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Original article

The heterogeneity of aging brain: altered functional connectivity in default mode network in older adults during verbal fluency tests

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Keywords: mild cognitive impairment; default mode network; verbal fluency tests; heterogeneity of aging; functional connectivity

Background Successful aging (SA) and mild cognitive impairment (MCI) are heterogeneous groups of aging. To explore the heterogeneity, the functional connectivity was studied in these populations.

Methods The present study utilized functional connectivity magnetic resonance imaging (fcMRI) to investigate default mode network (DMN) in 8 healthy subjects of SA, 8 subjects of usual aging (UA), and 8 MCI patients during verbal fluency tests (VFTs). Functional connectivity (based seeds) of different groups was analyzed by using statistical test.

Results Compared with SA and UA groups, MCI subjects exhibited decreased functional connectivity in the DMN regions, including the inferior parietal lobule and left angular gyrus ($t=3.53$, $P<0.001$). Compared with UA and MCI groups, the SA elderly exhibited increased functional connectivity in the precuneus ($t=3.53$, $P<0.001$).

Conclusions These findings suggested that abnormalities of functional connectivity in DMN might be related with semantic memory impairment in aging. Left angular gyrus and precuneus might be the potential imaging-based biomarker for distinguishing heterogeneous process of elderly.

The default mode network (DMN) has been identified as a rest state of brain function, which includes the bilateral posterior cingulate cortex (PCC)/precuneus, medial prefrontal cortex (MPFC), inferior temporal lobe, and inferior parietal lobule (IPL). These brain areas were active not only when subjects were at resting state, but also when individuals were engaged in internal tasks, such as autobiographical memory retrieval, envisioning the future. Default mode abnormalities have been reported in Alzheimer’s disease (AD) and working memory tasks. As a memory task, verbal fluency tests (VFTs) were widely used as neuropsychological tests whose fundamental component was the retrieval of semantically or lexically associated words from long-term memory storage. Related with semantical memory retrieval, it also has been applied in studies of mild cognitive impairment (MCI) and AD.

MCI refers to a transitional state between normal aging and AD; it does not notably interfere with activities of daily life. Successful aging (SA) is a heterogeneous category of normal aging. Features of SA include well reserved cognitive function, independent activities of daily living, satisfied mood status (absence of any depressive or anxious symptoms) and no disability. Apart from SA, another category is usual aging (UA) in which aging changes do not lead to pathology. Previous studies have suggested that normal aging is associated with alterations in the connectivity of brain within the DMN. Our study differs from previous studies in the discrepancies between diseased and healthy; we focus on the heterogeneous groups of aging — SA and MCI elderly.

During VFTs, it was expected that an intrinsic, functional connectivity also existed. It was mediated through spontaneous low-frequency blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) signal fluctuations for the network of brain regions that support the default mode.
to research the heterogeneity of aging during VFTs, under the hypothesis of differences existed in aging. On the basis of above hypotheses, with seed at PCC and MPFC, DMN spatial patterns of all subjects were observed by cross-correlation functional connectivity analysis, which could be another useful perspective to study the aging and MCI.

METHODS

Subjects
All subjects were randomly selected (psudorandom, uniformed distribution) from samples of cluster sampling (235 SA samples, 151 UA samples, 59 MCI samples, and a total of 445 samples) in Shiqian and Ganquan Community, Putuo District, Shanghai, China, in 2003. A survey, Health Survey of Elder in Community in Shanghai, 2000, consisted of mini mental state examination (MMSE), activity of daily living scale (ADL) and index of physical disease (IPD) etc were carried out with the criteria for each group.11

Totally 24 older adults were enrolled, 8 subjects met the standards of SA, 8 subjects met the criteria of UA, and 8 subjects were in the group of MCI. The study was approved by the Ethics Committee of the Tongji Hospital of Tongji University, and was performed according to the standards set by the Declaration of Helsinki. Written informed consent was obtained from each participant. All subjects were right-handed, normal hearing, and were normal level through the physical examination and routine laboratory tests, and without significant physical disease or disability. Detailed demographics findings are shown in Table 1. And there was no significant difference of age among three groups.

