Long-term environmental enrichment induces CA1 enlargement in APPswe/PS1dE9 transgenic mice: A deformation-based morphormetry study

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Introduction Alzheimer's disease (AD) is associated with Aβ-pathology, neuronal loss and atrophy in the hippocampus and cortex. It is also well-established that enriched environment (EE) can induce plastic changes in the hippocampus and cortex throughout the life span. Previous studies have demonstrated that, compared to their littermates raised in a standard laboratory environment (SE), APPswe/PS1dE9 transgenic mice raised in EE immediately after weaning showed reduced cognitive impairment, and improved neurogenesis and synaptic plasticity in the hippocampus [1]. In this study, we aimed to investigate whether EE treatment will cause macrostructural changes in the brain of APPswe/PS1dE9 transgenic mice. Deformation-based morphormetry (DBM) was used to analyze the anatomical magnetic resonance imaging data.

Materials and methods Male wild type (WT) and APPswe/PS1dE9 transgenic (Tg) mice, all in a C57/BL6 genetic background, were used. The pups stayed with their mother before they were assigned, at postnatal day30, to receive either EE treatment or SE treatment. The SE-treated mice were housed in mouse cages (230× 130× 120 mm^3) with 4-5 animals per cage. The EE-treated mice were raised in group of 7 in larger (430 × 300 × 170 mm^3) and toy-containing cages. A running wheel remained in the cage all the time. The other toys, including wooden bricks, glass beads and plastic tubes, were changed every week. All animals had access to food and water *ad libitum*. No replenishment animal was introduced to the cage in cases some mice died before the imaging experiments. A total of 29 animals, including WT-EE (n=7), Tg-EE (n=9), WT-SE (n=7) and Tg-SE (n=6), were imaged on a 7 T/20 cm Bruker Biospec scanner at 6 months of age under 0.5-1% isoflurane anesthesia. A 72-mm diameter volume coil was used for transmission, and a quadrature surface coil for signal reception. T_2-weighted images were acquired with a RARE sequence, TR 2300 ms, effective TE 24 ms, RARE factor 4, slice thick 0.5 mm, FOV 1.5 cm×1.7 cm, matrix size 256×256 and 38 averages. The custom-built template for spatial registration had an isotropic spatial resolution of 59 μm×59 μm×59 μm, and represented the average of 29 2-6 months old wild type C57/BL6 mice. With the ANTs software (http://www.picsl.upenn.edu/ANTS/), the raw images from each individual mouse were registered to the template first with a rigid transformation algorithm, and subsequently with a diffeomorphic transformation algorithm. Jacobian determinants were calculated from the diffeomorphic deformation fields. Log-Jacobian images were reconstructed, smoothed with a 0.18-mm FWHM Gaussian kernel, and analyzed statistically in SPM8 with voxel-wise two-factor ANOVA and independent samples t-tests.

Results The voxels showed significant main effect of environment, as revealed by two-factor ANOVA, mainly located in the hippocampal CA1 subregion, with little involvement of other hippocampal subregions (Fig. 1A). Inter-group DBM comparisons (Fig. 1B-D) showed that both WT and Tg animals had significantly enlarged CA1 after 6 months of EE treatment. The extent of EE-induced CA1 enlargement, however, appeared to be modulated by the genotype. With an uncorrected statistical threshold of p≤0.001, the EE-induced CA1 enlargement was confined to a small cluster of voxels in the left hippocampus of the WT mice (Fig. 1B). The Tg mice showed more prominent and extended EE-induced CA1 enlargement under the same statistical threshold, involving bilateral hippocampus (Fig. 1D). Neither the WT mice nor the Tg mice showed EE-related structural changes in CA3.

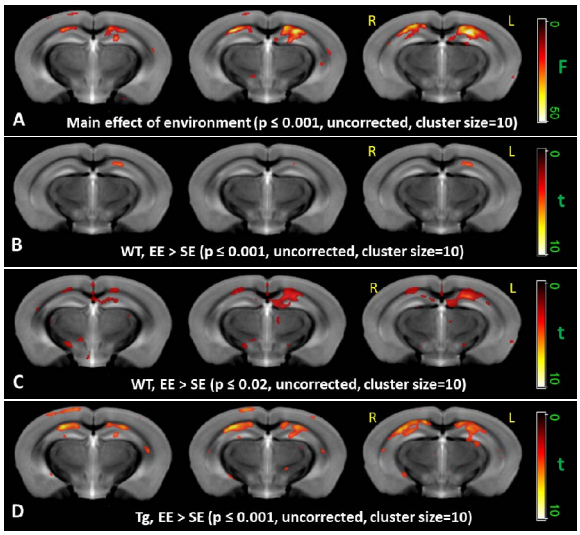

Figure 1. DMB results. A: main effect of environment by two-factor ANOVA. B and C: inter-group comparisons between the WT-EE group and the WT-SE group by voxel-wise t-tests. D: inter-group comparison between the Tg-EE group and the Tg-SE group by voxel-wise t-tests.

Discussion The main finding is that long-term (i.e., 6 months) EE treatment, starting at postnatal day30, induces macrostructural changes (i.e., enlargement) in CA1, and such changes appeared to be more prominent in the APPswe/PS1dE9 mice than in the WT mice. These results are consistent with previous observations that long-term environmental enrichment alter synaptic transmission and plasticity in CA1 [2]; and that the animals housed in EE showed increased connectivity between CA1 and cortical areas compared to those raised in SE [3]. The CA3 pyramidal neurons of the APPswe/PS1dE9 transgenic mice were shown to be more susceptible to Aβ-related damages than the CA1 pyramidal neurons [4]. EE may prevent degeneration of CA3 neurons through rescuing neurogensis in DG [5]. The more prominent CA1 enlargement observed in the Tg mice may, therefore, be contributed to the synergistic effects of EE-induced plasticity in the CA1 *per se* and better preserved CA3-CA1 synaptic transmission in the Tg-EE mice, as compared to the Tg-SE mice.

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