CORTEX XXX (2012) 1–20

Available online at www.sciencedirect.com

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Research report

Effects of working memory training on functional connectivity and cerebral blood flow during rest

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ARTICLE INFO

Article history:
Received 7 March 2012
Reviewed 20 June 2012
Revised 29 July 2012
Accepted 13 September 2012
Action editor Bradley Postle
Published online xxx

Keywords:
Working memory training
Functional connectivity
Cerebral blood flow
Rest
Plasticity

ABSTRACT

Working memory (WM) training (WMT) alters the task-related brain activity and structure of the external attention system (EAS). We investigated whether WMT also alters resting-state brain mechanisms, which are assumed to reflect intrinsic brain activity and connectivity. Our study subjects were subjected to a 4-week WMT program and brain scans before and after the intervention for determining changes of functional connectivity and regional cerebral blood flow during rest (resting-FC/resting-rCBF). Compared with no-intervention, WMT (a) increased resting-FC between the medial prefrontal cortex (mPFC) and precuneus, which are key nodes of the default mode network (DMN), (b) decreased resting-FC between mPFC and the right posterior parietal cortex/right lateral prefrontal cortex (LPFC), which are key nodes of the EAS, and (c) increased resting-rCBF in the right LPFC. However, the training-related decreases in resting-FC between the key DMN node and the nodes of EAS were only observed when the whole brain signal was regressed out in individual analyses, and these changes were not observed when the whole brain signal was not regressed out in individual analyses. Further analyses indicated that these differences may be mediated by a weak but a widespread increase in resting-FC between the nodes of EAS and activity of multiple bilateral areas across the brain. These results showed that WMT induces plasticity in neural mechanisms involving DMN and the EAS during rest and indicated that intrinsic brain activity and connectivity can be affected by cognitive training.

1. Introduction

Working memory (WM) is the limited capacity storage system involved in the maintenance and manipulation of information over a short time period (Baddeley, 2003). WM is a functionally important system that underlies a wide range of higher-order cognitive activities (Baddeley, 2003; Osaka and Nishizaki, 2000). Reduced working memory capacity (WMC) is associated

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http://dx.doi.org/10.1016/j.cortex.2012.09.007

with neurological and psychiatric disorders (Baddeley, 2003; Goldman-Rakic, 1994; Rose and Ebmeier, 2006) as well as normal aging (Wingfield et al., 1988).

Some aspects of functional connectivity (or correlation of activities between different brain regions) and cerebral blood flow (CBF) during rest [resting-FC/resting-regional CBF (rCBF)] are associated with conditions with reduced WMC. Regions in the external attention system (EAS), which is dedicated to external attention (Buckner et al., 2008; Corbetta and Shulman, 2002; Laird et al., 2005), such as the lateral prefrontal cortex (LPFC) and posterior parietal cortex (e.g., the inferior/superior parietal lobule), are involved with WM and are activated during WM performance (Baddeley, 2003). The EAS is divided into two networks; the dorsal attention network and the ventral attention network (Fox et al., 2006). In contrast, regions in the default mode network (DMN), such as the medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC), and precuneus, are deactivated during WM performance. Activities in brain regions that belong to the same network correlate with one another during rest, and a strong anticorrelation is observed between the abovementioned two networks during rest (Fox et al., 2005). In other words, when one network is activated, the other is deactivated. The strength of resting-FC among regions in DMN is correlated with individual WMC (Hampson et al., 2006). The strength of anticorrelations between the two networks is generally reduced under conditions with reduced WMC (Broyd et al., 2009; Sambataro et al., 2010). It was shown resting-rCBF in PFC or anterior regions was decreased under conditions with reduced WMC (Martin et al., 1991; Weinberger et al., 1986) described above. These brain activities during rest are assumed to reflect brain's intrinsic activity and connectivities (Fox and Raichle, 2007).

