

Change in Tissue Microstructure and Resting-state Functional Connectivity in Hippocampus during Pregnancy

Russell W. Chan^{1,2}, Leon C. Ho^{1,2}, Iris Y. Zhou^{1,2}, and Ed X. Wu^{1,2}

¹Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Hong Kong SAR, China, ²Department of Electrical and Electronic Engineering, The University of Hong Kong, Hong Kong SAR, China

INTRODUCTION: Pregnancy is associated with hormonal changes which remodel the maternal brain structurally and functionally [1]. It was reported that the hippocampal dendritic spine density increased during pregnancy [2]. Moreover, previous studies showed that pregnancy improved spatial learning and memory [3] and, reduced anxiety and stress responsiveness [4-5]. Furthermore, it was documented that the hippocampus could be altered by learning, memory, anxiety and stress [6]. Therefore, we hypothesized that pregnancy induces microstructural and functional connectivity changes in the hippocampus. Diffusion tensor imaging (DTI) provides microstructural information and has been used to probe neural plasticity [7]. Resting-state functional connectivity MRI (RSfMRI) is the measure of spontaneous fluctuations in the BOLD signal. Recently it has been demonstrated that individual differences in functional connectivity were correlated to individual differences in behavior and performance [8-9]. In this study, we aimed to study the rat pregnancy model by DTI and RSfMRI to investigate the microstructural changes, functional connectivity changes, as well as the parallel relationship between microstructural changes and functional connectivity changes in the hippocampus.

MATERIALS AND METHOD: Animal Preparation: Pregnant female Sprague-Dawley rats (N=5, 16 weeks) were induced. The subjects were examined with MRI 4 days before mating and gestational day 17 (G17). The subjects were under mechanical ventilation with 1.5% isoflurane anesthesia during MRI experiments. **MRI Protocols:** All MRI measurements were acquired utilizing the 7T Bruker scanner with a quadrature surface coil. Diffusion-weighted images were acquired using 4 shot SE-EPI sequence with 30 diffusion gradient directions, TR/TE=6000/31.612ms, $\delta/\Delta=5/17$ ms, NEX=4, FOV=46x46mm², MTX=128x128, twenty four 0.36- mm-slices without gaps and b-value=1000. Five additional images with b-value=0 (b₀ images) were also acquired. RSfMRI were acquired using a single-shot GE-EPI sequence with TR/TE=1000/20ms, flip angle=56°, FOV=32x32mm², MTX=64x64, 10 1mm slices without gaps and a total of 440 data points. RARE T2W images were acquired with TR/TE=3500/36ms as anatomical reference for EPI images. **Data Analysis:** All diffusion-weighted images were registered to the respective b₀ image. Fractional anisotropy (FA) map was calculated. Subsequently, voxel based morphometry (VBM) was applied. Lastly, with reference to VBM results, two 3x3 voxels region of interest (ROI) in the dentate gyrus was selected for quantitation. All RSfMRI data were compensated for slice timing, detrended, realigned as well as temporally low-pass filtered to obtain low frequency fluctuations. Subsequently, inter-animal co-registration was performed using respective anatomical references. Z-score maps of the hippocampal network were obtained using GIFT v1.3h (Group ICA Toolbox) and masked with the two 3x3 voxels ROIs in the hippocampus. Correlation coefficients were calculated using seed-based analysis (SBA) with a pair of 3x3 seed voxels and 3x3 ROI in the hippocampus, same as the ROIs used in ICA. Statistic evaluation was conducted using paired t-test and results were considered significant when p<0.05 (*, ** denote p<0.05 and p<0.01 respectively.)

RESULTS: Fig.1 shows the t-score map resulted from VBM as well as the ROI selection for tissue microstructural quantitation. Fig.2 shows the mean z-score map resulted from independent component analysis (ICA). Fig.3 shows a typical correlation coefficient (CC) map resulted from seed-based analysis (SBA). The statistical evaluations are summarized in Fig.4. The functional connectivity measured using SBA and ICA both showed significant increase. The tissue microstructure measured using FA also showed significant increase. The relationship between FA and CC in the hippocampus of each individual subject is shown in Fig.5. Both the FA and CC values were observed to increase during pregnancy in each individual subject. More importantly, each subject exhibited similar trend.

