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 (RSfcMRI) 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 RSfcMRI 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, MTX=128x128, 20 four 0.36-mm-slices without gaps and v-value=1000. Five additional images with v-value=0 (b=0 images) were also acquired. RSfcMRI were acquired using a single-shot GE-EPI sequence with TR/TE=1000/20ms, flip angle=55°, FOV=232x232mm, T2-weighted 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 b=0 image. Fractional anisotropy (FA) map was calculated. Subsequently, voxel based morphometry (VBM) was applied. Lastly, with reference to VBM results, two 3×3 voxels region of interest (ROI) in the dentate gyrus was selected for quantification. All RSfcMRI data were compensated for slice timing, detrended, realigned as well as temporally low-pass filtered to obtain low frequency fluctuations. Subsequently, inter- and intra-co-registration was performed using respective anatomical references. Z-score maps of the hippocampal network were obtained using GIFT v1.3b (Group ICA Toolbox) and mask with the two 3×3 voxels ROIs in the hippocampus. Correlation coefficients were calculated using seed-based analysis (SBA) with a pair of 3×3 seed voxels and 3×3 ROI in the hippocampus, same as the ROIs used in ICA. Statistical evaluation was conducted using paired t-test and results were considered significant when 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 RSfcMRI 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.