Functional Brain Mapping in ADHD Rats using Manganese-enhanced MRI

Chieh-Yin Chang[1], Chi-Ru Lai[1], Bor-Show Tzang[2], Vincent Chin-Hung Chen[3], Yeu-Sheng Tyan[1,4], and Jun-Cheng Weng[1,4]

[1]School of Medical Imaging and Radiological Sciences, Chung Shan Medical University, Taichung, Taiwan, [2]Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taichung, Taiwan, [3]Department of Psychiatry, Chung Shan Medical University Hospital, Taichung, Taiwan, [4]Department of Medical Imaging, Chung Shan Medical University Hospital, Taichung, Taiwan

Introduction

The spontaneously hypertensive rat (SHR) is the best-validated animal model of attention-deficit hyperactivity disorder (ADHD) based on behavioral, genetic, and neurobiological data [1]. The symptoms of this common disorder include difficulty controlling behavior and over-activity. Manganese-enhanced MRI (MEMRI) uses manganese ion (Mn^{2+}) as the contrast agents by shortening the spin-lattice relaxation time constant (T1) and entering the voltage-gated calcium channels in active neurons. It enables visualization of neuronal tracks, and enhance the capacity of MRI to provide functional information of the localization of brain activity [2]. In the study, we tried to establish a working protocol to map the motor cortex of ADHD rats, and compared the functional brain mapping between ADHD and normal Wistar-Kyoto (WKY) rats by MEMRI method. In the results, we have mapped ADHD motor cortex using MEMRI and have shown the difference of the manganese enhanced cortical and thalamic regions between ADHD and WKY rats.

Materials and Methods

Eleven ADHD and six normal WKY rats were initially anesthetized with 2% isoflurane mixed O_2 gas. $MnCl_2$ was given, 30 mM in 10 μl, directly into primary motor cortex (M1) of left brain by stereotaxic instrument and microinjection systems. The injection rate was 0.16 μl/min and total injection time was 63 min. All rats were scanned three days after $MnCl_2$ administration. During the MR scanning, each rat was anesthetized with 2% isoflurane mixed with O_2, maintained with 1.5% isoflurane. Rat body temperature was maintained at 37°C using warm water circulation.

The experiment was performed on a 1.5T MRI (Sonata, Siemens MAGNETOM, Germany) with a surface coil for RF reception. Two imaging sequences were performed to acquire T1-weighted images and R1 mapping. Multi-slice spoiled gradient echo (GE) sequences were performed to obtain T1-weighted images with TR/TE = 17/7.57 ms; flip angle = 70°; in-plane resolution = 195μm x 390μm; slice thickness = 1.5 mm. To improve detection sensitivity over the full extent of Mn^{2+} concentrations, image data for R1 mapping were acquired [3]. To obtain R1 mapping, multi-slice turbo spin echo (TSE) sequences were performed with half in-plane resolution; slice thickness = 1.5 mm; TE = 15 ms. The TSE sequence was performed to acquire 8 sets of images corresponding to 8 different TRs, ranging from 490 to 5000 ms, to sample along the recovery of longitudinal magnetization.

Given the current field strength and scan time, cortical enhancement could not be visualized clearly in each individual rat; the enhancement could only be detected by averaging the rat images of the same group of rats. The post-processing of the image data entailed stereotactic coregistration, pixel intensity normalization and inter-subjects averaging using FMRIB Software Library (FSL) and homemade software. To highlight the activity-related functional enhancement between ADHD and WKY rats, the averaged Mn^{2+}-enhanced T1WIs in the ADHD group were subtracted from that in the WKY group after brain extraction and coregistration. To provide statistical difference between two groups, two-sample t-test was performed using Statistical Parametric Mapping (SPM) and voxel-based t-value and p-value mapping were obtained.

