Decreased functional connectivity of the amygdala in Alzheimer's disease revealed by resting-state fMRI

Hongxiang Yao a, Yong Liu b,c,* , Bo Zhou d, Zengqiang Zhang d, Ningyu An a, Pan Wang d, Luning Wang d, Xi Zhang d,* , Tianzi Jiang b,c,e,f

a Department of Radiology, Chinese PLA General Hospital, Beijing, 100853, China
b Brainnetome Center, Institute of Automation, Chinese Academy of Sciences, Beijing, 100190, China
c National Laboratory of Pattern Recognition, Institute of Automation, Chinese Academy of Sciences, Beijing, 100190, China
d Department of Neurology, Institute of Geriatrics and Gerontology, Chinese PLA General Hospital, Beijing, 100853, China
e Key Laboratory for NeurolInformation of Ministry of Education, School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu, 610054, China
f The Queensland Brain Institute, The University of Queensland, Brisbane, QLD 4072, Australia

ARTICLE INFO

Article history:
Received 21 September 2012
Received in revised form 20 March 2013
Accepted 24 March 2013

Keywords:
Amygdala
Resting-state fMRI
Alzheimer's disease
Mild cognitive impairment

ABSTRACT

Alzheimer’s disease (AD), the most common cause of dementia, is thought to be a progressive neurodegenerative disease that is clinically characterised by a decline of memory and other cognitive functions [1]. Furthermore, AD is characterised by neurofibrillary tangles, β-amyloid plaques and neuronal and synaptic losses with neuropathology [2]. Mild cognitive impairment (MCI) is considered to be an intermediate state between normal ageing and AD [3]. In particular, amnestic MCI (aMCI), which is a subtype of MCI characterised by the main complaint of memory disorders, appears to represent an early stage of AD [4–6]. With the development of neuroimaging, many brain networks, including the default mode network, have been identified. Generally, the core regions of the default mode network include part of the medial prefrontal cortex, part of the medial temporal lobe, the posterior cingulate cortex (PCC), the tempo-parietal junction and the precuneus [7–9]. Functionally, the default mode network is characterised by high activity when the brain is not engaged in specific behavioural tasks and deactivated/suspended during the performance of external tasks [7,9,10]. In the past 10 years, many studies have demonstrated disrupted default mode network architecture in neurodegenerative disorders, such as Parkinson’s disease [11], AD [10] and Huntington’s disease [12]. For details, please refer to some recently published excellent reviews [7–9,13,14]. In AD patients, pathological alterations have been found predominantly in medial temporal lobe structures, including the hippocampus and amygdala [15]. The amygdala, a component of the limbic system, is located in the ventral temporal lobe and directly mediates emotional learning and memory operation functions in certain brain regions, including the hippocampus and prefrontal cortex [16]. The amygdala is involved in emotion and a
variety of cognitive functions, such as attention, perception, emotional memory, declarative memory and explicit memory [17]. The amygdala plays an important role in the interaction of emotion and cognition because of its extensive connections with many brain areas, such as the sensory cortices, hippocampal complex, and prefrontal cortex [18]. Many previous studies have shown significant atrophy of the amygdala in AD patients [19,20]. Meanwhile, it was reported that the volume reductions in the amygdala correlate with the decreased MMSE scores that are seen in AD [21]. Furthermore, the amygdala and the hippocampus are the regions that are firstly damaged in AD [20], and this damage could be a predictor of AD. In terms of the functional connectivity pattern, the hippocampus has been widely studied, and a consistent alteration pattern has been found in MCI and AD [22,23]. However, the pattern of functional connectivity of the amygdala in AD or MCI has not been well studied.

We hypothesised that the functional connectivity pattern of the amygdala was impaired in AD and that the altered functional connectivity pattern of the amygdala should have alterations related to disease severity. To prove this hypothesis, we used resting-state functional MRIs to investigate the altered amygdala connectivity patterns in 35 AD patients, 27 MCI patients and 27 age- and gender-matched normal control (NC) subjects. We investigated the altered functional connectivity patterns of amygdala in AD compared with the NC group. In addition, the MCI subjects allowed for an evaluation of how different illness stages affect the functional connectivity pattern of the amygdala. Finally, a correlation analysis was performed between the strength of the functional connectivity of the identified regions and the various clinical variables (MMSE and AVLT immediate and delayed recall scores) to evaluate the relationship between the strength of functional connectivity and the cognitive abilities of the MCI and AD subjects (Fig. 1).