Data acquisition

Stimulation pattern
VFTs were chosen as stimulation paradigm, and led to block-design (eyes closed). The block-design consisted of six blocks, and each of them lasted 60 seconds. After inputted in the computer, the sound of command was auto-played to the headphone of subjects through E-prime software. When the subject heard the instruction, the volunteer was tasked to covertly retrieve as many words as possible that belonged to the given category (consisted of animals, vegetables and fruits).

Scanning process and parameters
Before scanning, subjects were required to fulfill simulated training for the compliance, check the handedness, and read the experimental instruction. Next, the behavioral data was obtained after scanning under the same environment. MRI scanning was performed on a 1.5 T scanner (Marconi USA). Anatomical T1-weighted images were acquired using a three-dimensional (3D)-spoiled gradient recalled (SPGR) sequence. Generating 25 axial slices, the imaging parameters were as follows: TR/TE=500 ms/12 ms, thickness/interval: 5.0 mm/1.0 mm, FOV=22 cm, matrix=256×256, flip angle=90°. Functional images were collected by using a gradient-echo echo-planar imaging (EPI) sequence, and the imaging parameters were as follows: TR/TE=3000 ms/40 ms, thickness/interval: 5.0 mm/1.0 mm, FOV=24 cm, matrix=64×64, flip angle=90°; 25 axial slices were acquired using a 3D-spoiled gradient recalled (SPGR) sequence, and 130 volumes were obtained during 390 seconds of a subject run.

Data preprocessing

Preprocessing of fMRI data was conducted through the SPM2 software package (statistical parametric mapping, http://www.fil.ion.ucl.ac.uk/spm, UK) including slice time correction, 3D motion detection and correction, and spatial normalization. To prepare the data for the main analyses, images were sampled to 3 mm×3 mm×3 mm and smoothed with a 6-mm full-width at half maximum of an isotropic Gaussian filter.

DMN identification

A pair of spherical regions (radius 10 mm) were selected as seeds, and they were positioned in the PCC/precuneus area (0, –52, 30) (Talairach coordinate)17,18 and MPFC (–1, 47, –4) (Talairach coordinate).19 Mean BOLD signal intensity time course were extracted from the seeds. Before computing the time series extracted from each brain voxel, two procedures were needed to remove possible variances. First, temporal band-pass filtering (0.01–0.08 Hz) was conducted through a phase-insensitive filter.18,19 Second, through linear regression, six head motion parameters obtained from the realigning step were utilized to correct the time series.18,19 Then, the cross-correlation functional connectivity analysis was performed by computing temporal correlation, between the seeds and all brain voxels.

The correlation coefficient of each voxel was the average of the correlations (or anticorrelations) with the two seed regions,19 and it was later normalized to Z-scores with Fisher’s r-to-z transformation. Therefore, an entire brain Z-score map was acquired for each subject. To achieve statistical significance of functional correlations at the group level, SPM8 (http://www.fil.ion.ucl.ac.uk/spm, UK) was utilized for the statistical test.

Linear trend cross groups

To investigate the underlying linear trend of altered

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age (years)</th>
<th>Female/male</th>
<th>MMSE scores</th>
<th>Animals</th>
<th>Vegetables</th>
<th>Fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA</td>
<td>76.0±5.5</td>
<td>5/3</td>
<td>27.75±1.98</td>
<td>17.2±2.1</td>
<td>16.4±2.6</td>
<td>17.6±2.4</td>
</tr>
<tr>
<td>UA</td>
<td>76.0±2.7</td>
<td>4/4</td>
<td>22.63±3.16</td>
<td>13.8±2.0</td>
<td>12.5±1.4</td>
<td>15.9±2.2</td>
</tr>
<tr>
<td>MCI</td>
<td>77.0±5.6</td>
<td>4/4</td>
<td>17.87±4.64</td>
<td>10.1±2.3</td>
<td>9.0±1.2</td>
<td>9.4±1.5</td>
</tr>
</tbody>
</table>

Table 1. Information of demography, MMSE scores and performances (number of words)
functional connectivity across MCI, UA and SA groups, the Pearson’s correlation and linear regression between correlation coefficients and MMSE scores were calculated, with threshold $P < 0.05$ (uncorrected).