Results and Discussions

In the WKY group, most of the cortical enhancement was localized in the left M1 area, only little cortical enhancement extended to the left thalamic regions (Fig. 1a). In the ADHD group, the enhancement extended form M1 to the left thalamic regions obviously (Fig. 1b). To highlight the activity-related areas, R1 mapping was made. In the WKY group, the R1 mapping showed gradients of R1 with their peaks located at the left M1 areas (Fig 1c). In the ADHD group, the R1 mapping showed the enhancement extended to right M1 and left thalamic regions (Fig. 1d). Subtracted images (Fig. 1e), voxel-based statistical t-value mapping (Fig. 1f) and p-value mapping (Fig. 1g) between two groups showed additional enhancement concentrated in the both sides of M1 and left thalamic regions. After giving a threshold of p-value < 0.05 to the t-value mapping, the statistical significance could be observed (Fig. 1h).

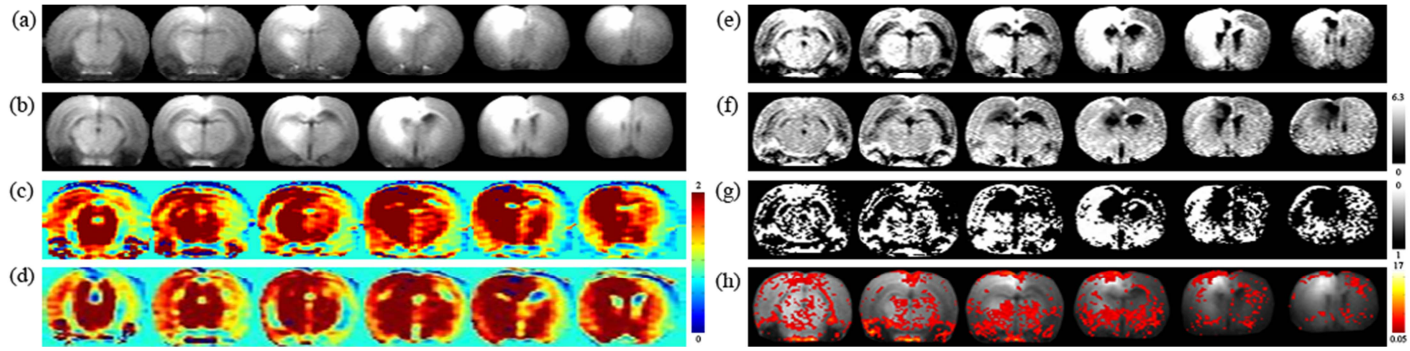

Fig. 1 Six consecutive slices of the averaged Mn^{2+}-enhanced T1WIs in (a) WKY and (b) ADHD groups. R1 maps in (c) WKY and (d) ADHD groups. (e) Image subtraction, (f) voxel-based t-value mapping, (g) p-value mapping and (h) threshold t-value mapping between two groups.

When the activity of a particular region of the brain increased, the accumulation rate of the manganese ions in the cells is directly proportional to rate of the calcium into the neurons. Hence, the manganese ions of the activation regions shorten the T1 as to increase the signal intensity and tend to extend to others regions. The cortical enhancement in the left M1 of the ADHD group was significantly higher than the WKY group. This observation demonstrated that the rats in ADHD group had difficulty controlling and over-activity behavior, and may have more activated neurons allowing the manganese ions passed through the left M1 via the calcium voltage-gated channels. In addition, the ventrolateral thalamus, a connection between the primary motor cortex and the left thalamic regions, caused the enhancement extended form M1 to the left thalamus as well as the basal ganglia [4]. The enhancement in the right M1 were also observed because two cerebral hemispheres are connected by the corpus callosum.

Conclusions

We have mapped the motor cortex using the MEMRI on a clinical MR system, and have shown the difference of the manganese enhanced cortical and thalamic regions between ADHD and WKY rats. Our results indicate that the MEMRI method is potentially useful to study plasticity in surgically or genetically manipulated rat brains.

References

[1] Sagvolden T, et al., Neuropharmacology. 2009; 57: 619-626. [2] Massaad CA, et al., Methods in Molecular Biology. 2011; 711: 145-174.
[3] Chuang KH, et al., Magn Reson Med. 2006; 55: 604-611. [4] Middleton FA, et al., Brain Res Rev. 2000; 31: 236-250.