Fig. 1. Stretch of the pipeline in the present study. Firstly, we investigated the altered amygdala connectivity patterns in AD compared with the NC group. Secondly, the MCI subjects allowed for an evaluation of how different illness stages affect the functional connectivity pattern of the amygdala. Finally, a correlation analysis was performed between the strength of the functional connectivity of the identified regions and the various clinical variables (MMSE and AVLT immediate and delayed recall scores) to evaluate the relationship between the strength of functional connectivity and the cognitive abilities of the MCI and AD subjects.

2. Materials and methods

2.1. Subjects

Written consent forms were obtained from all subjects or their legal guardians. All of the participants were recruited by advertisement (http://www.301ad.com.cn, Chinese version) and evaluated at the Chinese PLA General Hospital, Beijing, China. Before being selected for this study, all of the participants received general physical, psychological and laboratory examinations [24]. None of the subjects were taking any medication that may have influenced cognition during the scans. All of the subjects were right handed and underwent a neuropsychological test battery, including the Mini-Mental State Examination (MMSE), Auditory Verbal Learning Test (AVLT), Geriatric Depression Scale (GDS), Clinical Dementia Rating (CDR) and Activities of Daily Living (ADL) scale. In brief, in the present study, the AVLT consisted of one learning trial in which a list of 10 Chinese double-character words was read, and the subject was asked to immediately recall as many of the items as possible. The trial was repeated twice, and the immediate recall score was the average accurate recall of the three trials. After a 5-min delay, each subject was asked to recall the words from the initial list (AVLT-delayed recall). The subjects were then told to identify the 10 studied words, which were mixed with 10 new words (AVLT-recognition).

The MCI diagnostic criteria that was used in this study was described by Peterson et al. [3] and included the following components: (1) memory complaints lasting at least 6 months, (2) CDR = 0.5, (3) intact functional status and ADL ≤ 26 and (4) the absence of dementia according to the International Classification of Diseases, 10th Revision (ICD-10). The following inclusion criteria were followed: (1) AD was diagnosed using the ICD-10 criteria for AD, (2) CDR = 1 or 2, (3) no nootropic drugs, such as anticholinesterase inhibitors, and (4) the ability to perform the neuropsychological test and tolerate the MRI scanning. The AD and MCI patients also met the core clinical criteria for probable AD dementia and MCI according to the recently published recommendation for new diagnostic criteria [1]. The NC criteria were as follows: (1) normal general physical status, (2) CDR = 0 and (3) no memory complaints.

The study exclusion criteria included the following: (1) metabolic conditions, such as hypothyroidism or vitamin B12 or folic acid deficiencies, (2) psychiatric disorders, such as schizophrenia or depression, (3) infarction or brain haemorrhage, as indicated by MR/CT imaging or (4) Parkinson’s disease, epilepsy and other nervous system diseases that could influence cognitive function. Any person with a metallic foreign body, such as a cochlear implant, heart stent or other MR scanning relevant contraindications, was also excluded from the study.

For each subject, T2-weighted images were collected during the scan. All images were evaluated by senior radiologists (Dr. Yao [the first author] together with another radiologist). Patients with white matter alterations were excluded from the current study. Note that the cognitive function of the healthy volunteers was rated in the normal range using the MMSE, and the mean age and gender distributions of the control group were well-matched with the MCI and AD patient groups (Table 1).

2.2. Data acquisition

MR scans were performed at the Chinese PLA General Hospital, Beijing, China, with a 3.0 T GE MR system using a standard head coil. Resting-state fMRI data were acquired using an echo planar imaging (EPI) sequence with a repetition time (TR) = 2000 ms, echo time (TE) = 30 ms, flip angle (FA) = 90°, matrix = 64 × 64, field of view (FOV) = 220 mm × 220 mm, slice thickness = 3 mm and slice
Chi-square was used for gender comparisons, one-way ANOVA with Bonferroni post hoc test was used for age, and neuropsychological tests comparisons. MMSE: Mini-Mental State examination; CDR: Clinical Dementia Rating; AVLT: Auditory Verbal Learning Test.

