fMRI revealed neural substrate for reversible working memory dysfunction in subclinical hypothyroidism

De-Fa Zhu,1,* Zhao-Xin Wang,2,4,* Da-Ren Zhang,2 Zhong-Lin Pan,3 Sheng He,5 Xiao-Ping Hu,6 Xiang-Chuan Chen2 and Jiang-Ning Zhou2

1Department of Endocrinology, Anhui Geriatric Institute, The First Affiliated Hospital of Anhui Medical University, 2Hefei National Laboratory for Physical Science at Microscale and School of Life Science, University of Science and Technology of China, 3Department of Radiology, PLA 105 Hospital, Hefei, Anhui, 4Guangdong Key Lab of Medical Molecular Imaging, Shantou University Medical College, Shantou, China, 5Department of Psychology, University of Minnesota, Minneapolis, MN and 6Department of Biomedical Engineering, Georgia Tech and Emory University, Atlanta, GA, USA

Correspondence to: Xiang-Chuan Chen or Jiang-Ning Zhou, Department of Neurobiology and Biophysics, School of Life Science, University of Science and Technology of China, Huangshan road 443, Hefei 230027, Anhui, China
E-mail: chxc@ustc.edu.cn or jnzhou@ustc.edu.cn
*These authors contributed equally to this work

Cognitive impairments have been found in thyroid hormone-related diseases (e.g. hyperthyroidism and hypothyroidism) for a long time. However, whether and how subclinical hypothyroidism (SCH) causes any deficits in brain functions, and whether a hormone-replacement treatment is necessary for SCH patients, still remain controversial subjects. In the present study, functional MRI (fMRI) was used to measure brain functions by asking euthyroid subjects, hyperthyroid patients and SCH patients to perform the widely used digit n-back working memory task. After having been treated with L-thyroxine for ~6 months, the SCH patients were asked to do the same fMRI experiment. The hypothyroid and SCH patients scored significantly lower in the 2-back task than either the hyperthyroid patients or the euthyroid subjects (P < 0.012). The fMRI showed that a common frontoparietal network, including bilateral middle/inferior frontal gyri (M/IFG), bilateral dorsolateral prefrontal cortex (DLPFC), bilateral premotor areas (PreMA), the supplementary motor area/anterior cingulate cortex (SMA/ACC) and bilateral parietal areas (PA), was activated by the n-back task in all the subjects. Further quantitative analysis showed that the load effect of blood oxygen level-dependent (BOLD) response appeared in all the five regions of interest (ROIs) in the euthyroid and hyperthyroid subjects. In the pre-treatment SCH patients, however, the load effect of BOLD response was only found in the PA and PreMA, but not in other frontal cortex ROIs [general linear model (GLM), F < 2.6, P > 0.1]. After an ~6 month treatment with LT4, the SCH patients exhibited the same load effects in all five ROIs as the euthyroid subjects (GLM, F > 6, P < 0.05) along with an improvement of performance in n-back task. These results suggest that working memory (but not other memory functions) is impaired in SCH patients, mainly as far as disorders of the frontoparietal network were concerned. Both the memory performance and frontal executive functions were improved after an L-thyroxine-replacement treatment.

Keywords: working memory; functional MRI (fMRI); frontal cortex; subclinical hypothyroidism (SCH); L-thyroxine treatment

Abbreviations: BOLD = blood oxygen level-dependent; DLPFC = dorsolateral prefrontal cortex; fMRI = functional MRI; GLM = general linear model; M/IFG = middle/inferior frontal gyrus; MQ = memory quotient; PA = parietal areas; PreMA = premotor areas; ROIs = regions of interest; SCH = subclinical hypothyroidism; SMA/ACC = supplementary motor area/anterior cingulate cortex; TSH = thyroid-stimulating hormone; WMS-CR = Wechsler Memory Scale—Chinese Revision