Previous studies have shown the effects of WM training (WMT) on psychological measures and neural systems (Klingberg, 2010; Takeuchi et al., 2010b; Uchida and Kawashima, 2008). Moreover, changes in brain activity, gray/white matter structures, and dopamine D1 receptor density in LPFC and posterior parietal regions after WMT have been demonstrated (Klingberg, 2010; Takeuchi et al., 2010b). Nevertheless, to our knowledge, no previous study has investigated the effect of WMT on resting-FC and resting-rCBF. Using these analyses of rest-related neural mechanisms, we were able (a) to determine how brain regions interact not only with other brain regions to which they are directly connected structurally but also with regions to which they are not structurally connected and (b) to investigate the state of DMN and the WMN during the cognitive processes involved during rest. Given that altered resting-FC underlies conditions with reduced WMC and that resting-FC underlies individual WMC, both of which are of scientific and clinical interest, it is important to investigate the extent of plasticity in resting-FC and resting-rCBF. Our previous study demonstrated that regional gray matter volume (rGMV) in EAS was decreased after a 5-day intensive (4-h training per day) WMT program involving mental calculations (Takeuchi et al., 2011d). We previously proposed that this type of intensive and concentrated training leads to decreased rGMV, possibly after an initial increase in rGMV, whereas training that is not so extensive (e.g., up to 1 h per day for 4 weeks) leads to increased rGMV, which may be followed by a decrease in rGMV after further training (Takeuchi et al., 2011b, 2011d). This proposal is based on the observation of many studies. We further suggested usage dependent synaptic formation and reduction may underlie these rGMV changes (Takeuchi et al., 2011b, 2011d). It is unclear whether this relatively mild form of WMT leads to increased rGMV.

Here young adult subjects underwent a 4-week WMT (up to 1 h per day). Before and after the intervention, they underwent scanning sessions in which resting-FC associated with DMN and the EAS, resting-rCBF and rGMV were measured. Subjects in the WMT group completed a 4-week intensive adaptive WMT program, and subjects in the control group did not participate in any such training during the study period. The lack of an active control group (placebo training) is common to almost all imaging studies of cognitive training (Dahlin et al., 2008; McNab et al., 2009; Olesen et al., 2004; Takeuchi et al., 2010a, 2011b; Westerberg et al., 2004), while an active control group has been widely used in psychological studies of cognitive training (Klingberg et al., 2005, 2002). It is an appropriate approach for this type of study as the effects of placebo training in a cognitive training study of this type, which involves normal adults, are not known and cannot be detected statistically (Takeuchi et al., 2011d). In addition, the real effects of some cognitive intervention that can be used as an active control intervention cannot be ruled out (Takeuchi et al., 2011b). Our subjects also participated in psychological experiments in which they completed a number of cognitive tests. We hypothesized that WMT would increase resting-FC within DMN, increase anticorrelations between DMN and EAS, increase resting-CBF in PFC, and increase rGMV in EAS. The hypotheses relating to resting-FC and resting-CBF are based on the abovementioned previous studies that showed that conditions with reduced WMC are generally characterized by a decrease in resting-FC within DMN, a decrease in anticorrelations between DMN and EAS, and a decrease in resting-CBF in PFC. The hypothesis relating to rGMV is based on our previous proposition described above.

2. Methods

2.1. *Subjects*

We enrolled 81 healthy, right-handed university students [59 men and 22 women; mean age 21.1 years, standard deviation (SD) 1.9]. They had normal vision and no history of neurological or psychiatric illness, which was assessed using a routine questionnaire. Handedness was evaluated using the Edinburgh Handedness Inventory (Oldfield, 1971). Written informed consent was obtained from each individual for the projects in which they participated. The Ethics Committee of Tohoku University approved all procedures. This study was performed together with another intervention study involving multitask (MT) training. The different subjects underwent the different training protocols (WMT and MT training). We aimed to determine the following: (1) effects of WMT on resting-FC, resting-rCBF, rGMV and performance on the belowmentioned cognitive tests, (2) effects of WMT on social/emotional/self/behavioral variables, (3) cognitive and neural factors affecting the WMT outcome, and (4) whether polymorphism or genetic factors affects the WMT outcome. Given these

