Mapping Brain Activations Induced by Exposure to Enriched Environment with Manganese-Enhanced Magnetic Resonance Imaging

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Introduction It is well-known that exposure to an enriched environment (EE) can induce plastic changes in the hippocampus and improves learning and memory in hippocampus-dependent tasks[1]. The commonly used techniques in the previous studies on the effects of EE on brain include behavioral tests, electrophysiological recording, microdialysis and morphological evaluations of neurons and synapses. Few previous studies have utilized neuroimaging techniques to map brain activities associated with EE exposure. In this study, we attempted to map accumulative brain activations in rats exposed to the EE for a period of 24 hrs with manganese-enhanced magnetic resonance imaging (MEMRI).

Materials and methods Nine male SD rats, weighing 180-220 g, received a single dose of i.p. injection of $MnCl_2$ solution (120 mmol/L, 3 ml/kg). The animals were then randomly assigned into two groups— the standard housing group (n=5) and the EE-treated group (n=4). The rats in the former group were returned to their original plastic cage (40 cm×30 cm×20 cm (height)) immediately after $MnCl_2$ injection, while the rats in the later group were transferred into an EE cage (80 cm×60 cm×45 cm (height)), made of stainless steel, that they never encountered. The EE cage contained platforms at different levels, six toys of different shapes and colors, two wheels, and 3 tunnels with shading.

All animals were imaged at 24 hrs after $MnCl_2$ injection on a 4.7 T/30 cm Bruker Biospec scanner with a 5 cm-diameter volume coil. A 3D T_1-weighted gradient echo pulse sequence was used with FOV 3 cm×3 cm×3 cm, matrix size 128×128×64, TR 35 ms, TE 5 ms, flip angle 30° and 16 averages. The total imaging time to acquire a 3D dataset was 1 hr 16 min. The MEMRI datasets were analyzed with a voxel-based method and a region of interest (ROI)-based method. In the voxel-based analysis, a customized template was first created using the datasets from all the animals. The image volume for each individual rat was then aligned to the template. Pixel signal intensity was normalized to the averaged intracranial signal intensity in the corresponding slice. The normalized image volumes were spatially smoothed with a 0.47mm full-width at half-maximum isotropic Gaussian kernel and analyzed voxel-wise by SPM2 within the framework of general linear model. The significance threshold was set to be $p<0.005$ (uncorrected) with a cluster size of 10. For ROI-based analysis, the ROIs representing different anatomical structures were traced manually on the raw images with the guide of a digital atlas[2]. The averaged signal intensity of each ROI was calculated and normalized to the averaged signal intensity of the whole slice (including the ROI) in which the ROI was positioned. Inter-group comparisons were performed with one-way ANOVA followed by post hoc Tukey's test.

Results The results of voxel-based analysis revealed significant activations in the sub-regions (DG, CA1 and CA3) of anterior hippocampus (aHPC), nucleus accumbens (NAc), and the 9th cerebellar lobule (9Cb) of the rats exposed to EE, relative to the rats housed in the standard environment (Fig. 1). The results of ROI analysis also demonstrated significant activations in the aHPC of the rats exposed to EE (Fig. 2). Little activations were found in the posterior hippocampus (pHPC).

Discussion Generally the rats housed in the EE showed greater curiosity and more exploratory activities than the rats housed in the standard environment. Interestingly, the MEMRI data showed significant activations only in the aHPC, NAc and some cerebellar structures, but little activations in the pHPC and the structures belonging to the motor system. In the aHPC, the spatial activation patterns were somewhat different among the DG, CA1 and CA3 sub-regions. There results are consistent with the notions that 1) hippocampus subserves processes associated with the encoding of novel information [1]; 2) the aHPC is functionally distinct from the pHPC[3], and 3) different sub-regions of the hippocampus may have different functions in detecting spatial novelty[4]. It is also known that exposure to novelty may activates the mesolimbic dopamine system of the brain, including the NAc[5]. Our finding that the NAc became activated during EE exposure is in line with the previous observations. In summary, the results of this study demonstrated that it is feasible to use MEMRI to map brain activations associated with EE exposure.

Acknowledgements Supported by a 973 grant (2006CB705600) from the Ministry of Science and Technology of China and a grant (30870674) from Natural Science Foundation of China.

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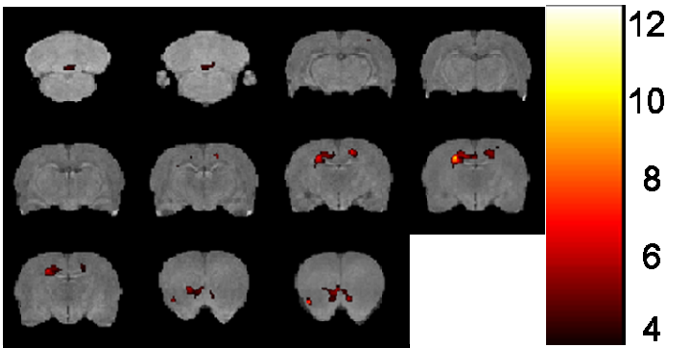

Figure 1 Functional brain activations in the rats exposed to the enriched environment for 24 hrs, revealed by voxel-based analysis of the MEMRI datasets. The color bar codes the significance *t* values.

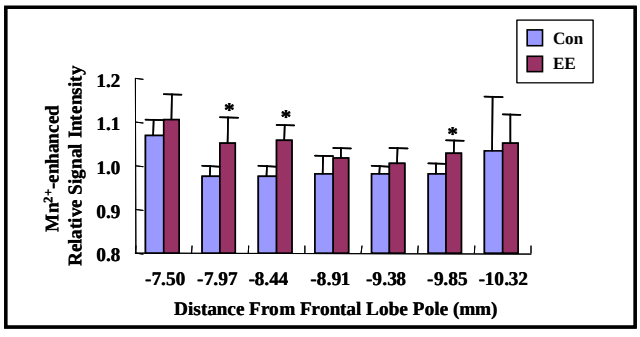

Figure 2 Manganese-enhanced relative signal intensity in the CA1 sub-regions of the hippocampus of the rats exposed to the standard environment (Con) and enriched environment (EE) for 24 hrs after receiving $MnCl_2$ injection. *$p<0.05$ compared to Con.