Introduction

Thyroid hormones are very important for human beings, not only for maintaining normal physiological functions; they are also thought to be related to high-level cognition (Timiras and Nzekwe, 1989; Loosen, 1992). Epidemiological surveys using the revised Wechsler Adult Intelligence Scale (WAIS-R) and the Mini-Mental State Examination (MMSE) suggest a positive correlation between plasma thyroid hormone level and cognitive function in people with normal plasma thyroid levels (Prinz et al., 1999; Volpato et al., 2002). Abnormal plasma concentrations of the thyroid hormone are thought to be a risk factor for dementia (Bulens, 1981; Smith and Granger, 1992; Kalmijn et al., 2000). Hypothyroidism and hyperthyroidism are the two most frequently diagnosed endocrinological diseases (Cooper, 2000; Roberts and Ladenson, 2004). Previous studies in hypothyroid patients usually reported cognitive impairments (Mennemeier et al., 1993; Dugbartey, 1998; Burmeister et al., 2001; Roberts and Ladenson, 2004). For patients with hyperthyroidism the data are ambiguous, in that cognitive deficits were shown in some studies, but not in others (Zeithofer et al., 1984; Cunha, 1990; Schloete et al., 1992; Stern et al., 1996). Inconsistent results can also be found in patients with subclinical hypothyroidism (SCH), which is characterized by elevated plasma thyroid-stimulating hormone (TSH) concentration with normal thyroid hormone level (Evered et al., 1973; Cooper, 2001; Col et al., 2004). Some investigators have suggested that SCH is associated with cognitive decline, because SCH patients performed worse than euthyroid subjects in some neuropsychological tests such as the Wechsler Adult Intelligence Scale (WAIS), the Wechsler Memory Scale (WMS) or verbal fluency (Monzani et al., 1993; Baldini et al., 1997; del Ser Quijano et al., 2000). However, other investigators found the neuropsychological performance of SCH patients to remain within the normal range (Bono et al., 2004). Moreover, little is known about the neural substrate for the possible influences of thyroid hormone on the cognitive functions of SCH patients (Anderson, 2001).

Cognitive functions of SCH patients have been evaluated with various psychometric and psychopathological tests in many previous studies (Monzani et al., 1993; Baldini et al., 1997; Bono et al., 2004), but none of these tests was designed to examine working memory function specifically. Given that working memory may be the core of many cognitive functions and may be vital for general human intelligence (Baddeley, 1992; Wickelgren, 1997), it should be investigated whether working memory function is affected by SCH or not. Working memory refers to short-term storage and online manipulation of information (Baddeley, 1992; Cohen et al., 1997). In the present study, working memory function was evaluated with a digit n-back task (Fig. 1; n = 0, 1 and 2). N-back tasks are widely used to investigate the neural substrates of working memory (Caldicott et al., 1999; Nystrom et al., 2000; Honey et al., 2002; Jansma et al., 2002). In a 2-back task trial, a series of digits were presented one by one at the same location on a computer screen. Subjects were asked to report the second digit that was presented before the current one. Because the digits appeared continually, subjects had to temporarily store three digits in memory, make their reports as required and then delete the reported digit from memory before the next digit appeared. Obviously, subjects kept updating the memorized digits during this process. In a 1-back task trial, the first digit presented before the current one was reported, and in a 0-back task trial, the current digit was reported. This last task is a simple identification task. As a consequence, the storage and manipulation demands, or the task loads, varied across the three n-back levels.

In these functional brain imaging studies on healthy subjects, working memory-related brain areas exhibited varied neural responses at different n-back levels (load effect); brain areas involved in other processes, such as perception of input stimulus or performance of output report, did not demonstrate the load effect on neural response. Thus, the load effect can be used to examine whether the working memory-related brain areas function normally or whether they are not functioning (Caldicott et al., 2000; Perlstein et al., 2001).

The present study used functional MRI (fMRI) to evaluate the brain functions in the digit n-back task for euthyroid subjects, hyperthyroid patients and SCH patients. Blood oxygen level-dependent (BOLD) signals were measured at three n-back levels (0-, 1- and 2-back) so that the load effect of BOLD response could be examined in the working memory-related brain areas. In this way we were able to examine whether or not the hyperthyroid and SCH patients exhibited working memory deficits in the n-back task, and which brain areas might be affected. Meanwhile, the Wechsler Memory Scale—Chinese Revision (WMS-CR) was used to investigate...
whether or not there were any changes in other memory functions in these subject groups.