**RESULTS**

**Behavioral performance**

In line with expectations, the performance of SA group was better than UA group, while UA group was better than MCI group. Checking by one-way analysis of variance (ANOVA), there was significant difference between SA group and UA group in the performance of the first block (animals, $P < 0.01$). Notable discrepancies also existed between SA group and MCI group and between UA group and MCI group ($P < 0.01$). Details are also shown in Table 1.

**Functional connectivity of each group**

Through one-sample t-test ($P < 0.05$, false discovery rate correction, $k=10$ voxels), results of each group were achieved to illustrate DMN in normal aging. The connectivity patterns of positive correlations appeared to be similar during visual inspection of SA and UA group. Apart from the regions around with the two seeds, significant functional connectivity for seeds in SA group was primarily found in middle temporal gyrus (Broadmann areas BA19/39), angular gyrus (BA39/40), inferior parietal lobule (BA40), occipital lobe (BA19/17/18/37), anterior cingulate cortex (BA32), superior temporal gyrus (BA39/38), inferior temporal gyrus (BA20), thalamus, and cerebellum. In the group of UA, the positive correlation maps were observed in anterior cingulated cortex (BA24/32), inferior parietal lobule (BA40/39), angular gyrus (BA39), superior temporal gyrus (BA22/39), middle temporal gyrus (BA21/37), occipital lobe (BA17/18/19), and cerebellum. Except for the two seed regions, less notable regions of connection for seeds were shown in MCI group. Occipital lobe (BA18/19), middle temporal gyrus (BA21/37), and anterior cingulate cortex (BA10/32) were barely received.

**Alterations of DMN between groups**

To receive more significant and credible regions of alteration between groups, results were first checked by one-way ANOVA. Table 2 shows the main regions with significant differences between groups which contain IPL (BA39/40), lingual gyrus (BA19), posterior cingulated (BA29), precuneus (BA7) and postcentral gyrus (BA43). Meanwhile, the reliable effects of different levels (groups) are shown in Figures 1A and 2A.

Compared with MCI group, Figure 1B–2B shows the statistical map produced by post hoc t-test comparison of functional connectivity of the SA group. Increased

![Figure 1.](image)

**Table 2. Main effects of different groups in detail**

<table>
<thead>
<tr>
<th>Lobe</th>
<th>R/L</th>
<th>Brodmann</th>
<th>MNI coordinates</th>
<th>F values</th>
<th>Voxels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inferior parietal lobe</td>
<td>L</td>
<td>BA40/39</td>
<td>$-45$ $-63$ $39$</td>
<td>24.3</td>
<td>40</td>
</tr>
<tr>
<td>Superior parietal gyrus</td>
<td>L</td>
<td>BA39</td>
<td>$-51$ $-60$ $27$</td>
<td>21.74</td>
<td>40</td>
</tr>
<tr>
<td>Lingual gyrus</td>
<td>R</td>
<td>BA19</td>
<td>12 $-51$ $-6$</td>
<td>23.5</td>
<td>15</td>
</tr>
<tr>
<td>Posterior cingulate cortex</td>
<td>R</td>
<td>BA29</td>
<td>12 $-48$ $3$</td>
<td>12.63</td>
<td>16</td>
</tr>
<tr>
<td>Precuneus</td>
<td>L</td>
<td>BA7</td>
<td>$-9$ $-57$ $45$</td>
<td>18.6</td>
<td>16</td>
</tr>
<tr>
<td>Precuneus</td>
<td>L</td>
<td>BA7</td>
<td>$-18$ $-60$ $51$</td>
<td>12.84</td>
<td>16</td>
</tr>
<tr>
<td>Posterior cingulate cortex</td>
<td>R</td>
<td>BA29</td>
<td>15 $-39$ $12$</td>
<td>15.79</td>
<td>10</td>
</tr>
<tr>
<td>Postcentral gyrus</td>
<td>R</td>
<td>BA43</td>
<td>54 $-15$ $21$</td>
<td>14.41</td>
<td>15</td>
</tr>
<tr>
<td>Posterior cingulate cortex</td>
<td>L</td>
<td>BA29</td>
<td>$-3$ $-54$ $9$</td>
<td>13.41</td>
<td>12</td>
</tr>
</tbody>
</table>