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diverse aims and the possible infinite sources of variation, we focused on the first aim. This and the other study involving MT training shared the subjects of the control group, psychological and neuroimaging outcome measures, training period, and training frequency. Groups of participants completed the pre- and post-magnetic resonance imaging (MRI) studies and psychological experiments during different predetermined experimental periods (for example, one group participated in the project starting 4 weeks from November 4th, another group participated in the project starting 4 weeks from November 10th, etc.). Participants in one predetermined experimental period were randomly assigned to one of the two groups (e.g., the MT training group or the no-intervention control group). The two groups assigned were different for each predetermined experimental period. This means, for example, that participants in the 4-week period starting from November 4th were assigned to the WMT group or the no-intervention control group, but participants in the 4-week period starting from November 10th were assigned to the MT training group or the no-intervention control group. The participants selected the period they wished to participate in and were not notified that there were two intervention groups before the experiment. The characteristics of each group are shown in Table 1. The WMT and control groups did not differ significantly ($p > .1$, two-tailed t tests) in age, sex, and score on Raven's Advanced Progressive Matrices (RAPM) or other WM measures (visuospatial WM and digit span tasks). Subjects who misunderstood the rules for the psychological measures, tended to fall asleep during the psychological tests, or could not participate as planned were excluded from the relevant analyses.

2.2. Procedure

The WMT program consisted of computerized, in-house developed Borland C++ programs comprising four computerized tasks. Subjects undertook approximately 4 weeks (27 days) of training (each day, 20–60 min in most cases). However, the total training time depended on the level and time between trials. The subjects used the program provided to them on their personal computers. They were recommended to perform the WMT tasks every day; two training sessions for a week were conducted in the laboratory. When they could not perform the tasks because of computer problems, illness and other reasons, the subjects were allowed to miss a session. They could undergo WMT more than once a day. Performances in each block (a period when stimuli were presented sequentially) were logged in a computer file, and occasionally, the subjects were asked to mail the logs for compliance verification. The experimenter provided training feedback to the subjects as necessary. MRI scanning and psychological tests were performed immediately before and after the 4-week training. In other words, pre-training MRI scans and psychological tests were performed on day 1, training was provided from day 2 to day 28, and post-training MRI scans and psychological tests were performed on day 29.

Table 1 – The characteristics of subjects included in each of the three groups in this experiment.

	WMT ($N = 41$)	Control (N) ($N = 20$)	MT training ($N = 20$)
Age, mean (SD)	20.9 (1.6)	21.4 (2.2)	21.6 (2.1)
Female, number	14	5	3

Please note that the MT training intervention had the same control group as the WMT group. However, this study had nothing else to do with the study of MT training.

2.3. Training tasks

Four WMT tasks were presented during each training session. In all training tasks, difficulties (number of items to be remembered) were modulated based on subjects' performance. (a) A visuospatial WM task in which circles are presented one at a time at a 1/sec rate in an interface where 10 squares are distributed irregularly (circles are presented in one of these squares). After stimuli presentation, the subjects indicate the location and order of the presented stimuli by clicking on a computer screen with a mouse. (b) An auditory backward operation span task in which pairs of single digits (0–9) are presented verbally at a 1 pair/3-sec rate. Within this 3-sec period, one digit is presented per second but in the final second, no stimuli are presented (e.g., four pairs of digits would be presented as follows: 1, 3, , 4, 9, , 3, 7, , 2, 5,). The subjects have to remember the single digit of the sum of the presented pairs of digits and the order in which they are presented (in the above example, they would need to remember 4, 3, 0, 7) and then repeat the sequence by pressing numbered buttons on the screen in the reverse order (7, 0, 3, 4 in this order for the above example). (c) In the dual WM task, which is similar to the task implemented in the previous study (Dash et al., 2010), the subjects have to concurrently perform a visuospatial WM task and an auditory digit span task. Here circles are presented one at a time at a 1/3-sec rate in the same interface as that of the task (a). After stimuli presentation, the subjects indicate the location and order of the presented stimuli by clicking on a computer screen with a mouse. Simultaneous with the presentation of the circle, one single digit (0–9) is presented verbally. After stimuli presentation, the subjects indicate the digits and the order of the stimuli by pressing numbered buttons on the screen in the presented order. The subjects can perform either of the tasks first. In tasks [a] to [c], when the subjects solved the problems correctly, the task level increased by one, and when they failed to do so three times in a row, the level decreased by one. The difficulty level was varied by changing the number of presented stimuli in these tasks. In each session of each task [a] to [c], when subjects provided correct responses to a task with level N, this was logged in the computer and accumulated. When the sum of these Ns surpassed a predetermined number, each session of each task [a] to [c] ended. [d] In a dual WM task, which is similar to the task implemented in a previous study of WMT (Jaeggi et al., 2008), squares at eight different locations are presented sequentially on a computer screen at a 3-sec rate (stimulus length, 500 msec; interstimulus interval, 2500 msec). Simultaneous with the presentation of the squares, one of eight consonants is presented sequentially using headphones. A response is required whenever one of the presented stimuli matched the one presented n positions back in the sequence. The n value was the same for both streams of stimuli. Six auditory and six