Another important issue, whether or not SCH patients should be treated with L-thyroxine, still remains controversial (Schlinger et al., 2003; Guseklooo et al., 2004; Surks et al., 2004). Several studies have reported that an L-thyroxine therapy could reduce the symptoms of SCH, suggesting that most patients with SCH should be treated (Arem and Escalante, 1996; Cooper, 1998; Ayala et al., 2000; Bonito et al., 2004). Other investigators argued against this view, in that no clinic relevant benefits were found in SCH patients after a treatment, and it still needs to be proven that the treatment ameliorates the dysfunction caused by SCH (Chu and Crapo, 2001). To investigate this issue in the present study, the SCH patients also carried out the same fMRI experiment after having been treated with L-thyroxine for ~6 months. As we found that the SCH patients exhibited lower performance in the 2-back task than the euthyroid subjects and the hyperthyroid patients, and that their frontal areas functioned abnormally in the task, the data collected with the post-treatment SCH patients could help us verify whether these patients might recover from the working memory dysfunctions or not.

Material and methods

Subjects

Twelve euthyroid control subjects (11 females and 1 male, all right-handed, ages between 20 and 40), 16 hyperthyroid patients (14 females and 2 males, all right-handed, ages between 18 and 40), 9 hypothyroid patients (all females, all right-handed, ages between 17 and 43) and 11 SCH patients (10 females and 1 male, all right-handed, ages between 18 and 47) participated in the present study with informed consent. The experiments were performed with the approval of the First Affiliated Hospital of Anhui Medical University human subjects review committee. All the patients were recruited when they were first diagnosed and had not been treated with medicine or surgery. After having been treated with levo-thyroxine (LT4, APS Co., UK) for ~6 months, six SCH patients (all females) participated in the same experiments as the patients with pre-treatment conditions. The clinical manifestations of each subject are shown in Table 1. None of the subjects had neurological or psychiatric disorders and they were all matched for age and level of education [ANOVA (analysis of variance), F(4,50) < 1.780, P > 0.1]. Patients with hypothyroidism only performed the n-back task. Results showed that hypothyroid patients were unable to complete the 2-back task [accuracy is 38.8%; standard error (SE) = 11.6; shown in Fig. 2]. Another study has proved that individuals may disengage from the task if it is too difficult (Callcott et al., 1999). Hypothyroidism patients were, therefore, excluded from the subsequent fMRI study because it was expected that they might disengage from the 2-back in the fMRI session and thus render the results meaningless.

Assessment of hormone levels

Plasma hormone and thyroid peroxidase antibody (TPOAb) levels were measured with a chemiluminescence immunoassay for each subject. Apparatus and reagents used in the assay came from the DPC (Diagnostic Products Co., Immulite 2000, Los Angeles, CA, USA). Reference to normal range for each hormone is shown in Table 1.

Assessment of WMS-CR

Memory functions of each subject were assessed with the WMS-CR. The WMS-CR is designed to evaluate memory functions regarding information, orientation, mental control, figural memory, visual reproduction, associative memory, logic memory and numeric span. It also provides an overall ‘memory quotient’ (MQ) (Wechsler, 1987; Gong et al., 1989).

Paradigm of fMRI experiment

The digit n-back task used in the fMRI experiment is shown in Fig. 1. An n-back task block started with a 2 s cue that instructed the subjects how to perform the task (‘recall the current digit’, 0-back; ‘recall the first digit before the current one’, 1-back; ‘recall the second digit before the current one’, 2-back; all the cues were displayed in Chinese). After a 1 s delay, 10 digits were presented to the subjects serially. Each digit was displayed for 2 s, and then a fixation cross appeared for 1 s. During this 3 s period, the subjects made their report with the right hand to gesture the recalled digit, which was recognized and recorded by one of the authors sitting beside the scanner. A baseline control block was also adopted in the experiment, during which the fixation cross was displayed and the subjects were asked to look at it while taking a rest.

There were four epochs in an fMRI scan. Each epoch consisted of a 0-back, a 1-back and a 2-back task block, and followed by a baseline control block (Fig. 1). All the subjects underwent two fMRI scans. The sequence of the task blocks was 0-, 1-, 2-back in one scan, and 2-, 1-, 0-back in the other. These two task sequences were counterbalanced across all the subjects in each group. In addition to these functional scans, each subject also underwent high-resolution 2D and 3D anatomical scans for functional overlay and stereotaxic transformation.