**Table 1**

Demographic, clinical and neuropsychological data in normal control (NC), mild cognitive impairment (MCI) and Alzheimer’s disease patients (AD).

<table>
<thead>
<tr>
<th></th>
<th>NC (n = 27)</th>
<th>MCI (n = 27)</th>
<th>AD (n = 35)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>16/11</td>
<td>13/14</td>
<td>12/23</td>
<td>0.143</td>
</tr>
<tr>
<td>Age (year)</td>
<td>69.2±6.5</td>
<td>73.8±7.8</td>
<td>72.4±4.5</td>
<td>0.09</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.9±1.0</td>
<td>26.8±1.8a</td>
<td>19.7±4.1b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CDR</td>
<td>0</td>
<td>0.5</td>
<td>1.3±0.5b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AVLT-Immediate</td>
<td>5.9±1.1</td>
<td>4.6±1.5</td>
<td>2.6±1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AVLT-Delay</td>
<td>5.8±2.0</td>
<td>3.1±2.0a</td>
<td>0.6±1.2b</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

2.3. Data pre-processing

The data were pre-processed using statistical parametric mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm). To allow for magnetisation equilibrium, the first 10 volumes of each functional time series were discarded. The remaining 190 volumes were corrected for slice timing, realigned, spatially normalised to the standard Montreal Neurological Institute (MNI) EPI template and resampled to 2 mm × 2 mm × 2 mm cubic voxels. To further reduce the effects of confounding factors, six motion parameters, linear drift and the mean time series of all voxels within the white matter and the cerebrospinal fluid were removed from the data by linear regression. To reduce the effect of low-frequency drifts and high-frequency signals, a temporal filter (0.01–0.08 Hz) was performed using in-house MATLAB software. Finally, the regressed images were smoothed with a 6-mm full width at half maximum to reduce spatial noise [24,25].

Some recent literatures suggested that sub-millimetre head-motion during data scanning can have a substantial impact on some measurements of resting-state fMRI [26–28]. We also investigated the group differences in head-motion among the groups according to the algorithm introduced by Van Dijk et al. [28]. No significant differences in head motion among the three groups. In order to reduce the effect of head motion to the results, all the study participants satisfied the inclusion criteria of head movement <3 mm translation in any axis and <3° angular rotation in any axis during the fMRI scanning. Thirteen subjects (2 NC, 3 MCI and 8 AD) who exhibited frequent head motion during the scanning were excluded. The demographic and neuropsychological details for the remaining 89 subjects are shown in Table 1.

2.4. Definition of the amygdala

We chose the bilateral amygdala as the region of interest (ROI) using the WFU_PickAtlas tool software (www.ansir.wfubmc.edu). To guarantee that the selected voxels were located within the amygdala, we intersected the bilateral amygdala regions of the AAL and SPM brain templates. The selected volume of the left amygdala was 248 voxels and the right amygdala was 220 voxels, with voxel size 8 mm³ (Fig. 1).

2.5. Functional connectivity and statistical analysis of the bilateral amygdala

Functional connectivity analyses were performed for the left and right amygdala. For each seed region, a voxel-wise functional connectivity analysis was performed separately for each ROI. The time series from all of the voxels within the ROI were used as the seed reference time series, and the Pearson’s correlation coefficient (between the average time series for that seed and each voxel in the brain) was computed as the strength of functional connectivity.

For further statistical analysis, the correlation coefficients were transformed to z-values using the Fisher r-to-z transformation to improve the normality of the correlation coefficients [29]. Thus, a map that represented the functional connectivity strength and the seed region (in terms of the z-values for each subject) was obtained.

Within each group, the individual z-values were entered into a one-sample t-test in a voxel-wise manner to determine the brain regions that showed significant functional connectivity with the left amygdala. A combined threshold of contrast maps was set using clusters with a minimum volume of 100 voxels set at an uncorrected individual voxel height threshold of P < 0.001. We chose a relatively stringent threshold of the P value because of the null hypothesis of a correlation coefficient of zero and the relatively large normal control sample size.

