In Vivo Manganese-enhanced MRI of Conditioned Fear Response

I. Y. Zhou[1,2], A. Y. Ding[1,2], Q. Li[3,4], F. Y. Lee[1,2], S. J. Fan[1,2], K. C. Chan[1,2], G. M. McAlonan[3,4], and E. X. Wu[1,2]

[1]Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Hong Kong SAR, China, People's Republic of, [2]Department of Electrical and Electronic Engineering, The University of Hong Kong, Hong Kong SAR, China, People's Republic of, [3]Department of Psychiatry, The University of Hong Kong, [4]Centre for Reproduction Growth and Development, The University of Hong Kong

INTRODUCTION

Fear conditioning is widely used to study the neural basis of learning and memory [1]. It allows an organism to quickly assess and react to stimuli and predict danger. However, it was mostly restricted to behavioral or histological studies [2, 3]. Recent studies have been using BOLD-fMRI to detect the neural activity in living animals [4]. However it is technically difficult due to subject motion and is limited by poor spatial resolution. Paramagnetic manganese ion (Mn^{2+}) is known to enter synaptically activated neurons through voltage-gated calcium channels. Based on this, manganese-enhanced MRI (MEMRI) can be used to assess the neural circuitry involved in sensory paradigms [5] and to investigate more complex cognitive and emotional processes such as fear-conditioning longitudinally with higher spatial resolution. In this study, we aim to use *in vivo* MEMRI to detect the acute neuronal response after fear conditioning.

MATERIALS AND METHODS

Animal Preparation: Adult C57BL/6N mice (N=9, 90-100 days old, weighing 23-28g) were divided into 2 groups. Animals in *Group 1* (N=5) were subjected to fear conditioning (FC), followed immediately by i.p. injection of a 100 mM solution of $MnCl_2$ in isotonic saline at a dose of 45 mg/kg. *Group 2* (N=4) served as controls with no fear conditioned stimulus but the same Mn administration dosage. MRI scans were performed on all the mice before and 24 hours after Mn injection. ***Conditioning protocol*** [6]***:*** On the training day, mice were placed individually into a conditioning chamber ($25\times25\times25cm^3$) for 6-minute acclimation, followed by 3 paired presentations of a clicker (30 sec, 4Hz, 80 dB) and footshock (2 sec, 0.5 mA). The inter-trial interval was 2 min and an additional 2-min rest after the final clicker/shock pairing in the chamber, yielding a total training time of 13min30s. The chambers were cleaned with 70% alcohol between each training session. ***MRI Protocols:*** All MRI experiments were performed on a Bruker 7 T scanner with a mouse brain coil. During the MRI scan, mice were anaesthetized with isoflurane (2.5% induction and 1.5% maintenance) with respiratory monitoring and kept warm under circulating water at 37 ^{o}C. T1WIs were collected with a RARE sequence using FOV = 25.6×25.6mm, MTX = 256×256, slice thickness = 0.5 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 = 4200/36ms, RARE factor = 8 and NEX = 2. ***Data Analysis:*** Pre- and post-injection T1WIs from different animals was first co-registered together with T2WIs as references using AIR5.2.5. Averaged ratio maps between the two groups were computed using the coregistrated image sets by Matlab7 for visualization and quantification of the signal intensity (SI) differences. 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 $p < 0.05$ which could be considered as statistically significant.

RESULTS AND DISCUSSION

With the present dosage of Mn administration, no animals were found to have significant Mn-induced behavioral disturbances due to its toxicity. Significantly decreased locomotor movement and increased freezing duration were found during FA training confirmed the mice acquired associative learning with aversive stimulus quickly (data not shown). The Mn-enhanced T1W images in Fig. 1 (left & middle) showed that the FC animals exhibited a higher Mn uptake in several brain regions as indicated by the pointing arrows, including amygdala, hippocampus, paraventricular nucleus of hypothalamus (PVH), and cingulate cortex comparing to the normal controls. The color-coded ratio map between the two groups (Fig.1, right) further delineated the differences of Mn-enhancement in these regions. All these structures were closely related to the process of conditioned fear [7]. For example, the amygdala is playing a key role in the formation and expression of fear while the fear memory is stored in the hippocampus. Fig. 2 showed a significantly higher Mn-uptake in FC animals in amygdala, hippocampus and PVH, possibly due to the fear-induced cellular hyperactivity in these regions. Exceeding the pain threshold [6] with the stimulation current during footshock may also result in the greater Mn-enhancement in PVH [8] in Group 1. Cingulate cortex, which plays a role in acquisition and extinction of conditioned-fear, also had higher post-Mn signal intensity with marginal significance (p=0.066). No distinct signal difference was found either prior to Mn-injection or in global enhancement after Mn-injection between the FC animals and controls, indicating the regional enhancement was specific to fear-conditioning. In conclusion, this study demonstrated that the neural response after the induction of conditioned-fear can be detected in most brain regions that are highly related to the process of fear by *in vivo* MEMRI. The results provide insights to neurocircuits involved in fear-conditioning and consolidate the capability of MEMRI as an in vivo probe for mapping neural activity.

