Altered resting-state functional connectivity and anatomical connectivity of hippocampus in schizophrenia

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Abstract

Hippocampus has been implicated in participating in the pathophysiology of schizophrenia. However, the functional and anatomical connectivities between hippocampus and other regions are rarely concurrently investigated in schizophrenia. In the present study, both functional magnetic resonance imaging (fMRI) during rest and diffusion tensor imaging (DTI) were performed on 17 patients with paranoid schizophrenia and 14 healthy subjects. Resting-state functional connectivities of the bilateral hippocampi were separately analyzed by selecting the anterior hippocampus as region of interest. The fornix body was reconstructed by diffusion tensor tractography, and the integrity of this tract was evaluated using fractional anisotropy (FA). In patients with schizophrenia, the bilateral hippocampi showed reduced functional connectivities to some regions which have been reported to be involved in episodic memory, such as posterior cingulate cortex, extrastriate cortex, medial prefrontal cortex, and parahippocampus gyrus. We speculated that these reduced connectivity may reflect the disconnectivity within a neural network related to the anterior hippocampus in schizophrenia. Meanwhile the mean FA of the fornix body was significantly reduced in patients, indicating the damage in the hippocampal anatomical connectivity in schizophrenia. The concurrence of the functional disconnectivity and damaged anatomical connectivity between the hippocampus and other regions in schizophrenia suggest that the functional–anatomical relationship need to be further investigated.

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Keywords: Schizophrenia; Hippocampus; fMRI; Diffusion tensor tractography; Functional connectivity; Anatomical connectivity

Abbreviations: fMRI, functional magnetic resonance imaging; DTI, diffusion tensor imaging; FA, fractional anisotropy; BOLD, blood oxygen level-dependent; DT-t, diffusion tensor tractography; PCC, posterior cingulate cortex; MPFC, medial prefrontal cortex; STG, superior temporal gyrus.

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1. Introduction

Convergent evidence from histological, molecular biology, structural, neuropsychological and functional imaging suggests that hippocampus involves in the pathophysiology of schizophrenia (Gothelf et al., 2000; Harrison, 2004). According to the opinion that the complex clinical presentations of schizophrenia are contributed to the abnormality in inter-regional interaction rather than the abnormality of single region (Friston, 1998), it is significant to investigate the interaction between the hippocampus and other brain regions from two different perspectives, i.e., functional and anatomical connectivity.

Recently, resting-state functional connectivity attracts more and more researchers’ attention. Spontaneous low-frequency (<0.08 Hz) fluctuation (SLFF) of the signal in the blood oxygen level-dependent (BOLD) MR imaging is often used to measure the resting-state functional connectivity (Biswal et al., 1995; Greicius et al., 2003; Lowe et al., 1998). The low-frequency BOLD coherence may be related to neuronal activity (Leopold et al., 2003). And many researchers have observed that in functionally related brain regions, even located remotely, these fluctuations are synchronous (Biswal et al., 1995; Lowe et al., 1998). This implies the existence of neuronal connections that facilitate coordinated activity in the human brain. In different neuropsychiatric diseases, including schizophrenia, the abnormalities in resting-state functional connectivity have been reported (Liang et al., 2006; Tian et al., 2006; Wang et al., 2006; Zhou et al., 2007a; Zhou et al., 2007b). Liang et al found that the temporal regions including hippocampus showed reduced functional connectivities with distributed brain regions during rest in schizophrenia (Liang et al., 2006). Because this study focused on the inter-regional functional connectivities of 116 regions of the whole brain, this preliminary study cannot reveal detailed information on the hippocampal connectivity patterns of schizophrenia.

In terms of the other two recent studies, one was interested in the functional connectivity pattern of the dorsolateral prefrontal cortex not that of the hippocampus (Zhou et al., 2007a), and the other focused on the inter-regional functional connectivities just within the default mode network and its anti-correlated network or between the networks (Zhou et al., 2007b). Thus exact information on functional connectivity of the hippocampus could not be obtained from the above studies. In order to obtain more precise and detailed information, it is necessary to analyze the resting-state functional connectivity patterns of the hippocampus in a voxel-wise matter.