$F=9.77$, $P < 0.001$, uncorrected, $k=10$ voxels. The coordinates were obtained in the Montreal Neurological Institute (MNI) space. R: right, L: left.
Figure 2. Comparison between groups in precuneus (BA7, \( t = 3.53, P < 0.001 \), uncorrected, \( k = 10 \) voxels). A: Significant effect was shown in precuneus through one-way ANOVA (\( F = 9.77, P < 0.001 \), uncorrected, \( k = 10 \) voxels). This picture exhibits that SA group carries the main functional connectivity, UA group contributes secondary effects, and MCI group contributes additional effects in this region. B: Compared with MCI group, SA group showed increased connection for seeds in precuneus. C: Compared to UA group, more significantly and importantly increased functional connectivity for seeds was found in SA group in precuneus. However, no significantly increased connection for seeds in precuneus was found in UA group, compared with MCI group.

Figure 3. Linear trend of altered functional connectivity across MCI, UA and SA groups. The horizontal axis is MMSE score, and vertical axis is correlation coefficient. The right edge is relative region and its MNI coordinate.

DISCUSSION
Function connectivity of aging in DMN during VFTs
Functional connectivity MRI analysis was considered to be an efficient method to identify spatial patterns of spontaneous coherent activity in the fMRI BOLD signals.\(^{17-20}\) In the resting brain, functional connectivity, seeded at PCC and MPFC, has been adopted in the DMN.\(^{17,19}\) Greicius and his colleagues considered that the network, constituted by the brain regions, such as PCC and ventral anterior cingulate cortex, would be modulated by task demands, and also suggested that the retrieval and manipulation of episodic memories and semantic knowledge were likely elucidated by the DMN.\(^{21}\) Other researchers also mentioned that during the working memory task, functional connectivity in DMN also has been demonstrated in elders or adults in previous studies.\(^{5,15}\) It was expected that the functional connectivity in DMN would repeat again in aging during VFTs. In this study, it was well demonstrated that the strong functional connectivity between nodes had been discovered in the group of SA during VFTs. In the group of SA and UA, the functional connectivity in DMN was well found in PCC/precuneus (BA7/31), medial frontal gyrus (BA10) and angular gyrus (BA39/40), which contained most of DMN spatial patterns.\(^{2,17-19}\) Regions not in DMN, such as thalamus, cerebellum and some of occipital cortices, were also observed. These brain regions might also participate in the cognitive activity, and cerebellar contributions have been reported in Habas et al’s study.\(^{22}\) It meant that a complex functional connectivity existed in the brains of the aging.

It was thought that hippocampus contributed to the semantic memory.\(^{23,24}\) However, each group subjects did not show significant correlation between seeds and hippocampal regions in current study. It was reported that there was an age-related decreased activation in hippocampus in memory recognition.\(^{25}\) In hippocampus, atrophy of structure and decreased functional activity might be related with aging, and also affected by diseases, such as AD or MCI.\(^{25,27}\) These factors (e.g. aging and relative disease) might be some important reasons why we could not find significant functional connectivity
between hippocampus and seeds. Therefore, it does not mean that hippocampus does not contribute to semantic memory.

**DMN alterations between groups**

Previous studies have shown that altering functional connectivity or activity in DMN is related with AD or MCI.\(^{28,30}\) It was expected that a cognitive task demanding a high level of neuronal cooperation might be a sensitive diagnostic tool for MCI in aging. So we hypothesized that the differences of connectivity between various groups of aging would appear in the VFTs. And the fcMRI analysis was applied to distinguish MCI group from healthy aging elderly, during VFTs. Our hypothesis was confirmed when significant changes of DMN connectivity were observed (Table 2).

