Early Metabolic Changes in Hippocampus and Cingulate Cortex after Fear Conditioning

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INTRODUCTION

Various neuroimaging techniques have been employed to provide powerful and noninvasive measurements for structural and functional brain alterations in posttraumatic stress disorder (PTSD) [1]. However, the changes occurred in trauma exposure and during PTSD development can hardly be fully captured due to its complexity and high diversity in human [2]. Meanwhile, fear conditioning (FC) is a widely used procedure to study the neural basis of learning and memory and to reveal the pathomechanisms of PTSD. Proton MRS (^{1}H MRS) can be used to assess the metabolic changes in living brain and thus could provide biochemical evidence underlying the neural process, even when the anatomic changes are not apparent. Previously, ^{1}H MRS was employed to evaluate the neurochemical changes in hippocampus and other brain regions in PTSD patients [3, 4]. In this study, we aim to use in vivo ^{1}H MRS to investigate the metabolic changes in hippocampus and cingulate cortex of mouse brain after conditioned-fear training.

MATERIALS AND METHODS

Animal Preparation: Adult C57BL/6N mice (N=8) weighing 23-28g were subjected to fear conditioning and were MRI scanned before and one day after fear-conditioning training. ***Conditioning Protocol*** [5]***:*** On the training day, mice were placed individually into a conditioning chamber (25×25×25 cm^3) for 6-minute acclimation, followed by 3 paired presentations of a clicker (CS) (30 sec, 4Hz, 80 dB) and footshock (US) (2 sec, 0.5 mA). The inter-trial interval was 2 min and an additional 2-min rest was given 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 MR measurements were performed on a 7 T Bruker MRI scanner using a mouse brain coil. Under inhaled isoflurane anaesthesia, the animal was kept warm under circulating water at 37 ^{o}C with respiratory monitoring. RARE T2-weighted anatomical images were acquired for voxel localization in ^{1}H MRS. After shimming with FASTMAP, ^{1}H MRS was performed using a PRESS sequence combined with outer volume suppression (OVS) and with TR/TE=2500/17ms, 2048 data points and 256 averages. A 1×1.5×2.5mm^3 voxel was placed over the left hippocampus and another 1×1.2×2.5mm^3 voxel was placed over the cingulate cortex. ***Data Analysis:*** MR spectra were processed with jMRUI software using simulated metabolites in NMR-SCOPE as prior knowledge. The raw data was apodized with a 15-Hz Gaussian filter and phase-corrected. The residual water signal was filtered out with HLSVD algorithm. Various ratios of metabolites, NAA:Cr, Cho:Cr, Tau:Cr, Lac:Cr, and m-Ins:Cr, were statistically evaluated using two-tailed paired student's t-tests between the pre- and post-conditioning spectra with $p < 0.05$.

RESULTS

Significantly decreased locomotor movement and increased freezing duration were found during FA training confirmed the mice acquired associative learning with aversive stimulus (data not shown). No significant morphological changes were observed in T2W images after subjection to conditioned fear. Fig. 1 illustrates the changes of ^{1}H MRS Spectra in the two regions of interest, hippocampus (Hipp) and cingulate cortex (Cg), before and after the fear-conditioning session. The lowered NAA signal with respect to creatine (Cr) peak can be clearly observed in Hipp and Cg of post-conditioning animals. The statistical evaluation of the metabolites (Fig. 2) revealed that besides the distinct hippocampal NAA level reduction ($p<0.001$), the myo-inositol (m-Ins) signal also reduced significantly ($p<0.05$), while the choline (Cho) signal increased in Cg with the presence of lower levels of NAA.

DISCUSSION AND CONCLUSION

Reduction of NAA, a marker of neuronal density, integrity and health [6], indicates neuronal loss and cellular dysfunction [7]. The reduced NAA:Cr in Hipp and Cg following the fear conditioning could be mainly attributed to neuron cell dysfunction prior to neuronal loss. The significant decrease in m-Ins was not consistent with previous studies reporting no detectable m-Ins change [8], which could be due to the small voxel limiting the SNR and the large SD showing the variability in the detection of this relatively small peak. The increased Cho:Cr ratio in the presence of low levels of NAA usually indicates proliferation of neuroglia at the expense of neuronal number accompanied by grey matter atrophy [9], while the detectable Cho change in our results was seem to precede any significant morphological changes. In conclusion, this study showed that the early metabolic changes after fear-experiencing could be detected by in vivo ^{1}H MRS prior to any significant structural alterations, which can facilitate prompt intervention in neurobiological rehabilitation.

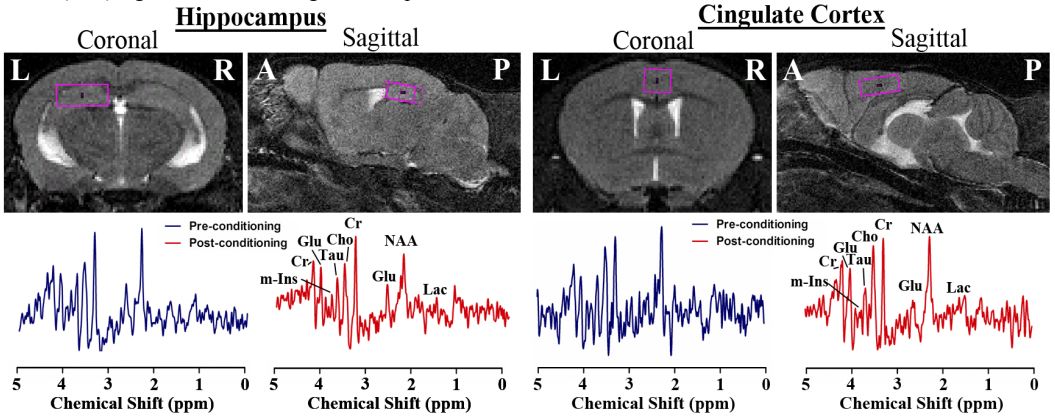

Fig.1 (Top row) Localization of the voxel of interest in hippocampus and cingulate cortex in the coronal and sagittal planes. (Bottom row) Representative ^{1}H MRS spectra from these two areas prior and after the induction of fear-conditioning.

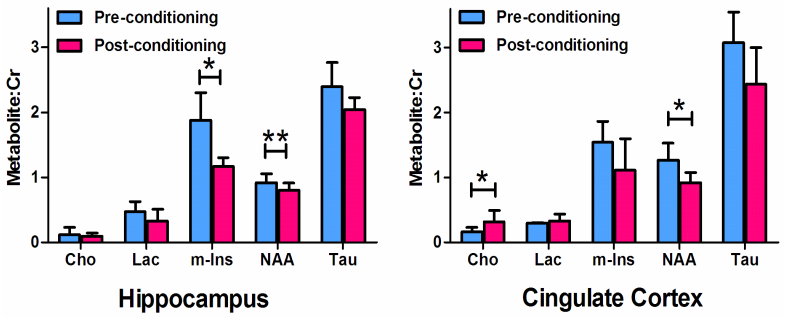

Fig.2 Comparisons of metabolite ratio changes with exposure to conditioned fear in hippocampus (left) and cingulate cortex (right). Paired t-tests were performed with * $p<0.05$, ** $p<0.01$.

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