Within each group, the individual z-values were entered into a one-sample t-test in a voxel-wise manner to determine the brain regions that showed significant functional connectivity with the left amygdala. A combined threshold of contrast maps was set using clusters with a minimum volume of 100 voxels set at an uncorrected individual voxel height threshold of P < 0.001. We chose a relatively stringent threshold of the P value because of the null hypothesis of a correlation coefficient of zero and the relatively large normal control sample size.

Within each group, the individual z-values were entered into a one-sample t-test in a voxel-wise manner to determine the brain regions that showed significant functional connectivity with the left amygdala. A combined threshold of contrast maps was set using clusters with a minimum volume of 100 voxels set at an uncorrected individual voxel height threshold of P < 0.001. We chose a relatively stringent threshold of the P value because of the null hypothesis of a correlation coefficient of zero and the relatively large normal control sample size. For each voxel and a cluster size of at least 50 voxels, which resulted in a corrected threshold of P_{min}=0.001, as determined by a Monte Carlo simulation (see http://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf). The parameters were FWHM = 6 mm using the AAL template as a mask. Subsequently, the regions that showed significant differences were extracted as the regions of interest (ROI), and the mean functional connectivity values of the MCI subjects were used for further analysis (Fig. 1). Statistical comparisons of the mean fitted strength of the functional connectivity between the groups were performed using a two-sample two-tailed t-test at a threshold of P < 0.05 (FDR corrected, with the number of groups multiplied the number of significant brain regions).

2.6. Relationship between functional connectivity and clinical variables

To determine whether the functional connectivity varied with disease progression in the patient groups (MCI, AD and AD plus the MCI groups), correlation analyses of the functional connectivity and the clinical variables (measured by the MMSE and AVLT-immediate and delayed recall scores) were performed after regressing out age and gender effects. As these analyses were exploratory in nature, we used a statistical significance level of P < 0.05 (uncorrected).

3. Results

Within the groups, we found significant functional connectivity between the bilateral amygdala and many brain regions, including the frontal lobe, parietal lobe, temporal lobe, and occipital lobe, using a one-sample, two-tailed t-test in a voxel-wise manner and combining the threshold clusters with 100 voxels (uncorrected, P < 0.001) (Fig. 2).

First, a two-sample, two-tailed t-test was used to determine the regions in which the functional connectivity was significantly different between the AD and NC groups. For the left amygdala,
a decreased functional connectivity was found in the left hippocampus/parahippocampus (HIP/PHIP), the bilateral caudate and the right superior temporal gyrus (STG) (Fig. 3, Table 2). In the right amygdala of the AD patients (compared with the NC group), decreased functional connectivity was found in left superior temporal gyrus (STG), the left precentral gyrus (PreCG), right MCC, right putamen (PUT), and inferior frontal gyrus (IFG), left insula/putamen (INS/PUT) and left superior/medial frontal gyrus (SFG/MFG) (Fig. 4, Table 3).

To evaluate the connection between disease severity and functional connectivity in AD patients, we investigated the functional connectivity pattern in MCI for all of the regions that were identified as altered in the AD group. We observed that in the bilateral amygdala, the functional connectivity strength of the MCI subjects was at the median level of the AD and NC subjects (Figs. 3 and 4, B). Importantly, for those regions that showed decreased functional connectivity in AD, we found that the functional connectivity strength between the bilateral amygdala and part of the identified regions correlated significantly and positively with the clinical scores (the MMSE and AVLT delayed recall) in the MCI and AD populations (Fig. 5, Table 4).

![Fig. 2. Brain areas with significant differences in functional connectivity of the amygdala in the NC, MCI and AD groups (P < 0.001, 100 voxels, AlphaSim corrected).](image)

**Table 2**

Decreased functional connectivity using the left amygdala as the ROI in the AD compared with the control group (cluster size > 50 voxels, $P_{\text{alpha}} < 0.001$, AlphaSim corrected).