On the other hand, a relative new neuroimaging technique, diffusion tensor imaging (DTI), affords the possibility to in vivo explore anatomical connectivity in the human brain. By measuring the degree of anisotropy in random motion of water molecules, DTI provides the information about cellular integrity and pathology (Eriksson et al., 2001; Le Bihan, 2003; Rugg-Gunn et al., 2002). A higher anisotropy of diffusion reflects a motion of water molecules favored in a specific direction, for example, parallel to highly structured white matter fibers. By tracking along the principal diffusion direction, the course of white matter fiber tracts may be visualized, which is known as diffusion tensor tractography (DTI). Fornix is a major pathway linking the hippocampus with other brain regions (Chance et al., 1999; Kuroki et al., 2006). Although the fornix integrity in schizophrenia has been assessed by ROI-based (Kuroki et al., 2006) and voxel-based (Kanaan et al., 2005; Kubicki et al., 2007; Kubicki et al., 2005) analysis, the results are inconsistent. In the present study, we investigated the fornix integrity using DTI, which has not been performed in schizophrenia although it has been used to visualize the fornix in healthy subjects (Catani et al., 2002; Wåkana et al., 2004) or epilepsy patients (Concha et al., 2005). We hypothesized that the resting-state functional connectivity of the hippocampus would be decreased; meanwhile, the integrity of the fornix measured by DTI would be damaged in the same patients with schizophrenia. In order to test our hypothesis, we investigated the hippocampal functional connectivity by using region-of-interest (ROI)-based correlation analysis (Biswal et al., 1995; Wang et al., 2006). We selected the anterior hippocampus as ROI based on the following considerations. 1) The anterior hippocampus may be relevant to hypotheses regarding the pathophysiology of schizophrenia (Szeszko et al., 2003). 2) MRI studies suggest that the subtle hippocampal changes in schizophrenia may primarily involve the anterior division (Csernansky et al., 2002; Heckers and Konradi, 2002; Narr et al., 2004; Pegues et al., 2003; Szeszko et al., 2003). 3) Abnormalities in anterior hippocampal regions in patients with schizophrenia are linked to deficits on neuropsychological tests related to frontal lobe function (Bilder et al., 1995), impaired ability to identify new items (Weiss et al., 2004) and verbal forgetting (Rametti et al., 2007). In addition, given the existence of extensive afferent and efferent neocortical connections in the anterior hippocampus, which integrates neural activity from widespread neocortical inputs and outputs (Sperling et al., 2003; Wang et al., 2006), this region is an appropriate candidate to link functional connectivity and anatomical connectivity.

By combining of DTI and functional connectivity analyses, the current study provides a particular perspective to understand the abnormality of the connectivity associated with the hippocampus in schizophrenia. This
investigation is expected to enrich our understanding to the role of the hippocampus in the pathophysiology of schizophrenia.

2. Materials and methods

2.1. Subjects

Patients with paranoid schizophrenia were recruited from the inpatient unit at the Institute of Mental Health, Second Xiangya Hospital of Central South University, from February 2005 to July 2005, and paid normal controls were recruited by advertisements. Subjects met the following criteria: age between 18 and 45 years, right-handed, no history of neurological or significant physical disorders, no history of alcohol or drug dependence, no history of receiving electroconvulsive therapy, completing at least high school, no history of neurological or significant physical disorders, no history of alcohol or drug dependence, no history of receiving electroconvulsive therapy, and no history of neurological or significant physical disorders. Normal controls were matched for age, gender, education level, and ethnicity.

2.2. Data acquisition

Magnetic Resonance Imaging was performed on a 1.5-T GE magnetic resonance scanner. Foam pads were used to reduce head motion and scanner noise. Three-dimensional T1-weighted images were acquired in a sagittal orientation employing a 3D-SPGR sequence (TR/TE=12.1/4.2 ms, flip angle=90°, FOV=24 cm). Whole-brain volumes were acquired with 20 contiguous 5-mm thick transverse slices, with a 1 mm gap and 3.75 x 3.75 mm² in-plane resolution. For each participant, the fMRI scanning lasted for 6 min. At the same locations as the functional images, the T1 anatomical image was obtained for each subject (TR/TE=2045/9.6 ms, flip angle=90°, FOV=24 cm).

Diffusion tensor imaging was acquired with single-shot echo planar imaging sequence in alignment with the anterior–posterior commissural plane. The diffusion sensitizing gradients were applied along 13 non-collinear directions (b=1000 s/mm²), together with an acquisition without diffusion weighting (b=0). Thirty contiguous axial slices were acquired with a slice thickness of 4 mm and no gap. The acquisition parameters were as follows: TR=12 000 ms; TE=105 ms; image matrix=128 x 128; FOV=24 cm; NEX=5. The parameters resulted in 1.875 x 1.875 x 4 mm³ acquisition voxel dimensions, interpolated to 0.9375 mm cubic size.