Table 1. Clinical information of the subjects (mean ± standard deviation)

<table>
<thead>
<tr>
<th>Group (number)</th>
<th>Age (years)</th>
<th>Education (years)</th>
<th>T3 (nmol/l)</th>
<th>T4 (nmol/l)</th>
<th>FT3 (pmol/l)</th>
<th>FT4 (pmol/l)</th>
<th>TSH (mIU/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroidism 12</td>
<td>29.67 ± 7.67</td>
<td>9.50 ± 3.45</td>
<td>1.92 ± 0.19</td>
<td>101.8 ± 7.29</td>
<td>4.49 ± 0.57</td>
<td>19.5 ± 1.67</td>
<td>1.73 ± 0.62</td>
</tr>
<tr>
<td>Hyperthyroidism 16</td>
<td>29.56 ± 7.93</td>
<td>8.87 ± 3.38</td>
<td>5.25 ± 1.63</td>
<td>253.02 ± 34.49</td>
<td>11.98 ± 3.49</td>
<td>46.63 ± 8.63</td>
<td>0.04 ± 0.04</td>
</tr>
<tr>
<td>SCH before 11</td>
<td>30.55 ± 9.68</td>
<td>9.09 ± 4.61</td>
<td>1.61 ± 0.32</td>
<td>85.74 ± 10.48</td>
<td>3.05 ± 0.45</td>
<td>13.01 ± 3.27</td>
<td>14.67 ± 7.13</td>
</tr>
<tr>
<td>SCH after 6</td>
<td>29.67 ± 10.61</td>
<td>8.83 ± 3.66</td>
<td>1.97 ± 0.23</td>
<td>108.5 ± 17.1</td>
<td>4.65 ± 0.58</td>
<td>19.53 ± 4.30</td>
<td>1.35 ± 1.05</td>
</tr>
<tr>
<td>Hypothyroidism 9</td>
<td>31.44 ± 9.29</td>
<td>5.67 ± 3.24</td>
<td>0.84 ± 0.17</td>
<td>42.89 ± 11.27</td>
<td>1.98 ± 0.53</td>
<td>5.91 ± 2.02</td>
<td>44.68 ± 19.60</td>
</tr>
<tr>
<td>Normal range</td>
<td>–</td>
<td>–</td>
<td>1.1–2.6</td>
<td>58–161</td>
<td>2.3–6.3</td>
<td>8.4–29.6</td>
<td>0.4–4.0</td>
</tr>
</tbody>
</table>

T3 = triiodothyronine; T4 = thyroxine; FT3 = triiodothyronine; FT4 = free thyroxine; TSH = thyrotropin-stimulating hormone; SCH = subclinical hypothyroidism.
Using a video projector (Epson EMP-530), the stimuli were presented on a translucent screen placed near the scanner bed. The fixation cross extended ~0.8’ × 0.8’, the cues extended ~8’ × 2’ and the digits extended ~2’ × 1’, all in visual angle. The centres of all the stimuli were displayed at the same location on the screen. Subjects viewed the stimuli through a mirror mounted on the head coil above their eyes.

**MRI data acquisition**

Imaging data were collected on a 1.5 T Philips Infinion MR System. During each functional scan, 179 volumes that consisted of 16 axial slices were obtained with a T2*-weighted echo-planar imaging (EPI) sequence [repetition time (TR) = 3 s, echo time (TE) = 40 ms, field of view (FOV) = 24 × 24 cm², matrix = 64 × 64, flip angle = 90°, slice thickness = 4 mm, gap between two adjacent slices = 1.2 mm]. Also, corresponding high-resolution T1-weighted imaging data were obtained with a spin echo (SE) sequence (2D anatomical data) and a spoiled gradient recalled echo (SPGR) sequence (3D anatomical data).

