DTI detection of fear conditioning induced microstructural plasticity

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Introduction

Fear conditioning (FC) 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. Neural and cellular changes associated with learning memory have been suggested in specific regions [2]. However, it was mostly restricted to behavioral or histological studies [3,4]. DTI is a powerful tool for detecting microstructural changes and may provide insights for the accompanied brain plasticity after FC in vivo. This study aims to employ DTI to assess the acute changes following fear conditioning in specific regions.

Methods

MRI Protocols: Eight C57BL/6N mice (90-95 days old) were scanned twice using a 7T Bruker scanner one day before and immediately after fear conditioning (FC) training. In vivo Diffusion-weighted (DW) images were acquired using a SE 8-shot EPI sequence with 15 diffusion gradient directions. Five additional images with b-value=0 (B_0 images) were also acquired. The imaging parameters were: TR/TE=3000/28.6ms, δ/Δ=5/17ms, NEX=4, FOV=2.8x2.8cm^2, acq matrix= 128x128 (zero-filled to 256×256), slice thickness=0.48mm (0.07mm gap), b-value =1000 s/mm^2. ***Fear conditioning protocol*** [5]**:** Mice were placed individually into a conditioning chamber (25×25×25 cm^3) for 6-minute habituation. During this time period, mice explored the chamber freely. Followed by 3 paired presentations of a clicker as the conditioned stimulus (30 sec, 4Hz, 80 dB, CS) and footshock as the unconditioned stimulus (2 sec, 0.5 mA, US). The inter-trial interval was 2 min and an additional 2-min rest after the final clicker/shock pairing in the chamber. The chambers were cleaned with 70% alcohol between each training session. Video monitoring was performed throughout the training and was used for later behavioral analysis. ***Data Analysis:*** Pre- and post-FC FA maps from all animals were first co-registered and normalized with a template B_0 image. Voxel-wised paired t-test was then performed between pre- and post-FC FA maps. The registration, normalization and statistical procedures were performed using SPM5. Total distance and freezing behavior (absence of movement except respiration) were automatically measured using EthoVision XT7. The data was analyzed using student t-test.

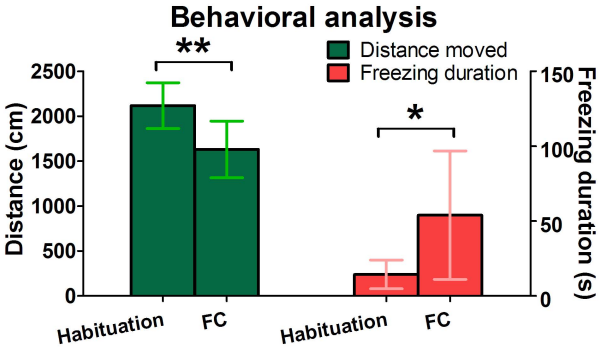

FIG.1 The significant difference of moved distance and freezing duration between habituation period (free exploration) and fear conditioning (FC) period (three paired CS with US) indicated associative learning acquired rapidly in mice. * p<0.05 ** p<0.005.

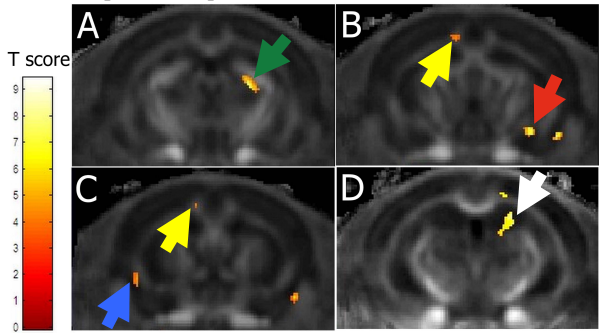

FIG.2 Regions with statistically significant changes between pre- and post-FC FA maps overlaid on the average FA map from all co-registered animals. **A-C**: Regions showing FA increases with threshold p<0.005 and extent threshold = 10 voxels. **D:** Region showing FA decrease with threshold p<0.001 and extent threshold = 25 voxels.

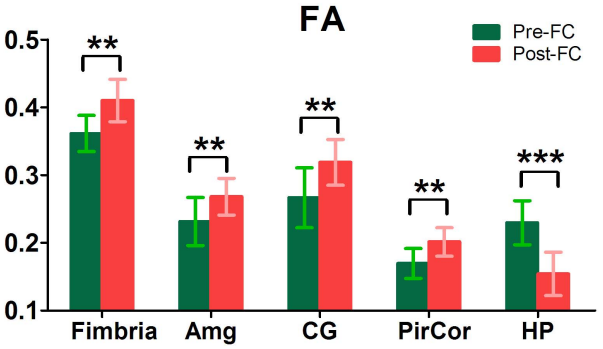

FIG.3 FA changes in the regions indicated in FIG.2 between pre- and post-FC. ** p<0.005, *** p<0.001.

Results

Significantly decreased locomotor movement and increased freezing duration were found during FC training as shown in FIG.1, confirming that the mice acquired associative learning with aversive stimulus quickly. Cued and contextual fear memories were confirmed latterly (data not shown). FIG.2 illustrates the regions significant changes found in the paired t-test analysis between pre- and post-FC FA maps. Significant FA increase was found in amygdala (Amg, FIG.2B red arrow), cingulum (CG, FIG.2B&C yellow arrows), fimbria (FIG.2A green arrow) and piriform cortex (PirCor, FIG.2C blue arrow). These regions are commonly recognized to be closely related associative learning and fear memory [2]. Interestingly, FA in hippocampus (HP, FIG.2D white arrow) was found to be significantly decreased after training, which was in agreement with the result from previous DTI study of spatial reference in rats [5]. FIG.3 shows the quantitative measurements of these FA changes between pre- and post-FC.

Discussions and Conclusions

Each mouse acquired associative learning during FC training period and showed fear memory afterwards. Significant FA changes were found in amygdala, cingulum, fimbria and piriform cortex, which are specifically related to fear learning. Previous studies have found synaptogenesis in amygdala, which is believed to be related to the formation of the conditioned fear response following fear conditioning [6], while hippocampus is reported to have a decreased neurogenesis rate following a sufficient stressor [7]. Our results are in parallel with those neurobiological findings, and suggest that the plasticity began shortly after fear learning in limbic and white matter regions. The regions exhibited changes were asymmetric in this study. Note that such asymmetry has also been reported by others in both human and animal psychiatric DTI studies [8,9]. The underlying biological origins of these microstructural changes as probed by in vivo DTI require further investigation. Nevertheless, our data show that DTI is a sensitive, non-invasive and in vivo tool for detecting microstructural plasticity caused by fear conditioning.

References

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