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visual targets existed per block (four appearing in only one modality and two appearing in both modalities simultaneously), and their positions were determined randomly. Here subjects responded by pressing "F" on a standard keyboard for visual targets and "J" for auditory targets. No responses were provided for non-targets. The difficulty level was varied by changing the *n* level. After each block, the subjects' individual performance was analyzed, and in the following block, the *n* level was adapted accordingly. For fewer than three mistakes per modality, the *n* level was increased by 1. For more than five mistakes, it was decreased by 1, and in all other cases, *n* remained unchanged. Here after each block, when *n* remained unchanged, it was logged in the computer and accumulated. When the *n* level increased by 1, the $n \times 3$ was similarly accumulated. A session of dual *N*-back tasks ended when the sum of this accumulation surpassed a predetermined number. Training was completed for the day after subjects completed all tasks.

2.4. *Psychological outcome measures*

Neuropsychological tests and questionnaires were administered for pre- and post-training evaluation. These included the following: [A] RAPM (Raven, 1998), a non-verbal reasoning task. [B] Bochumer Matrizen-Test (BOMAT) (Hossiep et al., 1999) (performed groupwise) and as described in Jaeggi et al. (2008). [C] A (computerized) digit span task, a verbal WM task (Takeuchi et al., 2011c). [D] A (computerized) visuospatial WM task in which circles are presented one at a time at a 1/sec rate in a four-by-four grid-like interface. After stimuli presentation, the subjects indicate the location and order of the presented stimuli by clicking the grid-like interface on a computer screen with a mouse in the presented (forward visuospatial WM task) or reverse order (backward visuospatial WM task). The number of items to be remembered was initially two and progressively increased. Three sequences were provided at each level, until the subjects responded incorrectly to all three sequences, at which point the task was ended. Each test score was equal to the sum of the number of items correctly repeated in the forward and backward visuospatial WM tasks. [E] Tanaka B-type intelligence test (Tanaka et al., 2003) type 3B. This non-verbal mass intelligence test, used for 3rd-year junior high school and older examinees, does not include story problems but uses figures, single numbers, and letters as stimuli. In all subtests, the subjects have to solve as many problems as possible before a certain time (a few minutes). This test consists of the maze test (trace a maze with a pencil from start to finish), counting cubes (count cubes piled up in a three-dimensional manner), a displacement task [substitute a figure (nine figures) with a number (1–9) according to a model chart], identification versus same–different judgments (Japanese kana characters; judge whether a pair of meaningless Japanese strings are the same), filling in a sequence of numbers (fill in the blanks of a number sequence with suitable numbers according to the rules of number arrangement), marking figures [select forms identical to three samples from a series (sequence) of eight different forms], and filling in figures (complete incomplete figures so that they are the same as the sample figures when rotated). [F] The Stroop task (Hakoda's version) (Hakoda and Sasaki, 1990), which measures response inhibition and impulsivity. Hakoda's version is a matching-type Stroop task requiring subjects to check whether their chosen answers are correct, unlike the traditional oral naming Stroop task. The test consists of two control tasks (Word–Color and Color–Word tasks), a Stroop task, and a reverse Stroop task. In the Word–Color task, a color name (e.g., "red") is presented in the leftmost of six columns. The other five columns are painted with five colors, and subjects have to check the column whose color corresponds to the color name in the leftmost column. In the Color–Word task, the leftmost of six columns is painted with a color and the other five columns contain color names. Subjects have to check the column with the name corresponding to the color painted in the leftmost column. In the reverse Stroop task, in the leftmost of six columns, a color name is printed in another color (e.g., "red" is printed in blue letters) and the other five columns are painted in five different colors from which subjects have to check the column whose color corresponds to the color name in the leftmost column. In the Stroop task, in the leftmost of six columns, a color name is printed in another color (e.g., "red" is printed in blue letters) and the other five columns contain color names. Subjects have to check the column with the name of the color in which the word in the leftmost column is printed. In each task, subjects have to complete as many of these exercises as possible in 1 min. Reverse Stroop and Stroop interference rates are calculated as follows:

$$\text{Reverse Stroop interference} = (\text{correct answers of Word} - \text{color test} - \text{correct answers of reverse Stroop test})/(\text{correct answers of Word} - \text{color test}) \times 100$$

$$\text{Stroop interference} = (\text{correct answers of Color} - \text{word test} - \text{correct answers of Stroop test})/(\text{correct answers of Color} - \text{word test}) \times 100.$$

[G] Arithmetic tasks. These tests measure multiplication performance consisting of two forms of one-digit times one-digit multiplication problems (a simple arithmetic task with numbers between 2 and 9) and two forms of two-digit times two-digit multiplication problems (a complex arithmetic task with numbers between 11 and 19). The two forms of each task are the same, but the numbers used in the problems are ordered differently. Each form of the simple and complex arithmetic tasks has to be completed in 30 and 60 sec, respectively. [H] Kyodai SX test's (Umemoto et al., 1963) subtests for numerical factors. The Kyodai SX test is a standardized paper-and-pencil type test used in Japan to assess psychometric IQ for subjects with higher cognitive abilities. It comprises several subtests. Here two subtests that involve

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complex mathematical problems and that are used for calculating numerical factors (numerical ability score) were used. In one subtest, in each question, one or two equations are presented (such as $15 \times 3 = 18$) and another equation in which one of the numbers is replaced by a square is presented ($54 \times 6 = \square$). In this subtest, the former equations become correct when symbols (except " = ") are replaced by another one (in case of the example above, $15 + 3 = 18$ is a correct equation). The subjects have to identify the number that should be in the square [$54 \times (+)6 = 60$, so the answer is 60]. In another subtest, in each question, arithmetic word problems are presented and subjects have to identify the combinations of symbols required to calculate the answer (e.g., +, −). In each question, five answer options are presented to the subjects. [I] The SA creativity test (Society_For_Creative_Minds, 1969; Takeuchi et al., 2010d, 2010e), which measures creativity through divergent thinking, involves three types of tasks (generate unique ways of using typical objects, imagine desirable functions for ordinary objects, and imagine the consequences of unimaginable things happening). The SA test scores the four dimensions of the creative process (fluency, originality, elaboration, and flexibility). Here the sum of the graded scores of the four dimensions was used for analysis.

Several questionnaires designed to assess the traits or states of the subjects were collected but are not described here. These choices were not arbitrary, and consistent with those reported in our previous studies (Takeuchi et al., 2010c, 2011d), results from all performance-type cognitive measures were reported. A tester blinded to the groups performed all neuropsychological assessments.