**MRI data analysis**

MRI data were analysed using AFNI (Analysis of Functional NeuroImages, a set of C programs for processing, analysing and displaying MRI data. http://afni.nimh.nih.gov/afni/) (Cox, 1996). After the first three volumes of each scan had been excluded, all functional data sets were processed to remove any linear drift and to correct motion and be normalized. Any scan in which the head motion was larger than 2 mm was excluded from further analysis. The data were then analysed in two ways: group and individual analysis. In the group analysis, all the subjects’ functional data sets of each group were concatenated together after being stereotaxically transformed into a common space (Talairach and Tournoux, 1988) and spatially smoothed with a Gaussian filter (FWHM = 6 mm). Correlation analysis based on the direct contrast between the task (including the 0-, 1- and 2-back block) and baseline control were then carried out to generate the activation map for each group (P ≤ 10⁻¹⁰, cluster size ≥ 4 voxels). This analysis revealed the brain activities related to the n-back task, but irrespective of the n-back task load. These activation maps were used to locate the regions of interest (ROIs), the n-back task-related brain areas, for the four subject groups.

For each subject, correlation analysis was performed on the functional data according to three contrasts: 0-back task versus control, 1-back task versus control and 2-back task versus control. As a result, three activation maps (P < 0.05, cluster size ≥ 4 voxels) were generated, and a combined activation map was obtained by the logical ‘OR’ of these maps. The ROIs for individual subjects were then identified on this combined map. After that, the amplitudes of the average BOLD responses at the three n-back level were calculated for each ROI. Whether or not the BOLD signal changed with the n-back load (the load effect) was further examined with statistical analysis.

**Statistical analysis**

ANOVA and independent sample t-test were used to compare the data of plasma hormone level, performances of the WMS-CR and the n-back task and age and level of education of all five subject groups. The load effects of performance accuracy and BOLD response in the n-back task were evaluated with a general linear model (GLM) for repeated measures. The statistical analysis was performed with SPSS for Windows (version: 13).

**Results**

**Hormonal data**

The hormonal data collected for all the subject groups are shown in Table 1.

As reported in previous studies (Evered et al., 1973; Cooper, 2001; Col et al., 2004), all the SCH patients who had not had any treatment had plasma thyrotropin-stimulating hormone (TSH) levels above the maximum normal values. Their plasma thyroid hormones (including T3, T4, FT3 and FT4) remained within the normal range. The T4 levels were not significantly (t-test, P = 0.307) lower, whereas the T3, FT3 and FT4 levels were significantly (t-test, P < 0.01) lower than those of the euthyroid control subjects.

After an ~6 months treatment with LT4, the plasma TSH concentrations of the SCH patients fell into the normal range. Compared with the pre-treatment condition, their T3 level did not significantly (t-test, P = 0.270) change, whereas the T4, FT3, FT4 levels significantly (t-test, P < 0.05) increased but still remained normal. No significant differences between the euthyroid subjects and the post-treatment SCH patients (t-test, P > 0.26) were found in plasma TSH and thyroid hormone levels.

The plasma TSH level of the hyperthyroid patients was under the lower normal value. Their thyroid hormone levels exceeded the upper normal limit. In contrast, the plasma TSH level of the hypothyroid patients exceeded the upper normal value. Their thyroid hormone levels were under the lower normal limit. The plasma hormonal data of all the euthyroid subjects were within the normal range.