<table>
<thead>
<tr>
<th>BA</th>
<th>Cluster size</th>
<th>T value</th>
<th>Z value</th>
<th>MNI coordinates (x,y,z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIP/PHIP.L</td>
<td>97</td>
<td>5.73</td>
<td>5.10</td>
<td>-28 -8 -28</td>
</tr>
<tr>
<td>Caudate</td>
<td>268</td>
<td>4.78</td>
<td>4.38</td>
<td>12 12 4</td>
</tr>
<tr>
<td>STG.R</td>
<td>38</td>
<td>4.14</td>
<td>3.87</td>
<td>326 -18</td>
</tr>
</tbody>
</table>

STG: superior temporal gyrus; PHIP: parahippocampal; HIP: hippocampal; L: left; R: right; BA: Broadmann Area.

![Fig. 3. (A) Brain areas with significant differences in functional connectivity of the left amygdala in the AD patients (P < 0.001, 50 voxels, AlphaSim corrected); (B) Bar of the functional connectivity strength of left amygdala in the NC (red), MCI (blue) and AD (black) groups in the identified regions.](image)

**Table 3**

Decreased functional connectivity using the right amygdala as the ROI in the AD compared with the control group (cluster size > 50 voxels, $P_{\text{alpha}} < 0.001$, AlphaSim corrected).

<table>
<thead>
<tr>
<th>Brain area</th>
<th>BA</th>
<th>Cluster size</th>
<th>T value</th>
<th>Z value</th>
<th>MNI coordinates (x,y,z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STG.L</td>
<td>21/38</td>
<td>66</td>
<td>5.15</td>
<td>4.67</td>
<td>-50 -2 -6</td>
</tr>
<tr>
<td>IFG/PUT.R</td>
<td>13/47</td>
<td>587</td>
<td>5.12</td>
<td>4.65</td>
<td>-38 -22 -12</td>
</tr>
<tr>
<td>SFG.L</td>
<td>104</td>
<td>4.87</td>
<td>4.45</td>
<td>-24 -4</td>
<td></td>
</tr>
<tr>
<td>INS/PUT.L</td>
<td>13</td>
<td>53</td>
<td>4.89</td>
<td>4.47</td>
<td>-36 -14</td>
</tr>
<tr>
<td>SFG/MFG.L</td>
<td>9</td>
<td>54</td>
<td>4.83</td>
<td>4.42</td>
<td>-38 -10</td>
</tr>
<tr>
<td>PreCG.L</td>
<td>44</td>
<td>85</td>
<td>4.19</td>
<td>3.91</td>
<td>-52 18</td>
</tr>
<tr>
<td>MCC.R</td>
<td>32</td>
<td>58</td>
<td>4.14</td>
<td>3.86</td>
<td>-60 14</td>
</tr>
<tr>
<td>PUT.R</td>
<td>92</td>
<td>3.75</td>
<td>3.55</td>
<td>30 -12</td>
<td></td>
</tr>
<tr>
<td>3.75</td>
<td>3.54</td>
<td>264 -2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.70</td>
<td>3.50</td>
<td>24 -6 -4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PreCG: precentral gyrus; MCC: middle cingulate cortex; SFG: superior frontal gyrus; MFG: medial frontal gyrus; IFG: inferior frontal gyrus; STG: superior temporal gyrus; PUT: Putamen; INS: insula; L: left; R: right; BA: Broadmann Area.
4. Discussion

In the current study, we have investigated the differences in functional connectivity patterns of the amygdala in AD, MCI and NC patients throughout the various cortical and subcortical brain regions using resting-state fMRI. Decreased functional connectivity was observed in both AD and MCI patients between the identified regions and the amygdala. Specifically, decreased functional connectivity was found in the regions that are included in the default mode network (DMN), context conditioning network and extinction network (Figs. 3 and 4). To the best of our knowledge, this is the first report on the alteration of functional connectivity in the amygdala of AD and MCI patients.