2.3. fMRI procedure

2.3.1. Data preprocessing

The data preprocessing was performed in MNI space. The first 10 volumes of each functional time series were discarded and the remaining images were preprocessed using the following steps: slice timing, motion correction, spatial normalization to the standard MNI space and resampling to 3 x 3 x 3 mm³, followed by spatial smoothing with 4 mm full-width at half maximum (FWHM) Gaussian kernel. All these processes were conducted by the statistical parametric mapping software.
package (SPM2, Wellcome Department of Imaging Neuroscience, London, UK).

According to the record of head motions within each fMRI run, all participants had less than 1 mm maximum displacement in x, y or z and less than 1° of angular rotation about each axis. As correlation analysis is sensitive to gross head motion effects, we further characterized the peak displacements as a measure of head motion for each subject (Jiang et al., 1995; Lowe et al., 1998), and no significant difference was found between groups (0.33 ± 0.19 mm for normal subjects, 0.35 ± 0.19 mm for paranoid patients, \( p = 0.8 \)). To further reduce the effects of confounding factors, six motion parameters, linear drift and the mean time series of all voxels within the entire brain were removed from the smoothed data through linear regress. Then the fMRI data were temporally band-pass filtered (0.01–0.08 Hz) using AFNI (http://afni.nimh.nih.gov/) (Fox et al., 2005; Greicius et al., 2003; Lowe et al., 1998).

In order to create a mask, firstly, the T1 anatomical images of all subjects were striped using the software MRICro (http://www.sph.sc.edu/comd/rorden/mricro.html), and then normalized to the standard MNI space. Finally, the mask was obtained by taking the intersections of the normalized T1 anatomical images of all subjects. Only the voxels within the mask were further processed.

In order to visualize the statistical results, a mean anatomical image was obtained by averaging the normalized high-resolution 3D T1-weighted images across all subjects.

2.3.2. Definition of regions of interest (ROIs)

We selected the anterior hippocampus as ROI based on the reasons mentioned in Introduction section. After spatial normalization, bilateral hippocampi were outlined manually on the coronal slice located at the dorsal margin of the mamillary body on high-resolution 3D images (Fig. 1). This has been used in a previous study (Wang et al., 2006). In brief, the dorsolateral border was delimited by the alveus; the ventral border was defined by the grey–white matter interface between the subiculum and the parahippocampal gyrus; and the medial border was differentiated from the adjacent cerebrospinal fluid. The margin of these boundaries and the amygdalohippocampal transition area were excluded from the ROIs (Wang et al., 2006).

Then the structural images of ROIs were coregistered to fMRI images applying 12-parameter affine transformation, and only voxels in fMRI images that were covered for more than 55% by the original structural ROI were used for further analyses.

2.3.3. Functional connectivity and statistics analysis

A voxel-wise functional connectivity analysis of ROI was used (Tian et al., 2006; Wang et al., 2006; Zhou et al., 2007a). The seed reference time series of each ROI was obtained by averaging the fMRI time series of all voxels within the ROI. Correlation analysis was carried out between the seed reference and the rest of whole brain in a voxel-wise manner using an in-house matlab program. Then, the correlation coefficients were transformed to \( z \)-values using the Fisher \( r \)-to- \( z \) transformation to improve normality. This produced spatial maps in which the values of voxels represented the strength of the correlation with the ROIs. Within each group, individual \( z \)-values were entered into a one-sample \( t \)-test in a voxel-wise manner to determine the brain regions showing significantly positive or negative connectivity to the left or right hippocampus. A combined threshold of contrast maps was set at \( p < 0.005 \) for each voxel and a cluster size of at least 432 mm\(^3\), which was equal to the corrected threshold of \( p < 0.005 \), determined by Monte Carlo simulation (see program AlphaSim by B.D. Ward in AFNI software. Parameters were: single voxel \( p = 0.005 \), FWHM = 4 mm, with mask, http://afni.nimh.nih.gov/). In order to compare the functional connectivity of left hippocampus between the control and patient group, the \( z \)-values were entered into a two-sample \( t \)-test in a voxel-wise manner to determine the brain regions that show significant differences in positive or negative connectivity to the left hippocampus. A combined threshold of contrast maps was set at \( p < 0.005 \) for each voxel and a cluster size of at least 432 mm\(^3\), which was equal to the corrected threshold of \( p < 0.005 \) determined by Monte Carlo simulation. Exactly the same statistical analysis was performed for the right hippocampus.
2.4. DTI procedure