**Psychometric evaluation**

Memory functions assessed with the WMS-CR for all the subject groups (Table 2) showed that there was no significant difference among them either in the total memory score [MQ: ANOVA, F(3,41) = 1.693, P = 0.184] or in the subtests [ANOVA, F(3,41) < 2.30, P > 0.09].
Table 2 WMS-CR performance of the subjects (mean ± standard deviation)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Euthyroid</th>
<th>Hyperthyroid</th>
<th>SCH (before)</th>
<th>SCH (after)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Information</td>
<td>5.00 ± 0.00</td>
<td>5.00 ± 0.00</td>
<td>4.82 ± 0.40</td>
<td>5.00 ± 0.00</td>
</tr>
<tr>
<td>Orientation</td>
<td>4.25 ± 0.97</td>
<td>4.50 ± 0.73</td>
<td>4.09 ± 0.70</td>
<td>4.67 ± 0.82</td>
</tr>
<tr>
<td>Mental control</td>
<td>30.54 ± 5.98</td>
<td>31.80 ± 5.47</td>
<td>28.23 ± 8.41</td>
<td>32.33 ± 4.96</td>
</tr>
<tr>
<td>Figural memory</td>
<td>10.42 ± 2.39</td>
<td>11.78 ± 1.74</td>
<td>10.64 ± 1.69</td>
<td>11.50 ± 1.76</td>
</tr>
<tr>
<td>Visual reproduction</td>
<td>20.67 ± 4.01</td>
<td>20.63 ± 4.66</td>
<td>17.82 ± 5.38</td>
<td>20.83 ± 3.06</td>
</tr>
<tr>
<td>Associative memory</td>
<td>9.17 ± 3.88</td>
<td>9.97 ± 3.76</td>
<td>7.18 ± 4.05</td>
<td>11.25 ± 2.48</td>
</tr>
<tr>
<td>Logic memory</td>
<td>10.92 ± 2.78</td>
<td>11.69 ± 2.30</td>
<td>10.14 ± 2.15</td>
<td>9.67 ± 3.27</td>
</tr>
<tr>
<td>Numeric span</td>
<td>10.50 ± 3.68</td>
<td>10.94 ± 3.53</td>
<td>8.18 ± 4.19</td>
<td>8.67 ± 3.01</td>
</tr>
<tr>
<td>Memory quotient</td>
<td>110.42 ± 21.86</td>
<td>114.06 ± 16.08</td>
<td>98.36 ± 19.54</td>
<td>111.17 ± 12.84</td>
</tr>
</tbody>
</table>

WMS-CR: Wechsler Memory Scale—Chinese revision; SCH: subclinical hypothyroidism.

Performance accuracy of n-back task

Performance accuracy of n-back task refers to the percentage of correctly reported digits in the total number of digits to be recalled in certain n-back conditions. Figure 2 demonstrates that the accuracies were not significantly different across all the subject groups in the 0-back tasks [ANOVA, F(4,49) = 0.412, P = 0.799]. In the 2-back task, these subject groups showed varied accuracies [ANOVA, F(4,49) = 23.772, P = 0.001] resulting from the lower accuracy of hypothyroid patients than other subject groups (t-test, P < 0.001); the pre-treatment SCH subject also performed poorly—worse in the 2-back task than euthyroid and hyperthyroid subjects (t-test, P < 0.012). In the 1-back task, these subject groups also showed varied accuracies [ANOVA, F(4,49) = 4.352, P = 0.004] resulting from the lower accuracy of the hypothyroid patients than the other subject groups (P < 0.05). In addition, the accuracies also changed with the n-back levels in the euthyroid, hyperthyroid, pre-treatment SCH and hypothyroid subject groups (GLM, F > 6.232, P < 0.01) but not in the post-treatment SCH patients [GLM, F(2,10) = 1.982, P = 0.188].

Functional MRI data

Regions of interest

The results from the analysis of the group and individual data showed that a common cortical network, including bilateral middle/inferior frontal gyri (M/IFG), bilateral dorsolateral prefrontal cortices (DLPFC), bilateral premotor areas (PreMA), the supplementary motor area/anterior cingulate cortex (SMA/ACC) and bilateral parietal areas (PA), was activated by the n-back task in all the subject groups (Fig. 3A). These results are consistent with previous reports about the involvement of this network in various n-back working memory tasks (Callicott et al., 1999; Jansma et al., 2000; Nystrom et al., 2000; Honey et al., 2002). The BOLD responses should also change with the n-back load (load effect) in these areas (Callicott et al., 1999; Jansma et al., 2000; Nystrom et al., 2000; Honey et al., 2002). Thus, all these areas were identified as ROIs. The left primary somatosensory/motor areas (SI/Motor) were also activated (Fig. 3A). It was set up as the control ROI because the load effect of BOLD response was predicted not to appear in this ROI.

Fig. 3 ROI and load effect of BOLD response. (A) Brain areas involved in the digit n-back task (ROIs). The activation map for the euthyroid subject was depicted. The other three subject groups displayed similar results. R = right hemisphere, L = left hemisphere. F1 = M/IFG, F2 = DLPFC, F3 = PreMA, F4 = SMA/ACC, P1 = PA, M1 = SI/Motor. (B) Load effect of BOLD responses in the left ROIs for the four subject groups. Similar results were demonstrated in the right ROIs, except that the right SI/Motor was not activated in the task. Red represents 0-back, green: 1-back, blue: 2-back. The plots show that only the pre-treatment SCH patients exhibited abnormal function in the frontal areas (shown in the black box). Error bars are I SE.

