MEMRI Study of Mice Cerebellar Activation after Voluntary Wheel Running

I. Y. Zhou[1,2], A. M. Chow[1,2], K. C. Chan[1,2], C. Lau[1,2], and E. X. Wu[1,2]

[1]Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Pokfulam, Hong Kong SAR, China, People's Republic of, [2]Department of Electrical and Electronic Engineering, The University of Hong Kong, Pokfulam, Hong Kong SAR, China, People's Republic of

INTRODUCTION:

There is increasing interest in studying functional and cellular effects of environmental stimuli for better intervention in neurological rehabilitation [1, 2]. Recent studies have been using BOLD-fMRI to detect the neural activity in living animals [3] and human [4]. However it is technically difficult to apply BOLD-fMRI to non-restrained animals due to its extreme susceptibility to subject motion. And with the limitation of spatial resolution, the implementation of BOLD-fMRI for brain imaging in mice, widely used experimental animal in neurobiology and behaving studies, is even more technically challenging. Paramagnetic manganese ion (Mn^{2+}) is known to enter synaptically activated neurons through voltage-gated calcium channels. Based on this ability, manganese-enhanced MRI (MEMRI) has been utilized to detect electrically or pharmaceutically induced activity in the rat cortex [5, 6] and validated by correlation with BOLD-fMRI [7]. The previous studies using this method mainly focused on passively-induced sensory activation in cortex such as exposure to auditory or olfactory stimulus [8, 9]. With less limitation than BOLD-fMRI, activity-induced manganese-dependant MRI can be feasibly used for freely behaving animals noninvasively to detect neural development longitudinally with higher spatial resolution. In the present study, we aim to use in vivo MEMRI to detect the cerebellar activation in mouse brain induced by voluntary wheel running.

METHODS:

Animals and Experimental Design: Adult male C57BL/6N mice (22-25g, N=12) were divided into 2 groups. Group 1 (N=6): voluntary wheel running; Group 2 (N=6): sedentary controls without exercising. In group 1, animals were housed in standard laboratory cage equipped with plastic running wheel (diameter 10 cm) from 24 hours before Mn administration. A 100 mM solution of $MnCl_2$ in isotonic saline (0.9% NaCl in water) was injected to animals in both groups at a dose of 45 mg/kg body weight intraperitoneally 24 hours before imaging. During the 48-hr period, mice in group 1 ran voluntarily. Animals in group 2 had same light/dark schedule with group 1 only without access to wheel.
MRI Protocols: All MRI experiments were performed on a Bruker PharmaScan 7 T scanner. During the MRI scan, mice were anaesthetized with isoflurane (3% induction and 1.5% maintenance) with respiratory monitoring and kept warm under circulating water at 37 ºC. T1WIs were collected from cerebellum region with a RARE sequence using FOV = 20.5×20.5mm, matrix resolution = 256×256, slice thickness = 0.3 mm, number of slices = 10, TR/TE = 420/7.5 ms, RARE factor = 4 and NEX = 64; T2WIs were acquired using the same voxel dimensions and slice geometry with TR/TE = 4200/38.7ms, RARE factor = 8 and NEX = 6.
Data Analysis: MR image data of different animals was first co-registered together using AIR5.2.5. Ratio maps between the exercising group and sedentary group were computed using the coregistrated image sets by Matlab7 for visualization and quantification of the signal intensity (SI) differences in the cerebellum. ROIs were manually defined according to mouse brain atlas and signal intensity was measured using ImageJ. Mann-Whitney test was performed between the two groups with $p < 0.05$ considered as statistically significant.