2.4.1. Data preprocessing

The DTI image preprocessing and fiber tracking were performed using DTI Studio software (free software from Radiology department, Johns Hopkins University, USA). The diffusion tensor elements were estimated by solving the Stejskal and Tanner equation (Basser et al., 1994), and then the reconstructed tensor matrix was diagonalized to obtain eigenvalues $\lambda_1$, $\lambda_2$, $\lambda_3$ and eigenvectors. The fractional anisotropy (FA) of each voxel was calculated according to the following formula:

$$FA = \sqrt{\frac{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_2 - \lambda_3)^2}{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}$$

2.4.2. Fiber tracking and definition of the fornix body

The fornix was reconstructed for each subject using the “fiber assignment by continuous tracking” method (Mori et al., 1999) which was implemented by DTI Studio software. All tracts in the dataset were computed by seeding each voxel with a FA greater than 0.15. Tractography was terminated if it turned an angle greater than 10° or reached a voxel with a FA less than 0.15. In order to obtain better anatomical correspondence across populations, we just analyzed the fornix body (which includes the fibers from the left and right hippocampal neurons) for each subject. Based on the anatomical knowledge of fiber projections, first, the whole left and right fornix tracts were respectively selected by placing left and right ROIs in the coronal slices of FA-weighted color map where the two separated crux can be clearly seen (Fig. 2A). Then we used two slices to define the fornix body: one is the axial slice where the furcated fornix column just can be seen on the FA map (Fig. 2B), and another is the coronal slice that passes the posterior commissure on the coregistered T1 image (Fig. 2C). In order to precisely locate the posterior commissure, the 3D T1-weighted image of each subject was coregistered to his b0 image by applying a 12-parameter affine transformation using SPM2. The part of fornix tracts locating both superior to the slice one and anterior to the slice two was defined as the fornix body to be analyzed (Fig. 3).

Fig. 2. Definition of the fornix body. (A) Region of interest (ROI) placement for tract selection of the fornix in a coronal slice of the color map. The two circles indicate the left and right ROI. (B) The axial slice where the furcated fornix column just can be seen on the FA map. The square indicates the furcated fornix column. (C) The coronal slice which passes the posterior commissure on the coregistered T1 image. The square indicates the posterior commissure.

Fig. 3. Fiber tracts reconstruction for the fornix between the axial slice of the furcated fornix column and the coronal slice of the posterior commissure, demonstrated in right-side view (A), left-side view (B) and top view (C). Blue represents the right side of the fornix body, yellow represents the left side and red represents the removed part of the fornix.
2.4.3. Diffusion tensor image statistical analysis

Mean FA was calculated for each subject by averaging the diffusion measurement across the voxels that form the fornix body derived from tractography. A two-sample t-test was performed to assess differences in mean FA of the fornix body between groups.

2.5. Correlation analysis

In the current study, we investigated the clinical correlates of two kinds of imaging measures, i.e. the strength of altered functional connectivities and the mean FA value of the fornix body in schizophrenia. Five clinical measures were included, i.e. PANSS total score (PANSS_P), score of PANSS positive subscale (PANSS_P), score of PANSS negative subscale (PANSS_N), medication does (excluding five non-medications), and illness duration. In terms of the clinical correlates of the functional connectivity strength, correlation analyses were performed between the z-values of the peak voxels of the altered functional connectivities and these clinical variables in patients with schizophrenia. If the number of the altered connectivity is n, then the number of correlation analyses is $5 \times n$. Following the Bonferroni correction for multiple comparisons, the level of significance was established at $p$ equal or inferior to $0.05/5 \times n$. In terms of the clinical correlates of the DTI measure, correlation analyses were performed between the mean FA value of the fornix body and these five clinical variables. The Bonferroni adjustment lowered the alpha to 0.01.

In addition, we investigated the correlations between the strength of the altered functional connectivities and the mean FA value of the fornix body. The Bonferroni adjustment lowered the alpha to $0.05/n$ for the $n$ correlation analyses.