BOLD response as a function of n-back load

As we predicted, the BOLD response did not vary with the n-back load in the SI/Motor for any of the subject groups (GLM, F < 1.09, P > 0.355; Fig. 3B). It was also expected that...
the load effect of BOLD response would appear in the M/IFG, the DLPFC, the PreMA, the SMA/ACC and the PA of both hemispheres for the euthyroid subjects. Our results confirmed this expectation [GLM, \( F(2,22) > 3.690, P < 0.043 \); Fig. 3B], indicating that the load effect in these five ROIs can be used as an index in analysing the data for the patient groups.

For the pre-treatment SCH patients, the load effect of BOLD response was only found in the PA and PreMA [GLM, \( F(2,18) > 4.444, P < 0.05 \)], but not in other ROIs in the frontal cortex [i.e. DLPFC, M/IFG, SMA/ACC; GLM, \( F(2,18) < 2.361, P > 0.145 \); Fig. 3B]. After being treated with LT₄, these patients exhibited the load effects in all the five ROIs, the same way the euthyroid subjects did [GLM, \( F(2,8) > 4.733, P < 0.05 \); Fig. 3B]. The hyperthyroid patients demonstrated similar results to those from the euthyroid subjects in these five ROIs [GLM, \( F(2,22) > 4.692, P < 0.032 \); Fig. 3B]).

Discussion

Working memory function is impaired by SCH and hypothyroidism

By using a digit n-back paradigm, the present study showed that the SCH patients without treatment obtained significantly lower scores in the 2-back task than the hyperthyroid patients and the euthyroid subjects, whereas no significant difference was seen between the latter two subject groups (Fig. 2). Interestingly, the hypothyroid subjects performed even worse than the SCH patients. Given that n-back task paradigms are widely used to evaluate working memory function (Owen et al., 2005), these results suggest that working memory is impaired by SCH, and more severely in hypothyroidism, but not in hyperthyroidism. Our data also showed that the results of the subtests and the MQ of the WMS-CR were not significantly different for the pre-treatment SCH patients and the euthyroid subjects (Table 2), suggesting that the effect of SCH on other memory functions is not as significant as that on working memory.

Performing the n-back task involves multiple processes, such as manipulating/updating items and storing them in working memory. Functional brain imaging studies suggest that these processes recruit differentiable but interacting cortical areas. For example, various areas in the frontal cortex have been demonstrated to be engaged in the executive functions (e.g. manipulating/updating memory items) (Braver et al., 1997; Cohen et al., 1997; Smith and Jonides, 1997, 1998; Fletcher and Henson, 2001), while memory items may be stored in the parietal cortex (Paulesu et al., 1993; Smith and Jonides, 1998). With the behavioural data mentioned above, it is impossible to tell which part of the cortical areas involved in the n-back task are impaired by SCH. The present study tries to answer this question through an fMRI approach.

Neural substrate for working memory dysfunction in SCH patient

The fMRI data showed that a frontoparietal network, including the M/IFG, the DLPFC, the PreMA, the SMA/ACC and the PA of both hemispheres, was involved in the n-back task in all the subject groups. Further quantitative analysis indicated that the BOLD response changed with the n-back load (the load effect) in both the frontal and the PA of the euthyroid subjects and the hyperthyroid patients, but only in the PA and PreMA areas of the pre-treatment SCH patients (Fig. 3B). The results from the euthyroid subjects and the hyperthyroid patients are consistent with many previous functional brain imaging studies on healthy subjects (Owen et al., 2005), suggesting that the frontal/parietal areas of these two groups functioned normally in the n-back task. In contrast, the pre-treatment SCH patients demonstrated abnormal functions in the frontal but not the PA, implying that the executive function of their working memory is impaired by SCH. On the basis of this interpretation one can infer that SCH patients would perform worse than euthyroid subjects in the tasks depending on the executive functions. Previous studies on the verbal fluency of SCH patients (del Ser Quijano et al., 2000; Bono et al., 2004) support this inference.