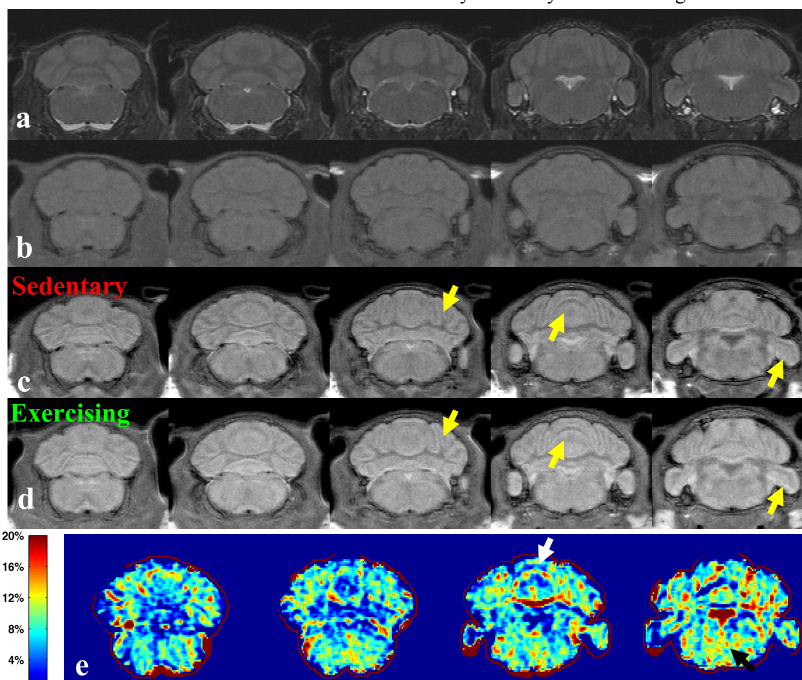

Fig. 1 (a) T2WIs and (b) T1WIs obtained before Mn-enhancement provide limited information of the mice cerebellum. With activity-induced Mn enhancement, cerebellar activation (yellow arrows) after voluntary wheel running (d) can be observed compared to sedentary animals (c) using T1WIs. Percentage difference map (e) gives direct visualization of the activated region such as lobules in vermis (white arrow) and caudal pontine reticular nucleus (black arrow).

RESULTS AND DISCUSSION:

With the present dosage of Mn administration, all the animals in group 1 were not facing Mn-induced motor disturbances due to its toxicity and kept the same degree of voluntary wheel running before and after Mn injection. Compared with T2WIs (Fig.1 [a]) and T1WIs without Mn-enhancement (Fig.1 [b]), MEMRI enhancement enables in vivo visualization of foliation patterns of mouse cerebellum in both groups (Fig.1 [c, d]). According to the percentage difference map (Fig.1 [e]), exercising mice in group 1 have better enhancement in the cerebellum (yellow arrows in Fig.1[c, d]) than those in sedenatry group. Note that cerebellar enhancement in MEMRI is likely due to uptake of Mn in the granule neurons [10]. Higher uptake of Mn in granule cell layer of exercising mice may lead to the above observation, probably resulting from exercise-induced cellular hyperactivity. Differences in signal intensity of T1WIs between the two groups are quantitatively measured by large ROIs covering vermis, ansiform lobule (Crus I & II), paraflocculus (PF) and caudal pontine reticular nucleus (PnC) where higher enhancement was observed in color map (Fig. 2). All of these areas of exercising animals show significantly higher signal intensity than the sedentary group except for PF, which still has marginally (P=0.0508) better enhancement. The vermis is known to regulate body and limb movements. Specialized cells in vermis, known as Purkinje cells, receive sensory information from the vestibular system of the inner ears and use this to compute information about the body's movement [11]. This is possibly the reason of vermis has the most remarkable enhancement in the exercising mice, while running wheel needs higher motor coordination. Crus I & II as well as PF of the hemisphere are mainly involved in planning movement and evaluating sensory information for action [12], while PnC has been known to mediate head movement, in concert with the nucleus gigantocellularis and the superior colliculus [13]. Therefore, all these regions in mice cerebellum are functionally activated after intensively voluntary wheel running.

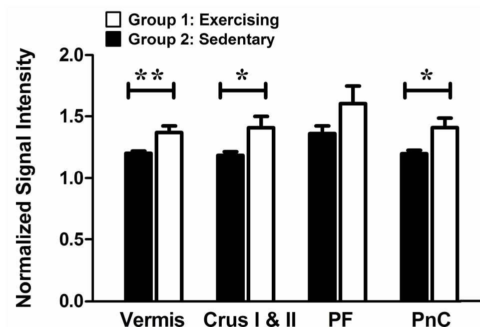

Fig. 2 Comparison of normalized signal intensity in vermis, ansiform lobule (Crus I & II), paraflocculus (PF) and caudal pontine reticular nucleus (PnC) between group 1 and 2. Mann-Whitney test was performed with * $p < 0.05$, ** $p<0.01$.

CONCLUSION:

The preliminary result of our study shows that the mouse cerebellum is largely activated compared to the sedentary animals after voluntary wheel-running exercise, providing insights of regional cerebellar activation upon behavioral change. This difference can be feasibly detected by MEMRI, suggesting the great potential of MEMRI as an in vivo probe for mapping neural activity.

REFERENCES: [1] Kronenberg G, et al. Neurobiol Aging 2006;27:1505-1513. [2] Cui L, et al. Neurobiol Aging 2009;30:903-909. [3] Van der Linden A, et al. NMR Biomed 2007;20:522-545. [4] Kennedy D P and E Courchesne Neuroimage 2008;39:1877-1885. [5] Lin Y J and A P Koretsky Magn Reson Med 997;38:378-388. [6] Lu H, et al. Proc Natl Acad Sci U S A 2007;104:2489-2494. [7] Duong T Q, et al. Magn Reson Med 2000;43:383-392. [8] Yu X, et al. Nat Neurosci 2005;8:961-968. [9] Chen W, et al. Neuroimage 2007;37:221-229. [10] Wadghiri Y Z, et al. NMR Biomed 2004;17:613-619. [11] Yakusheva T A, et al. Neuron 2007;54:973-985. [12] Apps R and R Hawkes Nat Rev Neurosci 2009;10:670-681. [13] Sasaki S, et al. Prog Brain Res 2004;143:383-389.