3. Results

3.1. Hippocampus functional connectivity analysis within the normal control group and within the patient group

In the normal control group, the left (Fig. 4A) and right (Fig. 4B) hippocampi showed significant positive functional connectivities with a number of regions, including bilateral hippocampi and parahippocampus gyri, bilateral...
temporopolar cortices and lateral temporal regions, posterior cingulate cortex (PCC), precuneus/cuneus, medial prefrontal cortex (MPFC), left precentral gyrus, anterior cingulate cortex, and several subcortical structures (striatum and midbrain). In addition, both the lateral prefrontal cortex and the cerebellum showed negative connectivities with the bilateral hippocampi. Most of these connectivities have been observed in healthy control subjects (Vincent et al., 2006; Wang et al., 2006) No asymmetry of functional connectivity was observed in the present study. In the patients with paranoid schizophrenia, the left (Fig. 5A) and right (Fig. 5B) hippocampi did not show any asymmetry.

Table 2

<table>
<thead>
<tr>
<th>Brain regions showing significant changes in bilateral hippocampus functional connectivity in schizophrenia</th>
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<tr>
<td>Brain regions</td>
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<tr>
<td>Decreased positive correlation with the left hippocampus</td>
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<tr>
<td>Posterior cingulate cortex extending to right cuneus</td>
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<tr>
<td>Right superior temporal gyrus</td>
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<tr>
<td>Right parahippocampus gyrus</td>
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<tr>
<td>Left medial temporal pole</td>
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<tr>
<td>Left parahippocampus gyrus</td>
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<tr>
<td>Left cuneus</td>
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<tr>
<td>Decreased negative correlation with the left hippocampus</td>
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<tr>
<td>Left cerebellum</td>
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<tr>
<td>Decreased positive correlation with the right hippocampus</td>
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<tr>
<td>Right middle occipital gyrus/cuneus</td>
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<tr>
<td>Right medial prefrontal cortex</td>
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<tr>
<td>Decreased positive correlation with the right hippocampus</td>
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</table>

*(x,y,z)* coordinates of primary peak locations in the Montreal Neurological Institute space.
3.2. Altered functional connectivity of bilateral hippocampus in the patient group

When comparing the pattern of functional connectivity of the left hippocampus between the control and patient groups, a set of regions, including PCC extending to right cuneus, left cuneus (bilateral cunei are located at the dorsal stream of the extrastriate cortex in this study), bilateral parahippocampus gyri, right superior temporal gyrus (STG), and left medial temporal pole, showed significantly reduced positive connectivity (i.e. correlation coefficient of the control group larger than that of the patient group) to the left hippocampus in patients with paranoid schizophrenia. And one region in left cerebellar posterior lobe showed reduced negative connectivity (i.e. absolute value of correlation coefficient of the control group larger than that of the patient group) to the left hippocampus in patients with paranoid schizophrenia (Table 2, Fig. 6A). No enhanced positive or negative connectivities were observed in patient group compared to the normal group.

In the case of the right hippocampus, right middle occipital gyrus/cuneus and right MPFC showed reduced positive connectivity to the right hippocampus in patients with paranoid schizophrenia. No reduced negative connectivity or enhanced positive or negative connectivities were observed in patients with paranoid schizophrenia (Table 2, Fig. 6B). Visual inspection of the connectivity maps revealed that more regions showed altered functional connectivities with the left hippocampus in schizophrenia, compared with those connected to the right hippocampus.

3.3. DTI measures of the fornix

The mean FA value of the fornix body in patients with schizophrenia (0.32 ± 0.03) was significantly lower ($t=3.19, p=0.003$) than the normal controls (0.35 ± 0.03).

3.4. Correlation analyses

In the current study, we found nine clusters showing altered functional connectivity with the bilateral anterior hippocampi (Table 1). According to the alpha adjusted via Bonferroni correction for multiple comparisons, we

![Fig. 6. Differences of functional connectivity of left (A) and right (B) hippocampus between the control and the patient groups. The left side of image represents the left side of brain. Color bar indicates the T-score. Warm color illustrates the regions showing reduced positive connectivity in the patient group. And cool color illustrates the regions showing reduced negative connectivity in the patient group. No regions showing increased positive or negative connectivity were found. Abbreviations: (a) cerebellar posterior lobe, (b) medial superior temporal gyrus, (c) parahippocampus gyrus, (d) superior temporal gyrus, (e) cuneus, (f) posterior cingulate cortex extending to right cuneus, (g) medial prefrontal cortex.](#)
Table 3
The clinical correlates of the strength of the altered functional connectivities in schizophrenia and the correlation between the strength of these functional connectivities and the mean FA value of the fornix body within each group.

