Neuroadaptation to Single Traumatic Stressor Revealed by Resting-state fMRI in Awake Rats

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Introduction: Alterations of resting-state functional connectivity (RSFC) have been implicated in a wide range of psychiatric disorders. However, RSFC studies focusing on animal models of psychiatric disorders have been sparse, possibly due to confounding effects of widely used anesthesia on evaluating brain function in animals. To bridge the gap between basic biomedical and human imaging research, in the present study we utilized the awake animal imaging approach established in our lab (1-5) to evaluate an animal model of post-traumatic stress disorder (PTSD) (6,7). We revealed long-lasting impairment of RSFC within the amygdala-mPFC circuit and heightened anxiety level assessed by behavioral measurement after a single-episode predator odor exposure in animals.

Methods: 32 male Long-Evans adult rats (250 – 350 g) were used for behavioral experiments, and among them 16 rats were imaged. For behavioral testing, each rat was habituated for the exposure environment for 10 min for 2 consecutive days. On Day 3, rats were exposed to a piece of cat collar (worn by a cat for 3 months for the trauma group and new for the control group) in an inescapable chamber for 10 min. 7 days after the exposure, the anxiety level of rats was assessed by using the elevated plus maze (EPM) test for 5 min. The ratio between the time spent in open arms and the time spent in open+closed arms was calculated. For the imaging experiment, RSFC was measured immediately following the EPM experiments in 16 rats that were previously acclimated. MRI experiments were conducted on a Bruker 4.7 T magnet. Rats were all fully awake during imaging sessions. Gradient-echo images were then acquired to collect rsfMRI data using the echo-planar imaging (EPI) sequence with the following parameters: TR = 1s, TE = 30ms, flip angle = 60°, matrix size = 64×64, FOV = 3.2cm×3.2cm, slice number=18, slice thickness = 1mm. 200 volumes were acquired for each scan, and nine scans were obtained for each animal. rsfMRI analysis followed the conventional routine: co-registration to a segmented template, motion correction, spatial smoothing (FWHM = 1mm), regression of motion parameters and the signals of white matter and ventricles, and 0.002-0.1Hz band-pass filtering. Scans with excessive motion (>0.25 mm) were discarded. Functional connectivity was estimated by seed-based correlational analysis. The infralimbic (IL) portion of mPFC was selected as the seed region. Correlation coefficients were transformed to z scores using Fisher's z transformation and then averaged across scans and animals in the same group. Subsequently, the averaged z values were transformed back to r values. RSFC maps were displayed by thresholding the correlation coefficients at 0.21 and a cluster size of 10 voxels ($p<0.001$). For the ROI analysis (Fig. 4), correlation coefficients were calculated between regionally averaged ROI time courses.

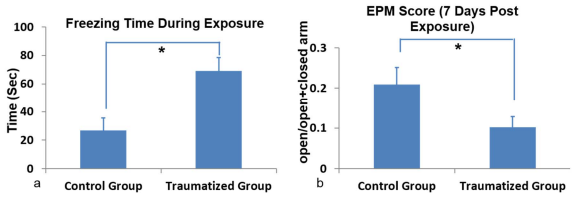

Fig. 1. Behavior measures. a, traumatized group (n=16) showed significantly more freezing behaviors during predator odor exposure compared to control group (n=16). Seven days after the traumatic event, these rats still showed a significantly higher anxiety level in the EPM test. Error bars in SEM. *: $p<0.05$.

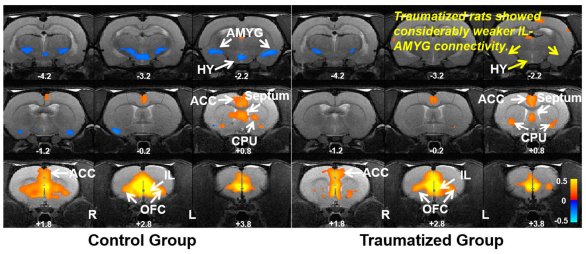

Fig. 2. Long-lasting impact of the trauma stressor on the mPFC-AMYG circuit. ACC: anterior cingulate cortex; AMYG: amygdala; CPU: caudate-putamen; OFC: orbital cortex; HY: hypothalamus.

Results: We found that the freezing behavior was significantly increased during predator odor exposure (Fig. 1. a) and the anxiety level in the rat was also significantly heightened seven days after the exposure (Fig. 1b). Intriguingly, we also found that the functional connectivity within the AMYG-mPFC circuit was considerably compromised seven days after the traumatic event (Fig. 2 and 3). In contrast, no significant difference of functional connectivity between bilateral visual cortices was observed (Fig. 3). ROI-wise analysis also revealed altered RSFC in other regions including hippocampus and ACC (Fig. 4).

Conclusions: The results of the present study provided neuroimaging evidence indicating neuroadaptation induced by traumatic stress in awake rodents. These data also support the face validity and construction validity of using the paradigm of single trauma exposure in an inescapable environment as an animal model of post-traumatic stress disorder.

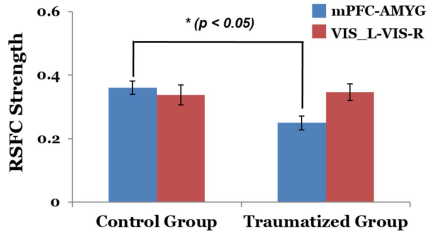

Fig. 3. RSFC strength in the mPFC-AMYG circuit was significantly weaker in traumatized rats compared to control rats ($p < 0.0003$, $n = 8$ for each group). By contrast, trauma exposure did not induce any changes of RSFC in the visual system. Error bars in SEM.

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References: 1. Liang et al., NeuroImage 83:237-44. 2. Liang et al., Neuroimage 59(2):1190-9. 3. Zhang et al., J Neuroscience Met: 189(2): 186–196. 4. Liang et al., J Neuroscience: 31(10):3776 –3783. 5. Liang et al. J Neuroscience: 32(30): 10183-10191. 6. Cohen and Zohar, Ann N Y Acad Sci. 1032:167-178. 7. Cohen et al., Biol Psychiatry. 53:463-473.

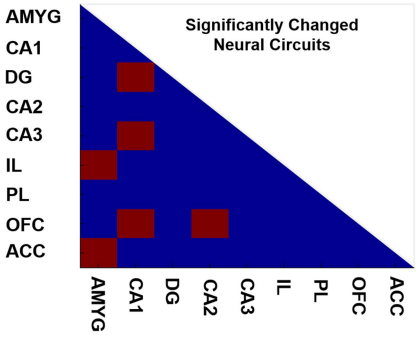

Fig. 4. Long-lasting impact of a single trauma exposure on the neural circuits of mPFC, AMYG and HP. Significant changes in RSFC were observed within neural circuitries between dentate gyrus (DG) and CA1, CA3 and CA1, IL (but not PL) and AMYG, OFC and CA1, as well as ACC and AMYG ($n = 8$, $p < 0.05$, FDR corrected). Regions of mPFC included ACC, IL and PL; Regions in HP included CA1, CA2, CA3