4.1. Decreased functional connectivity of the amygdala in AD

We found significantly decreased functional connectivity between the amygdala and the other regions, including the superior temporal gyrus (STG), hippocampus (HIP) and the parahippocampal gyrus (PHIP) in AD. In these regions, the hippocampus plays a critical role in learning and memory, including short-term memory, long-term memory and spatial processing [30]. The parahippocampal gyrus is thought to be related to memory encoding and retrieval [30]. The amygdala has been shown to modulate the hippocampus during episodic memory tasks and to facilitate the parahippocampus during emotionally influenced memory storage [31]. Moreover, the amygdala is considered to play an important role in enhancing explicit memory for both pleasant and unpleasant emotional stimuli and modulating memory storage in other brain regions [32,33]. Previous studies also demonstrated that the hippocampus and parahippocampal gyrus are involved in the neural circuit for amygdala-mediated emotional memory enhancement [34,35]. Additionally, the amygdala modulates hippocampus-dependent memory [36], and its activity facilitates the induction and expression of hippocampal long-term potentiation, which may be the underpinning mechanism of memory consolidation [36,37]. This role is inconsistent with the decreased memory ability and disrupted hippocampal connectivity in AD/MCI [22,23,38].

Note that the hippocampus, parahippocampal gyrus and SFG/MFG are important nodes in the default mode network. The default mode network is involved in many cognitive functions, such as episodic memory, theory of mind, self-evaluation and introspection [9]. Interestingly, specific regions of the default network, including the precuneus and the posterior cingulate cortex (PCC), are selectively vulnerable to early amyloid deposition in AD. Amyloid deposition accumulates in the regions that are associated with the default mode network even before cognitive dysfunction symptoms emerge [39]. In addition, the PCC and precuneus present early metabolic and perfusion abnormalities and abnormal deposition of A-beta in AD [9,40]. Disrupted connectivity between the hippocampus/entorhinal cortex and the PCC/precuneus is found in AD [22,38,41]. These regions are also cortical hubs in the brain, and impaired connectivity between these regions may be responsible for hypo-metabolism and hypo-perfusion in the PCC/precuneus in early AD [9,40]. The decreased functional connectivity between the amygdala and the regions in the default mode network may be caused by disruption of the default mode network in AD. Previous studies demonstrated that functional connectivity in the default

Table 4

Summary of correlation between the mean fitted strength of functional connectivity between amygdala and the identified regions and various clinical variables (MMSE, AVLT Delay recall) in both MCI and AD (P < 0.05).

<table>
<thead>
<tr>
<th>Brain area</th>
<th>Brain area</th>
<th>AD and MCI</th>
<th>MCI</th>
<th>AD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amy.R</td>
<td>STG.L</td>
<td>0.378</td>
<td>0.002</td>
<td>-0.062</td>
</tr>
<tr>
<td>INS/PUL.L</td>
<td>0.315</td>
<td>0.013</td>
<td>0.056</td>
<td>0.783</td>
</tr>
<tr>
<td>SFG/MFG.L</td>
<td>0.045</td>
<td>0.731</td>
<td>-0.057</td>
<td>0.777</td>
</tr>
<tr>
<td>PreCG.L</td>
<td>0.420</td>
<td>0.001</td>
<td>0.056</td>
<td>0.782</td>
</tr>
</tbody>
</table>

For abbreviations see detail in Tables 2 and 3.
mode network is disrupted in aMCI [42,43]. Additionally, we found the strength of the altered functional connectivity in MCI subjects just located in the middle of the AD and NC samples. Taken together, these findings suggest that decreased functional connectivity between the amygdala and the default mode network may underlie the decline of episodic memory in aMCI and AD as well as the symptom of absent-mindedness in AD.

The middle cingulate cortex showed decreased functional connectivity to the amygdala (Figs. 3 and 4). Previous studies have demonstrated that the middle cingulate cortex was involved in many complex functions, such as emotion, fear and cognition [44]. In addition, extensive connectivity was found between the middle cingulate cortex and the amygdala [29]. A functional brain imaging study showed that the amygdala is necessary for emotional memory enhancement. In addition, the cingulate cortex was shown to be vulnerable in the early stage of AD [15]. Taken together, our findings suggest that decreased functional connectivity between the amygdala and the middle cingulate cortex may explain the decreased cognitive function in AD. This result can also be strengthened by the correlation between the functional connectivity between the middle cingulate cortex and the amygdala and the MMSE of the AD and MCI patients (Fig. 5).