2.5. *Image acquisition and analysis*

MRI data acquisition was conducted using a 3 T Philips Intera Achieva scanner. Using an magnetization-prepared rapid gradient echo (MPRAGE) sequence, high-resolution T1-weighted structural images (240 × 240 matrix, TR = 6.5 msec, TE = 3 msec, FOV = 24 cm, 162 slices, 1.0-mm slice thickness) were acquired. For the resting-state functional MRI (fMRI), 34 transaxial gradient-echo images (64 × 64 matrix, TR = 2000 msec, TE = 30 msec, flip angle = 70°, FOV = 24 cm, 3.75-mm slice thickness) covering the entire brain were acquired using an echo planar sequence. For this scan, 160 functional volumes were obtained while subjects were resting. During the resting-state scanning, the subjects had to close their eyes but not move, sleep, or think about anything (Greicius et al., 2003). Arterial spin labeling (ASL), an MR imaging method, was used to measure resting-rCBF non-invasively. ASL was performed with quantitative signal-intensity targeting by alternating radiofrequency pulse labeling of arterial regions, a pulsed ASL method (Petersen et al., 2006a, 2006b). Details of the sequence and the calculation method for the perfusion parameters have been described elsewhere (Petersen et al., 2006a, 2006b). The actual imaging parameters were as follows: 64 × 64 matrix, TR = 300 msec, TE = 22 msec, FOV = 24 cm, seven slices, 7.0-mm slice thickness (2.0-mm gap), SENSE = 2.5, 84 averages, scan duration, 5 min 52 sec. We determined the position of the slice by putting the fourth of seven slices on the body of the corpus callosum in the coronal scout view (Taki et al., 2011). During the ASL scan, the subjects had to remain still with their eyes closed and could not sleep or think about anything. Three images with no diffusion weighting (b value = 0 sec/mm^2) ($b = 0$ images) and one $b = 0$ image were acquired from 52 and 9 subjects, respectively, using a spin-echo echo-planar imaging (EPI) sequence (TR = 10,293 msec, TE = 55 msec, FOV = 22.4 cm, 2 × 2 × 2 mm^3 voxels, 60 slices). The mean image of three $b = 0$ images (for 52 subjects) or one $b = 0$ image (for seven subjects) was used in the preprocessing of imaging data. Our study subjects also participated in other studies or projects. Subjects completed the fMRI paradigms of the N-back WM task (2-back WM condition and 0-back control condition) before and after training. Details relating to these N-back fMRI paradigms can be found in our previous report (Takeuchi et al., 2011c). We used the fMRI data from the 2-back task of 80 subjects who participated in the fMRI study to show the brain regions activated during the 2-back WM task before training (Fig. 2b). Only some of the MRI scans performed in this study were described here. Other scans included scans for diffusion tensor imaging and fMRI scans for the emotion task.

2.6. *Preprocessing and individual-level statistical analysis of resting-FC data*

Preprocessing and analysis of FC data were performed using SPM5 implemented in Matlab. Before analysis, blood oxygen level-dependent (BOLD) images from the pre- and post-training scans were corrected for slice timing, re-aligned, and re-sliced to the mean image of the BOLD images from the pre-training scan. As has been done in our previous study (Takeuchi et al., 2011c), the skull and skin parts of all BOLD images of each subject were then stripped by masking the raw BOLD images of each subject with a threshold of given signal intensity in the spatially smoothed [8-mm full width at half maximum (FWHM)] mean image of all BOLD images of each subject. By doing this, we were able to delete the skin and skull parts of the images but not the brain parenchyma parts. This is because the skin and skull parts of the BOLD images have little signal intensity immediately over or under themselves. Thus, by spatial smoothing, the signal intensity of the skin parts is decreased compared with the brain parenchyma parts (signals of small portions of voxels that have an unusually high signal intensity compared with the voxels around them also decrease). The threshold for performing skull stripping was the same for all subjects, and it was determined by visual inspection ensuring that the skulls of the subjects were stripped but their brain parenchyma parts were not deleted. The first smoothing for skull stripping was performed to make mask images for skull stripping the unsmoothed BOLD images. Thus, the processed BOLD images that were used in the next processing step were unsmoothed images. Furthermore, using the movement-related parameters obtained in the realignment procedure described above, we calculated an estimate of the total amount of movement in the resting-state fMRI session. This was done by calculating the sum of $\sqrt{[(x_{vol\_n} - x_{vol\_n-1})^2 + (y_{vol\_n} - y_{vol\_n-1})^2 + (z_{vol\_n} - z_{vol\_n-1})^2]}$ ($n = 2, 3, 4, \ldots 160$) for each session.

