# DTI Investigation of Short-term Plasticity in Amygdala and Hippocampus Induced by Fear Conditioning

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## Introduction

Fear conditioning (FC) is widely used to study the neural basis of learning and memory (1). Considerable evidences support that amygdala serves as the central circuit, both amygdala and hippocampus contribute to memory formation and consolidation following FC. However, studies of the biological mechanism underlying FC were mostly restricted to immunohistochemistry methods (2, 3). DTI is emerging as a powerful tool for probing neural plasticity. Its noninvasive nature allows the exploration of plasticity network between various brain regions simultaneously and longitudinally. This study aims to assess the microstructural plasticity using DTI, which may provide insights into the plasticity process during FC.

## Methods

***MRI Protocols:*** Eighteen C57BL/6N mice (90-95 days old) were scanned using a 7T Bruker scanner 1 day before, 1 hr after, and 24 hrs after fear conditioning (FC) training. 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*:** Mice were placed individually into a conditioning chamber (25×25×25 cm$^3$) for 6-min habituation. Followed by 3 paired presentations of a clicker as the conditioned stimulus (CS, 30 sec, 4Hz, 80 dB) and footshock as the unconditioned stimulus (US, 2 sec, 0.5 mA). The inter-trial interval was 2 min and an additional 2-min rest was allowed 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 behavioral analysis. ***Data Analysis:*** Each mouse brain volume was normalized to a custom $B_0$ template generated from a representative animal. Voxel-wised paired t-test was first performed between pre- and 1 hr post-FC FA maps, the resulting significant voxels were projected onto each individual animal's DTI index maps at each time point for quantitation comparisons. The normalization and statistical procedures were performed using SPM5. ***Histology:*** Mice trained with the same FC protocol were selected as FC group (N=4). Age-matched mice (N=4) that underwent identical FC paradigm except for neither CS nor US presentation were selected as sham/control group. One hr after training, the brain tissues were collected. Immunohitochemical (IHC) analysis was performed on 30 μm thick coronal sections with anti-synaptophysin, a maker for synapses (4).

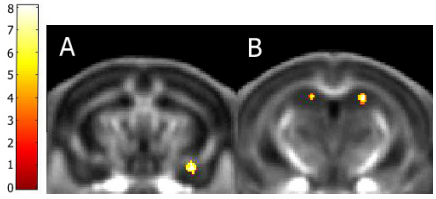


**FIG.1** Voxels with statistically significant changes between pre- and 1 hr post-FC FA maps overlaid on the average FA map from all animals. **A**: FA increase with threshold p<0.001 found in amygdala (36 voxels, n=18). **B:** FA decrease with threshold p<0.005 found in hippocampus (24 voxels, n=9). Color bar illustrates T score of the statistical map.

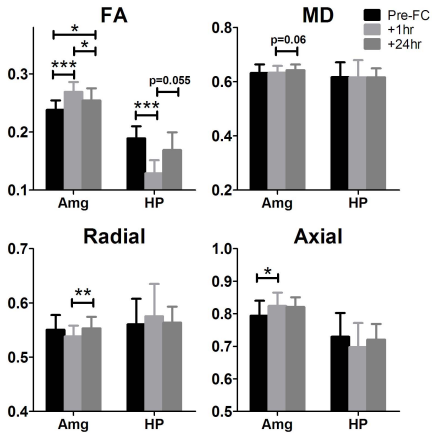


**FIG.2** DTI index changes in the voxels shown in FIG.1 between pre-FC, 1 hr post-FC and 24 hrs post-FC. * p<0.05, ** p<0.005, *** p<0.001.

## Results

Associative learning and fear memories were confirmed by the behavioral analysis as well as cued and contextual tests (data not shown). FIG.1 illustrates the significant voxels found in the paired t-test analysis between pre- and 1 hr post-FC FA maps. One hr after FC training, significant FA increase was found in amygdala (FIG.1A), whereas FA in hippocampus (FIG.1B) was found to decrease significantly. In addition, significant FA increase in cingulum and decrease in posterior thalamus were also detected (data not shown). These regions are well documented to be closely related to associative learning and fear memory (5). Although the sample size in hippocampus is smaller than that in amygdala (n=9 versus 18 due to exclusion of datasets with motion artifacts in the posterior brain), the consistency among animals were examined and confirmed that the effect of fear conditioning on FA changes in specific regions were reproducible. FIG.2 shows the DTI index measurements at all time points from the significant voxels shown in FIG.1 for amygdala and hippocampus. A consistently reverse or recovery trend was observed at 24 hrs post-FC in both regions in terms of FA, radial and axial diffusivity. At 1 hr post-FC, FA increase in amygdala resulted from the radial diffusivity decrease and axial diffusivity increase. On the contrary, FA decrease in hippocampus was accompanied by radial diffusivity increased and axial diffusivity decrease. MD exhibited little change in both regions. FIG.3 demonstrates that synaptophysin expression increased in the central nucleus of amygdala, decreased in polymorph layer of dentate gyrus (DGpo) and CA3 of hippocampus after FC.

## Discussions and Conclusions

The in vivo data at 1hr post-FC showed significant and distinctly different FA changes in amygdala and hippocampus. These changes were observed to reverse at 24 hrs post-FC. Such FA changes were also consistent with radial and axial diffusivity changes. These in vivo measurements indicated that FC induced microstructural plasticity was dynamically regulated, and can be monitored by quantitative DTI. On one hand, our results were consistent with the present consensus that amygdala and hippocampus are the key regions for memory formation and consolidation following classical FC paradigm (6, 7). On the other hand, our results indicated that microstructural plasticity in hippocampus following FC was distinctly different from that in amygdala. This was supported by our IHC results that showed the synapse up-regulation in amygdala while down-regulation in hippocampus, which paralleled with our FA findings. Previous studies using gene and protein expression methods suggested that neuronal morphogenesis and structural plasticity up-regulation in amygdala versus down-regulation occurred in hippocampus half an hour after FC training (8, 9). Electrophysiological study also showed that basolateral amygdala activity peaked at 30-50 min after one-trial fear learning task (10). These short-term changes could lead to a bulk effect on synaptogenesis, dendritic growth and axon branching that are involved in neural plasticity (11). Therefore, it is possible that significant FA changes could be detected in the key regions such as amygdala and hippocampus shortly after FC that could directly arise from fear learning induced memory acquisition and processing. Our in vivo DTI results indicated that microstructural plasticity could induce differential changes in various DTI indices. Although the exact biological processes underlying the DTI changes remain to be elucidated, this study demonstrated that DTI is a sensitive and non-invasive in vivo tool that can provide insights into the microstructural plasticity during fear conditioning.

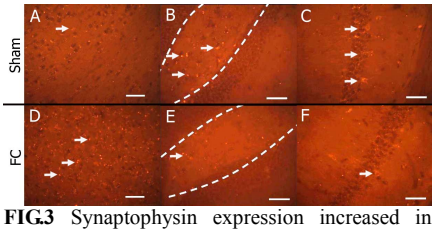


**FIG.3** Synaptophysin expression increased in amygdala and decreased in hippocampus in FC group compared with sham group. Bright dots indicate positive staining of synaptophysin (marker of synapses) as pointed by arrows. **A-C**: Central nucleus of amygdala (CEA), polymorph layer of dentate gyrus (DGpo), and CA3 of hippocampus in a sham animal. **D-F**: Regions in an FC animal that correspond to regions shown in A-C. Scale bars represent 100 μm.

## References

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