DISCUSSION AND CONCLUSION: It was reported that the hippocampal dendritic spine density increased in female rats during late pregnancy when compared to nulliparous female rats [2]. It was also suggested that this additional neural plasticity facilitated learning and memory [2-6]. Previously, it was proposed that RSfMRI could be related to individual behavior and performance [10]. Hence, the increased FA was likely related to the increased hippocampal dendritic spine density [2] and the increased interhemispheric functional connectivity in hippocampus was probably contributed to the improvement in spatial learning and memory as well as reduction in anxiety and stress responsiveness [3-6]. Furthermore, the strong correlation observed between FA and CC suggested that the FA and CC values of individual subjects were coupled during pregnancy. It has been proposed that functional connectivity is partly reflective of interactions between structurally connected neuronal populations, but strong functional connections still exists between regions with no direct structural connections [11]. Hence, the mechanism coupling the structural and functional connections is still unclear. The current rat pregnancy model provides direct manipulations on structural and functional changes. It may serve as a valuable tool for investigating the structural and functional relationships. In conclusion, our results supported that pregnancy induced tissue microstructural and functional connectivity changes in the hippocampus. Furthermore, the results indicated that fractional anisotropy and functional connectivity were coupled during pregnancy. Hence, allowing the rat pregnancy model to serve as a tool for studying the structural and functional relationships. Further studies combining fiber tracking, histology and/or electrophysiology are required to confirm the coupled relationship, as well as to elucidate the mechanism and causality of these coupled changes.

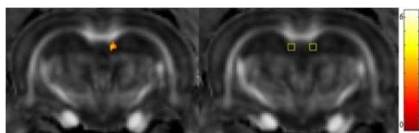


Fig.1: The t-score map overlaid on fractional anisotropy (FA) shown on the left indicates voxels with significant FA increase during pregnancy. The threshold is p=0.05. The region of interest (ROI) shown on the right was applied for quantitation.

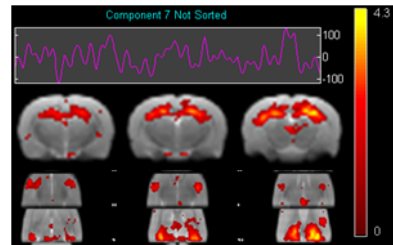


Fig.2: The mean z-score map of the interhemispheric hippocampal network obtained with independent component analysis (ICA).

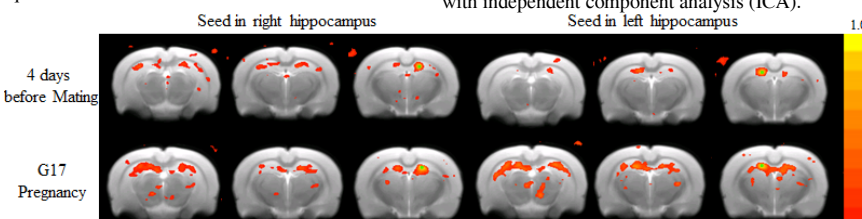


Fig.3: The correlation coefficient maps obtained using based analysis (SBA). It shows the functional connectivity is higher and more dispersed during pregnancy. The threshold is 0.5.

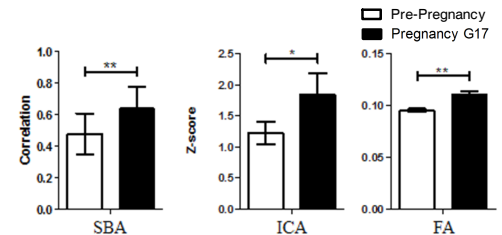


Fig.4: Comparison of functional connectivity 4 days before mating and G17 pregnancy in the hippocampus using seed based analysis (SBA) and independent component analysis (ICA). Comparison of microstructural integrity 4 days before mating and G17 pregnancy in the hippocampus using FA. Two-tailed paired t-test was applied.

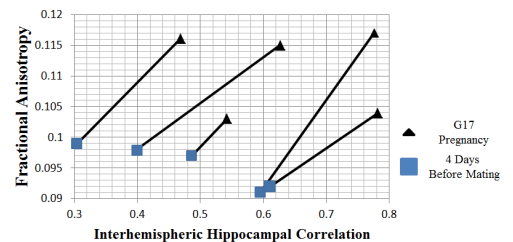


Fig.5: The relationship between FA (as a measure of microstructural integrity) and correlation coefficient (as a measure of functional connectivity) in the hippocampus of individual subjects 4 days before mating and G17 pregnancy.

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