<table>
<thead>
<tr>
<th>Brain regions</th>
<th>PANSS_T</th>
<th>PANSS_P</th>
<th>PANSS_N</th>
<th>Medication does*</th>
<th>Illness duration</th>
<th>FA within patients</th>
<th>FA within controls</th>
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<tbody>
<tr>
<td>Decreased positive correlation with the left hippocampus</td>
<td></td>
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</tr>
<tr>
<td>Posterior cingulate cortex extending to right cuneus</td>
<td>0.611**</td>
<td>0.633**</td>
<td>0.479</td>
<td>0.233</td>
<td>0.006</td>
<td>−0.105</td>
<td>−0.098</td>
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<tr>
<td>Right superior temporal gyrus</td>
<td>0.093</td>
<td>0.006</td>
<td>0.284</td>
<td>−0.11</td>
<td>−0.14</td>
<td>−0.112</td>
<td>0.398</td>
</tr>
<tr>
<td>Right parahippocampus gyrus</td>
<td>0.223</td>
<td>0.46</td>
<td>0.371</td>
<td>0.452</td>
<td>0.012</td>
<td>−0.109</td>
<td>0.137</td>
</tr>
<tr>
<td>Left medial temporal pole</td>
<td>0.146</td>
<td>0.136</td>
<td>0.101</td>
<td>0.421</td>
<td>0.137</td>
<td>−0.042</td>
<td>0.328</td>
</tr>
<tr>
<td>Left parahippocampus gyrus</td>
<td>−0.16</td>
<td>−0.215</td>
<td>0.171</td>
<td>0.049</td>
<td>−0.12</td>
<td>−0.425</td>
<td>0.07</td>
</tr>
<tr>
<td>Left cuneus</td>
<td>0.583*</td>
<td>0.482</td>
<td>0.428</td>
<td>0.615*</td>
<td>−0.01</td>
<td>0.196</td>
<td>−0.155</td>
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<td>Decreased negative correlation with the left hippocampus</td>
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<tr>
<td>Left cerebellum</td>
<td>−0.29</td>
<td>−0.223</td>
<td>−0.273</td>
<td>−0.56</td>
<td>−0.28</td>
<td>0.457</td>
<td>−0.457</td>
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<tr>
<td>Decreased positive correlation with the right hippocampus</td>
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<tr>
<td>Right middle occipital gyrus/cuneus</td>
<td>0.517*</td>
<td>0.544*</td>
<td>0.341</td>
<td>0.41</td>
<td>−0.08</td>
<td>−0.004</td>
<td>−0.086</td>
</tr>
<tr>
<td>Right medial prefrontal cortex</td>
<td>−0.33</td>
<td>−0.273</td>
<td>−0.335</td>
<td>−0.04</td>
<td>0.145</td>
<td>0.168</td>
<td>−0.549*</td>
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<tr>
<td>Decreased positive correlation with the right hippocampus</td>
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The numbers in the table is the Pearson’s correlation coefficients. *p<0.05; **p<0.01; PANSS_P: positive symptom score obtained from PANSS; PANSS_N: negative symptom score obtained from PANSS; a: Chlorpromazine equivalent excluding 5 non-medications.

found no significant correlations between the strength of the altered functional connectivities and these clinical variables. However, we observed the trend towards significance between the PANSS total score and the strength of the left hippocampus-PCC/right cuneus connectivity, that of the left hippocampus-left cuneus connectivity and that of the right hippocampus-right cuneus connectivity, between the score of positive symptom and the strength of the left hippocampus-PCC/right cuneus connectivity and that of the right hippocampus-right cuneus connectivity, between medication does and the left hippocampus-left cuneus connectivity (p<0.05, uncorrected) (Table 3). No significant correlations or the trend towards significance were observed between these clinical variables and the mean FA value of the fornix body (p>0.05, uncorrected). No significant correlations but the trend towards significance between the FA value and the strength of the right hippocampus-right MPFC connectivity (Pearson’s r = −0.549, p = 0.042) was observed within the control group (Table 3).

4. Discussion

The distinctness between the current study and the previous studies (Liang et al., 2006; Zhou et al., 2007a,b) is that the current study focused on the neural network related to the anterior hippocampus. More importantly, the current study combined the resting-state functional connectivity and DT-t analyses. The multimodal imaging approaches can provide a particular perspective to understand the connectivity and abnormality of the connectivity in psychiatric diseases (Ramnani et al., 2004).