In the present study, we did not directly compare the data collected for different subject groups, because these data may be affected by some non-cognitive factors, such as the haemodynamic features and the status of the scanner. In contrast, the modulation effect of memory load on the BOLD response within a suitable range should not be qualitatively changed (e.g. eliminated) by these factors. For example, blood flow in a cortical area may be different for two different subject groups. This factor may increase (or decrease) the BOLD responses under the three n-back loads in this area. If only the BOLD responses in the 2-back condition of the two groups are compared, the result may be attributed to the change in blood flow, or the difference in memory functions, or both. If the BOLD responses are examined at the three load levels, the change in blood flow will not affect whether or not the BOLD response increases with the load. This load effect of BOLD response should be a more valid index to verify the difference in memory function between different groups (Callicott et al., 2000; Perlstein et al., 2001). In addition, the BOLD response in the left SI/Motor was also investigated in case the load effect of BOLD response was caused by the changes in scanner status. As predicted, this confounding factor can be ruled out because no load effect was found in the left SI/Motor (Fig. 3B).

Do SCH patients need L-thyroxine-replacement treatment?

Whether or not SCH patients need treatment is a basic and ongoing issue in previous studies (Gussekloo et al., 2000; Schlinger et al., 2003; Surks et al., 2004). In the present study, two lines of evidence suggest that treatment is necessary for SCH patients. First, both the behaviour and fMRI results for the n-back task showed that working memory, especially its frontal executive function, was impaired in the SCH patients without any treatment (see Discussion above).
Because the frontal executive function plays a central role in many cognitive functions (Chayer and Freedman, 2001), it should be of great benefit to the patients if they received a suitable treatment to ameliorate this working memory deficit. Secondly, our data showed that for the n-back task, not only the performance accuracy (Fig. 2) but also the load effect of BOLD response (normal response in healthy subjects) in the frontal areas (Fig. 3B) was recovered after an ~6 months treatment with L-thyroxine for the SCH patients. These results indicate that the impairment of the frontal executive function in the SCH patients is still reversible. This view is also supported by previous studies of frontal executive/working memory function in SCH patients. For example, the performances of SCH patients in a verbal fluency task improved after the patients were treated, suggesting that their frontal executive function was ameliorated (del Ser Quijano et al., 2000; Bono et al., 2004). Thus, the present study provides both behavioural and fMRI evidence for the view that SCH patients need an L-thyroxine-replacement treatment. We noted that the small sample size of treated SCH patients may diminish the force of the present study and that caution should be exercised in interpreting this part of the results. However, we have several reasons to believe that our results are robust. fMRI is more sensitive than the traditional method in both genetic and pharmacological studies. In particular, the study is the longitudinal within-subject design, which is well suited and powerful enough to address issues related to response to drug treatment in fMRI research. Therefore, a positive treatment effect has been found in this small sample in the present study. The results obtained from the hypothyroid patients who showed severe working memory deficits also support this notion and indicate that early intervention in SCH may prevent the cognitive impairment in the later stage of the disease, that is, hypothyroidism. However, as clinical cases may usually be quite variable, the large sample study is needed to examine the generalization of the treatment value in a further study.

Conclusion
In summary, our data showed that the pre-treatment SCH patients demonstrated a lower performance than the euthyroid subjects did in the 2-back task, whereas no significant difference in the subtest scores and the MQ of the WMS-CR was found between these two subject groups. These results suggest that only working memory function is impaired in SCH patients. Furthermore, the fMRI data revealed that the frontal areas were affected by SCH, indicating that the executive functions were abnormal in these patients. The deficits in both 2-back task performance and frontal executive functions were ameliorated by an L-thyroxine-replacement treatment. Thus, the present study provides evidence, in working memory function as well as in its neural substrate, for the view that SCH patients would benefit from clinical treatment.

Acknowledgements
This research was supported by the National Natural Science Foundation of China (30370478, 30328017 and 30470572) and Ministry of Science and Technology of China (2006CB500705). We are indebted to Mrs W. T. P. Verweij for correcting the English.

References
Col NF, Surks MI, Daniels GH. Subclinical thyroid disease: clinical applications. JAMA 2004; 291: 239–43.