The masked BOLD images were coregistered to a skull-stripped $b = 0$ image, which was created by a method similar to the one used for making skull-stripped BOLD images, and they were spatially normalized onto a skull-

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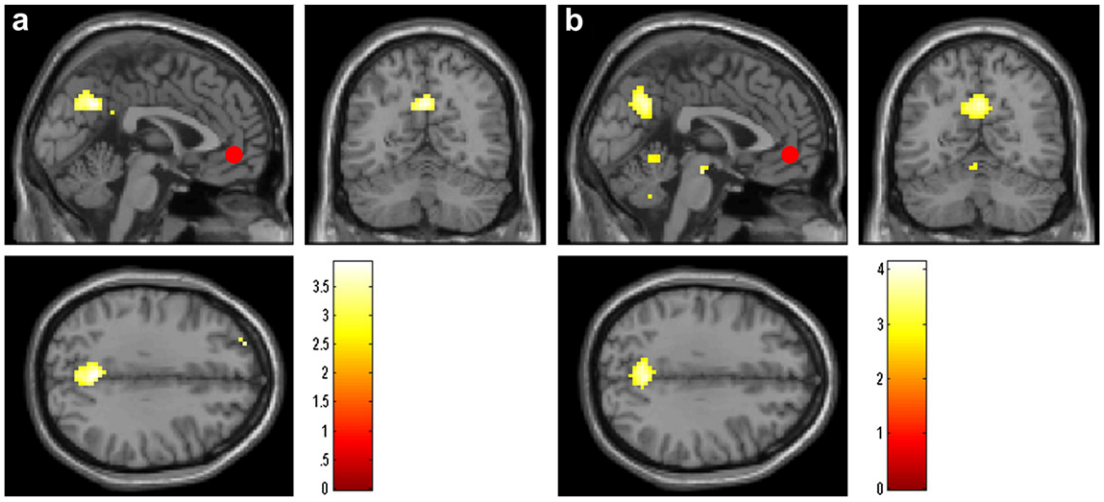

Fig. 1 – (a, b) WMT-related changes in resting-FC with the mPFC. Increase in resting-FC with mPFC in the WMT group compared with the control group. Results are shown with a threshold of $p < .005$, uncorrected. Compared with no-intervention, WMT resulted in an increase in resting-FC between mPFC and the precuneus. The red sphere is the schematic seed region in mPFC. (a) The resting-FC results when the time course of the whole brain signal was regressed out in individual analyses. (b) The resting-FC results when the time course of the whole brain signal was not regressed out in individual analyses.

stripped $b = 0$ image template, which was created from data obtained using our scanner (Takeuchi et al., 2010e). We did not use T1-weighted structural images for coregistering because coregisteration of the BOLD images taken in our studies to the T1-weighted structural images used in our laboratory often fails when visually inspected. This failure might possibly be caused by differences in the two images due to distortion of the BOLD images. We used $b = 0$ images for the normalization procedures for the following reasons. First, coregisteration of the BOLD images taken in our studies to the $b = 0$ images passes visual inspection of all images. This might be because both the images are taken using an EPI sequence and have similar characteristics including distortion (Reber et al., 1998). Second, $b = 0$ images have clearer anatomical characteristics that allow precise normalization. Third, the structure of the orbitofrontal cortex (OFC) is typically lost in BOLD images taken using the 3T scanner (Stenger, 2006). In a few cases, direct normalization of the BOLD images to the EPI template of SPM5 distorted the structures around OFC to compensate for the loss of OFC in the BOLD images taken in our study. On the