Significantly decreased functional connectivity between the amygdala and several regions, including the inferior frontal cortex, the insula, the putamen, was found in AD/MCI patients (Fig. 3). Interestingly, the amygdala, the hippocampus, the inferior frontal cortex, the insula, the ventral putamen, and the parietal cortex are included in the network of context conditioning and extinction [45]. This network contributes to the contextual modulation of conditioned responses and specifically reflects context-dependent recall of extinction memory [45]. In this network, the hippocampus plays a significant role for the encoding of contextual memories while the amygdala is considered to be important for active extinction learning [46,47]. Importantly, a previous study demonstrated that context extinction deficits preceded other cognitive impairments in patients with mild AD [48]. Hence, the decreased functional connectivity between the amygdala and the context conditioning and extinction network might be taken as a predictor of AD in the future.
The AD/MCI patients showed decreased functional connectivity between the amygdala and the sensorimotor network, which includes the precentral gyrus (Fig. 4). Early studies showed that the sensorimotor cortex was relatively spared and is only involved in the later stages of AD [15]. However, other studies suggested that sensorimotor dysfunction occurs earlier than previously thought in the progression of AD [45]. More importantly, the decreased functional connectivity between the amygdala and the above three regions has a robust positive relationship with the MMSE in AD and MCI (Fig. 5, Table 4). This result indicates cognitive decline and decreased sensorimotor ability that can be considered to be an early marker for abnormal brain function leading to AD.

We observed a significant correlation between connectivity strength and MMSE (Fig. 5, Table 4). The MMSE is used to diagnose and assess the progression and severity of the symptoms in AD/MCI. The correlation between the MMSE score and functional connectivity indicates a general relationship between abnormal functional connectivity and cognitive impairment in patient groups. The AVLT is used to evaluate various functions, such as short-term auditory-verbal memory, learning rate, learning strategies, the presence of confabulation and confusion in memory processes. The decreased MMSE and AVLT scores reflect impaired function in daily life in AD/MCI. Therefore, our findings provide further evidence for impaired functional connectivity between the amygdala and sensorimotor network that may partially account for the cognitive and emotional symptoms as well as impaired function in the daily life of AD/MCI patients.

4.2. Methodological issues and further discussion

The function of amygdala is complex, and more importantly, the amygdala could be divided into several subdivisions that specifically contribute to the connection between the amygdala and the cortical and subcortical brain regions. In the current study, we considered the amygdala as a whole structure, which likely masked and overlooked the independent functions and patterns of connectivity of the individual subdivisions. Secondly, the amygdala has been found to be atrophied in many previous AD studies. However, the interaction between the structure atrophy and the alteration of the functional connectivity has not yet been investigated.

The ultimate goal of imaging studies is to identify markers to distinguish MCI/AD patients from normal controls. However, we could not directly use connectivity as a feature to distinguish these patients from the normal controls because of individual variability (Fig. 3), although we found a significant correlation between functional connectivity and MMSE (Fig. 5, Table 4). Additionally, we only identified altered regions in AD relative to normal controls. We tried to investigate differences in the connectivity to the bilateral amygdala in MCI relative to AD, but no significant alterations in MCI were found when we used the same statistical threshold. This might be because most of AD subjects still in early and mild stage of the disease (25 subjects with CD = 1 and 10 subjects with CD = 2). However, when we loosened the threshold of the statistical analysis, we found that the alterations in AD and MCI were similar. This reflects that the disease will destroy the functional connectivity of amygdala. The inability to detect significant alterations in MCI may be the result of individual variability in the small sample in the present study. A large sample size and other techniques, such as β amyloid assessment or FDC-PET and CSF-tau analysis, could be used to increase the diagnostic certainty of AD/MCI in future studies.

Acknowledgements

This research was partially supported by the Natural Science Foundation of China (Nos. 60831004, 81270020, 91132031), and the Natural Science Foundation of Tianjin (No. 11JCZDJC19500). We appreciate the generous assistance of Drs. Hengg Xie and Wei Wang with the data acquisition.

References