In the current study, we found both the reduced resting-state functional connectivities of the bilateral hippocampi and the impaired fornix integrity in patients with paranoid schizophrenia. These reductions in the functional interaction and the anatomical connectivity between the hippocampus and other regions provide further evidence for the implication of the hippocampus in schizophrenia.

The hippocampus plays an important role in memory, especially episodic memory (Eichenbaum and Fortin, 2005; Moscovitch et al., 2006; Suzuki, 2006). A recent study found that the healthy subjects’ hippocampus, several parietal region including PCC, MPFC and temporal lobe showed coherent spontaneous activity in the absence of task, stimuli, or explicit mnemonic demands, and thus proposed that the regions spontaneously correlated with the hippocampus constitute a memory network (Vincent et al., 2006). In the present study, we noticed that some regions in this memory network, such as posterior neocortex (including PCC and its adjacent cuneus), MPFC, temporal pole and parahippocampus gyrus, showed reduced functional connectivities to the left or right hippocampus in patient group. These regions also overlapped with the regions constituting the “default mode” network (Fox et al., 2005; Fransson, 2005; Greicius et al., 2003), which is implicated in retrieval and manipulation of episodic memory (Greicius et al., 2003; Vincent et al., 2006). PCC is the region
showing the highest metabolism level in the resting-state (Greicius et al., 2003; Raichle et al., 2001) and have been suggested to be involved in retrieval of episodic memories (Greicius et al., 2003; Vincent et al., 2006). Its adjacent cunei which are located in the dorsal stream of the extrastriate cortex in this study, has also been suggested to be involve in the retrieval of visual information from the episodic memory by observing their interactions with the medial temporal lobe during retrieval of information of spatial location (Kohler et al., 1998). The co-activity of MPFC and hippocampus has been found during an autobiographical retrieval task (Cabeza et al., 2004), and the enhanced activity of hippocampus, MPFC and temporal pole was observed during retrieval of personally relevant time-specific memories (Maguire and Mummery, 1999). The parahippocampal gyrus is thought to be involved in the translation of temporary hippocampal information storage to a more permanent storage in cortical association areas (Rolls, 2000). According to the finding of an experimental model study, the reduction in the connectivity between the two regions would result in a pattern of deficits that closely mimics the impairments of episodic memory in schizophrenia (Talairach et al., 2005). Combining these facts with our findings, we speculate that the reduced connectivity between the hippocampus and these regions during rest may reflect a breakdown of episodic memory network centered as hippocampus in schizophrenia, where the impaired episodic memory is often observed (Talairach et al., 2005). However, due to the lack of episodic memory performance in this study, this speculation needs to be validated in future study.

The fornix is the major linking pathway between the hippocampus and other regions. The damages in the fornix integrity undoubtedly will lead to the abnormality in the anatomical connectivity of the hippocampus. Unlike previous studies which conventionally assess the fornix integrity by ROI-based or voxel-based (Kanaan et al., 2005; Kubicki et al., 2007, 2005; Kuroki et al., 2006) analysis, which cannot give explicit information about the exact anatomical connection between regions, the present study investigated the fornix integrity by using diffusion tensor tractography (DT-t). This new method can visualize brain white matter in vivo by tracking fiber tracts along the preferred diffusion direction of water molecule and thus can provide exact and detailed information on the anatomical connectivity between regions (Mori et al., 1999). Consistent with the previous studies using conventional methods (Kubicki et al., 2005; Kuroki et al., 2006), by tractography we found reduced FA in the fornix of the schizophrenia, which is indicative of damages in fornix integrity (Kubicki et al., 2005; Kuroki et al., 2006). The damages may result from the abnormality in myelination of axons in the hippocampus, where the myelin-related gene expression abnormalities in schizophrenia were most pronounced (Katsel et al., 2005).

