Decreased functional connectivity in ApoE4 and ApoE-knockout mice revealed by resting-state fMRI at ultra-high field

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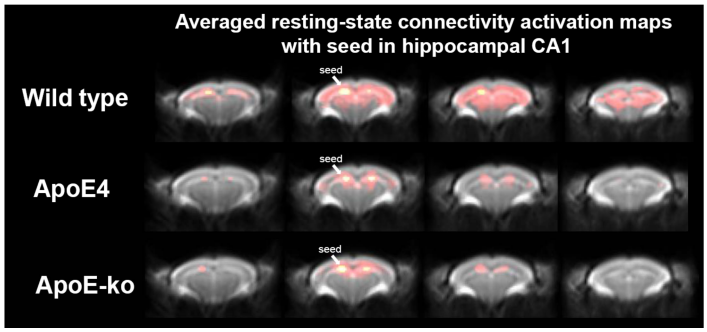
Purpose There exists a well-established association between the cholesterol-transporter apolipoprotein ε (apoE) genotype and the risk of developing Alzheimer's disease (AD). Cholesterol released from apoE-containing lipoprotein particles is used to support synaptogenesis and maintenance of synaptic connections. Compared to the apoE2 and apoE3 isoforms, the apoE4 isoform is a less potent cholesterol transporter, which in turn is linked to hypercholesterolemia, atherosclerosis and impaired synaptic connectivity[1-2]. Such abnormal connectivity has recently been explored by means of resting-state fMRI, showing marked differentiation in several functional networks in the brain compared with non-apoE4 carriers[3]. We tested here the hypothesis that dysfunctional apoE, and further its absence, are directly related to a loss of brain functional connectivity.

Target audience Researchers interested in the biological role of apoE genotype in AD. Researchers involved in the development of resting-state fMRI in mice.

Methods We used two mouse models (12 months of age, males), the apoE4 (n=6) created by targeting the murine APOE gene for replacement with the human APOE4 alleles and a knockout model for APOE (apoE-/-, n=10), and we compared the results with wild-type mice (C57BL6/J, n=9). MR measurements were performed on a 11.7T BioSpec Avance III small animal MR system (Bruker BioSpin, Ettlingen, Germany) equipped with an actively shielded gradient set of 600 mT/m. We used a circular polarized resonator for transmission and an actively-decoupled mouse brain receiver coil. Blood oxygenation level dependent (BOLD) data were acquired using a spin echo echo-planar imaging (SE-EPI) sequence with the following parameters: TE = 16.9ms, TR = 1.7s, voxel resolution = 0.19 × 0.26 × 0.5 mm, repetitions = 600, total acquisition time = 17min. During acquisition, mice were kept under low-dose anesthesia (Isofluran) to maintain a steady breathing rate at 180-200 breaths per minutes. The EPI data were first realigned using a least squares method and rigid-body transformation with SPM mouse toolbox (SPM5, Wellcome Department of Clinical Neurology, London). Individual EPI datasets were then spatially normalized to a study-specific template through linear affine and non-linear diffeomorphic transformation (ANTs. V1.9, http://picsl.upenn.edu/ANTS/). In plane smoothing and high-pass filtering were applied to compensate for imperfect registration and temporal low frequency noise. Functional connectivity maps were calculated using the REST Matlab toolkit[4], with seed ROIs in left and right auditory cortex (AuCtx), visual cortex (VCtx), cingulate cortex (CCtx), cornus ammonis 1 and 3 (CA1, CA3) and thalamus (Th). Correlation analyses were carried out between the seed ROIs and the whole brain for each animal and Pearson's correlation values were Fisher transformed to *Z*-scores.

Results Correlations maps in wild-type animals displayed a widespread functional connectivity distribution among brain areas, with stronger correlations between cortical and hippocampal regions and between hippocampal and thalamic regions, also inter- and intra-hemispheres. The Fisher's *Z*-correlation maps displayed an overall reduction in functional connectivity in apoE4 and apoE-/- mice in comparison with non-transgenic mice in several region of interest. In particular, apoE4 mice showed a significant reduction of functional connectivity between the AuCtx and the CA1 region (p=0.028), and between CA1 and CA3 region (p=0.022) and within CA3 (p=0.022). In ApoE-/- significantly lower connectivity was found between AuCtx and CA1 (p=0.040), between CA1 and CA3 (p=0.024), between Th and CA1 (p=0.039) and within AuCtx and VCtx (p=0.045 and p=0.021, respectively). Averaged maps of the Fisher's *Z*-correlation for a seed in the hippocampal left CA1 illustrate a widespread functional connectivity in cortical and thalamic regions in the wild-type mice that is almost absent in apoE4 and apoE-/- mice (threshold at Z=1, **figure 1**).



Discussion and conclusion In the present study we successfully implemented resting state functional connectivity measurements in wild-type, apoE4 and apoE-/- mouse brain at 11.7T. Our results indicate that at 12 months of age apoE4 and, more severely, apoE-/- mice have lower functional connectivity compared to wild-type. The difference between transgenic and non-transgenic mice is particularly significant in the hippocampus and in the visual and auditory cortex, consistently with human studies. In conclusion, we present a method to analyze resting-state functional connectivity in mice and we provide new evidences of a dependency between the apoE genotype and the functional connectivity, possibly mediated by apoE's presence and efficiency as cholesterol transporter in the brain.

References [1] Davignon J (2005) Apolipoprotein E and atherosclerosis: beyond lipid effect. *Arterioscler Thromb Vasc Biol* 25, 267-269.
[2] Mahley RW (1988) Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 240, 622-630. [3] Trachtenberg AJ, Filippini N, Ebmeier KP, Smith SM, Karpe F, Mackay CE. The effects of APOE on the functional architecture of the resting brain. *Neuroimage*. 2012 Jan 2;59(1):565-72. [4] Xiao-Wei Song, Zhang-Ye Dong, Xiang-Yu Long, Su-Fang Li, Xi-Nian Zuo, Chao-Zhe Zhu, Yong He, Chao-Gan Yan, Yu-Feng Zang. (2011) REST: A Toolkit for Resting-State Functional Magnetic Resonance Imaging Data Processing. *PLoS ONE* 6(9): e25031.