04	0.51±0.02	0.38±0.02	0.95±0.03	0.52±0.02
E22ΔAβ TG	0.34±0.02	0.89±0.03	0.51±0.02	0.41±0.02	0.98±0.03	0.51±0.02
E22ΔAβ WT	0.33±0.02	0.87±0.04	0.51±0.01	0.40±0.02	0.98±0.04	0.52±0.02
PSAPP TG	0.35±0.02	0.89±0.04	0.51±0.02	0.39±0.02	0.95±0.03	0.51±0.03
PSAPP WT	0.34±0.02	0.88±0.02	0.51±0.01	0.42±0.02	0.96±0.02	0.50±0.02

717

718 Table 2 | Water diffusion properties in white matter fibers

719 Descriptive statistics for fractional anisotropy (FA), axial diffusivity (D_{ax}), and radial diffusivity (D_{rad}) parameters are shown for
720 regions of interest positioned in the minor forceps and external capsule. Significant genotype effects for a strain are highlighted
721 in bold. Transgenic ArcAβ mice present a significant increase in the minor forceps for both FA (p=0.02) and D_{ax} (p=0.017).
722 Transgenic PSAPP present a decrease in FA in the external capsule (p=0.009), however no significant difference in either D_{ax} or
723 D_{rad}. D_{ax} and D_{rad} are indicated in [10⁻³mm²/s]. Values are given as as mean ± standard deviation.

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