Through one-way ANOVA, the significant alterations between groups were mainly found in parietal lobe and precuneus (Figures 1 and 2). Compared with the SA group and the UA group, significant decrease of functional connectivity in the MCI group was found in IPL (BA40) and especially in left angular gyrus (BA39, Figure 1). Using positron emission tomography (PET), Andreasen and his colleagues had reported that bi-parietal areas were activated in both short-term and long-term verbal memory, and suggested that these regions represented the distributed “storage” systems being referenced.\(^{31}\) Semantic, visual, and temporal checks were probably involved in this referencing process.\(^{31}\) In Woodard et al’s study,\(^{32}\) it was possible that PCC helped to establish and maintain memory traces to change them from short-term to long-term memory, and lesions in this region could produce retrograde memory impairment. Other studies have also demonstrated that PCC played a critical role in successful memory retrieval.\(^{33-35}\) So, it was reasonable that the maintenance of functional connectivity in the parietal lobe, especially the PCC, as a key “hub” of convergence being strongly intercommunicated with other memory systems,\(^{2}\) might contribute to the memory retrieval. In precuneus (BA7), a significant decrease of connectivity was found in the UA group and the MCI group compared with the SA group, and no significant discrepancies were observed between the UA group and the MCI group in this region (Figure 2). Precuneus, a region comprised of the posterior and dorsal portion of the medial parietal lobe, was adjacent to PCC. And the suggested pivotal role of the precuneus/posterior cingulate cortex in the default mode network was discussed in previous study.\(^{36}\) It was speculated that precuneus might be as similar and important as PCC role in memory retrieval. Furthermore, when observing the close relationship between DMN and semantic memory,\(^{37}\) one finds that the precuneus may play an important role in semantic memory. However, in AD, due to beta-amyloid deposition, DMN was related to episodic memory.\(^{38}\) To some degree, it might partly account for the better performance of SA group than the other two groups (Table 1). In our study, it was demonstrated that the decrease of functional connectivity within the DMN in aging might partly result in deactivation of memory network and the further decline of semantic memory. Normal aging might be associated with alterations in the connectivity of brain regions within the DMN,\(^{15}\) and memory networks should be less active in the MCI and AD groups.\(^{39}\) In addition, subtle decline in other areas of cognition (such as executive functions) during VFTs might coexist with memory deficits, and might also partly account for the different performances of groups.\(^{40,42}\) In some case, DMN alterations of functional connectivity, especially in left angular gyrus and precuneus, could be considered a biomarker for distinguishing heterogeneous elders,\(^{13,43}\) such as the elderly of the SA and MCI groups, from aging elderly.

Moreover, a significant link between altered functional connectivity (correlation coefficients) and levels of cognitive status (MMSE scores) has been provided (Figure 3) and it seems to show that functional connectivity in precuneus (BA7) was sensitive to the levels of cognitive status. These further support the idea that decreased functional connection might reflect the state of functional brain organization in aging.

**Limitation of this study**

Although limited by the small samples size, there was no significant functional activity estimated by generalized linear model (GLM) during the verbal fluency tests. It may have been caused by the reason that the BOLD signals related to the task were too low to be estimated. So we calculated the functional connectivity of groups to study the aging. However, heterogeneity of aging in resting state will be planned to further research this topic. In addition, the survey and criteria (especially MMSE) were the main means of defining the SA, UA and MCI groups. It is an inevitable limitation that the true states of subjects could not be absolutely detected through the proposed standard. Finally, the heterogeneity of the MCI group may have an effect on the outcome of our study. Here, we focused on the function of memory based on the memory task. The other cognitive alteration in MCI and aging will also be investigated by using resting-state or the special task relative fMRI in future.

**Conclusion**

Results from the present fcMRI study provided evidence for DMN maintenance and abnormalities in heterogeneous older adults with SA and MCI groups during VFTs. It was also suggested that areas in DMN might be utilized to participate in semantic process, and decrease of functional connectivity in DMN might partly lead to impairment of semantic memory. The current results also suggested that altered functional connectivity in DMN might reflect chronic, abnormal alterations on the early stage of AD in the brain, which could probably serve as a biomarker to distinguish between heterogeneous elders and usual aging elderly. Further investigations based on simultaneous EEG-fMRI\(^{44,45}\)
might reveal more detailed information about the impairment of DMN. Furthermore, this research might be a useful insight into questions about heterogeneity of aging brain.

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