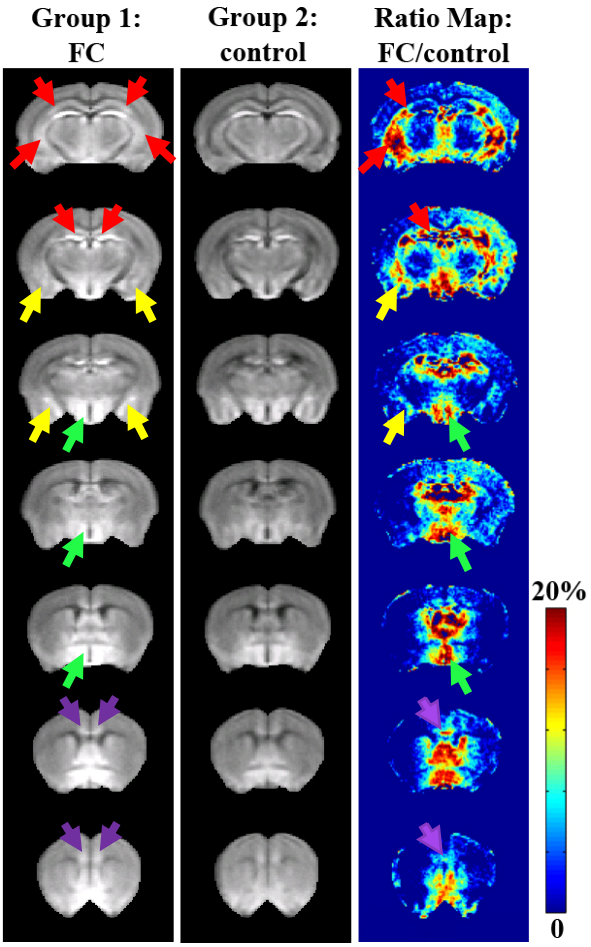

Fig.1 Averaged post-Mn T1WIs from Group 1 (fear-conditioning) and Group 2 (control) with the ratio maps showing the percentage signal differences between them. Enhanced Mn-uptake was observed in amygdala (yellow arrows), hippocampus (red arrows), paraventricular nucleus of hypothalamus (green arrows) and cingulate cortex (purple arrows) in FC animals.

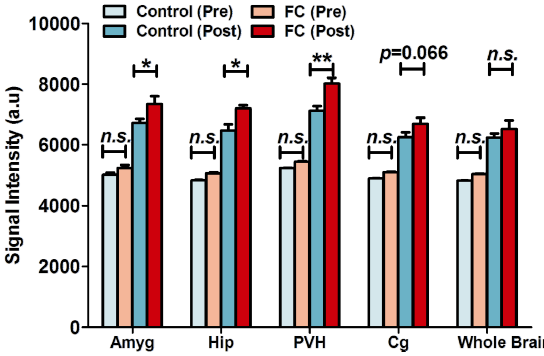

Fig.2 T1WI signal intensity changes (Mean ± SD) before and after Mn injection were compared between the two groups using ROIs covering amygdala (Amyg), hippocampus (Hip), paraventricular nucleus of hypothalamus (PVH), cingulate cortex (Cg) and the entire brain. Mann-Whitney test was performed with * $p < 0.05$, ** $p<0.01$.

REFERENCES

[1] Rodrigues S. M., Neuron 2004;44:75-91. [2] Campeau S. and M. Davis J Neurosci 1995;15:2301-11. [3] Bruijnzeel A. W., Brain Res 1999;819:15-22. [4] Van der Linden A., NMR Biomed 2007;20:522-45. [5] Yu X., Nat Neurosci 2005;8:961-8. [6] Siegmund A. and C. T. Wotjak J Psychiatr Res 2007;41:848-60. [7] Pezzone M. A., Brain Res 1992;597:41-50. [8] Gauriau C. and J. F. Bernard Exp Physiol 2002;87:251-8.