In the present study, some regions, showing reduced functional connectivities to the left or right hippocampus in schizophrenia, have direct or indirect anatomical connection with the hippocampus via the fornix, such as PCC (Vinogradova, 2001), MPFC (Cavada et al., 2000), and contralateral parahippocampus (Powell et al., 2004). The abnormality in the fornix integrity is thought to indicate the abnormal connectivity between the hippocampus and other regions (Kuroki et al., 2006). Our findings of both the reduced functional connectivities to the anterior hippocampus and damaged integrity of the fornix in the same group of schizophrenic patients supported this opinion. The concurrence of the abnormalities of functional and anatomical connectivity of the hippocampus suggests the anatomical–functional relationship to a certain extent, although the significant correlation between the two imaging measures measured by correlation analysis was absent in the current study. The anatomical–functional relationship is worthy to be investigated in future study with a larger sample size. In addition, in future study, there are several points to be kept in mind. First, the functional connectivity between two regions could occur due to the influence of a simultaneously activating third region. In future, partial correlation analysis, Structural Equation Modeling (Seminowicz et al., 2004) or Dynamic Causal Modeling (Friston et al., 2003) could be used to investigate the direct effect of one region to another region. Second, anomalies in other fiber bundles related to the hippocampus, such as the fibers within the hippocampus (Kalus et al., 2004; White et al., 2007), also contribute to the abnormality in the resting-state hippocampal functional connectivity. For further investigation of the functional–anatomical relationship, complete fiber tract map of the hippocampus obtained by probabilistic tractography may be needed.

In terms of the clinical correlates of these imaging measures, we only found that the strength of several connectivities showed the trend toward significance to correlate with the general symptom severity measured by PANSS total score, positive symptom severity measured by PANSS subscale score, or medication dose (p<0.05, uncorrected). These connectivities were related with PCC and bilateral cuneus. Similarly, in a recent study, positive symptoms measured by the Scale for the Assessment of Positive Symptoms correlate positively with connectivity between the PCC and several frontal and temporal regions (Bluhm et al., 2007). All these suggest that the resting-state functional connectivity may be clinically significant. We did not find significant correlations between the mean
FA value of the fornix body and any clinical invariables, as other studies which exploring the clinical significance of FA value (Buchsbaum et al., 1998; Foong et al., 2000; Kuroki et al., 2006). It is possible that the FA value is related to specific cognitive functions. In an exploratory study, the reduced fornix integrity has been suggested to correlate with lower memory scores and reduced executive functioning in chronic schizophrenia (Nestor et al., 2007). Further studies should include those patients who are evaluated with specific neuropsychological tests for hippocampal functions.

Several additional issues need to be addressed in the present study. First, the potential confound of respiratory and cardiac cycle artifacts is thought to possibly contribute to between-group differences in resting-state functional connectivity analysis. Under a relatively low sampling rate, such as TR=2 s used in the present study, cardiac and respiratory fluctuation effects could be aliased into the SLFF and could reduce the specificity of the connectivity effect (Lowe et al., 1998). However, Birn et al. has confirmed that the contributions of aliased cardiac and respiratory signal to resting-state functional connectivity are relatively minor (Birn et al., 2006). Thus, we believe that the between-group differences observed in this study are physiologically meaningful. Secondly, because functional connectivity only reflects the synchrony or consistency of activities of brain regions, not all of the regions anatomically connected to a prior region can be found by the kind of analysis based on ROI, especially in small sample study. Take the connectivity between the hippocampus and thalamus as an example. The direct connectivities between these regions have been described anatomically (Bertram and Zhang, 1999); however, it is absent whether in functional connectivity map of young healthy subjects (Vincent et al., 2006) or that of the elder healthy subjects (Wang et al., 2006). Finally, we observed the reduced negative correlations between the left hippocampus and the left cerebellum. The inter-regional negative correlation measured by SLFF is speculated to reflect competition between neuronal activities (Fox et al., 2005). Considering the potential role of the cerebellum in cognitive function, particularly learning and memory (Moscovitch et al., 1995), the reduced negative correlations between the hippocampus and cerebellum may be meaningful.

In conclusion, by combining functional connectivity and DT-t analyses, we found the reduction in the resting-state functional connectivities to the bilateral hippocampi and the damage in white matter integrity of the fornix in the same group of schizophrenic patients. The multimodal imaging approaches presently used provide a new avenue to understand the role of hippocampus in the pathophysiology of schizophrenia.

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Contributors
All authors were involved in the design and implementation of the study and the writing of the manuscript. Authors Yuan Zhou, Ni Shu and Tianzi Jiang devised the concept. Authors Yuan Zhou and Ni Shu carried out the analysis. Author Tianzi Jiang supervised the study. Authors Zhenhui Liu, Yihui Hao and Haihong Liu collected the imaging data and clinical information. Authors Yong Liu, Ming Song and Chunshui Yu joined in the interpretation of data.

Conflict of interest
None.

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Appendix A. Supplementary data
Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.schres.2007.11.039.

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