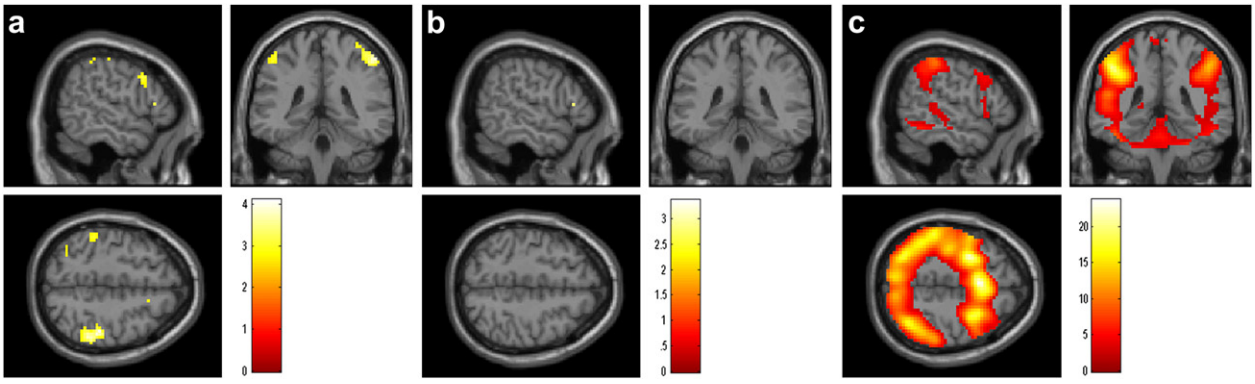

Fig. 2 – (a) WMT-related changes in resting-FC. Decrease in resting-FC with mPFC in the WMT group compared with the control group ($p < .005$, uncorrected). Compared with no-intervention, WMT resulted in a decrease in resting-FC between mPFC and the region in the right posterior parietal cortex (Please note this is the only significant result). Tendency of the results are seen in the bilateral LPFCs. These are the resting-FC results when the time course of the whole brain signal was regressed out in individual analyses. (b) These are the resting-FC results when the time course of the whole brain signal was not regressed out in individual analyses. Everything else is presented in the same manner as for (a). The significant results and tendencies observed in (a) were not observed in this analysis. (c) Brain regions that are activated during the 2-back task before the intervention period. Results are shown with a threshold of $p < .005$, uncorrected.

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other hand, $b = 0$ images appear to have relatively more of the structure around OFC on visual inspection, and this prevents this type of distortion and allows for better normalization. However, because $b = 0$ and BOLD images from the same subjects have similar characteristics, these images also probably match well in the coregisteration process, despite the difference in the structures around OFC. The normalization step resulted in BOLD images with $3 \times 3 \times 3$ mm voxels. They were then smoothed (8-mm FWHM).

Individual-level statistical analyses were performed using a general linear model. We removed low-frequency fluctuations with a high-pass filter cut-off value of 128 sec (1/128 Hz). Slow signal drifts with a period longer than this, probably not based on brain activities, are removed by this value. On the other hand, we did not use a low-pass filter. Serial correlations in the BOLD signal were accounted for by a first-degree autoregressive correction (Della-Maggiore et al., 2002) instead of being removed by a low-pass filter. The use of an autoregressive model (instead of low-pass filter) is recommended when the signals of the model frequently change over time, such as in rapid presentation event-related designs (Della-Maggiore et al., 2002). When the signals of the model do not change frequently neither an autoregressive model nor a low-pass filter is necessary to control for false positives (Della-Maggiore et al., 2002). In a study by Della-Maggiore et al. (2002), there were no circumstances in which a low-pass filter or the combination of an autoregressive model and a low-pass filter was better than an autoregressive model without a low-pass filter. This was so both in terms of the power of the analysis and in the control of false positives. Probably because there are no situations where a low-pass filter has been shown to be better than an autoregressive model, the low-pass filter is no longer available in the version of SPM that we used. Several sources of spurious variances were then regressed by putting these variances into the following regressors: (i) six parameters obtained by a rigid body correction of head motion, (ii) the whole brain signal averaged over a whole-brain mask, and (iii) time courses of brain signal changes in unsmoothed normalized images averaged over the brain masks of white matter and cerebrospinal fluid (CSF) areas. The brain masks of white matter and CSF consisted of areas of tissue probability $>.95$ in the SPM5 tissue probability maps for each tissue. This procedure removes fluctuations unlikely to be involved in specific regional correlations. Regressing out the global signal shifts the distribution of correlation coefficients, ensuring that they are approximately centered around zero in such a manner that their sum is less than or equal to zero. This method of data cleaning has been criticized as "artifactually" creating anticorrelations (Murphy et al., 2009), though, a recent study suggested that anticorrelations observed in resting-state connectivity are not an artifact introduced by global signal regression and might have biological origins (Chai et al., 2012). However, more importantly, regardless of the recent controversy about regressing out the whole brain signal, we believe that for the purposes of this study, it was important to regress out the global signal because of the following reasons. First, global signal correlation is observed partly because the brain is globally activated (Schölvinck et al., 2010). In this case, when these global brain activities are not regressed out, FC between two brain regions would show correlation of activity due to global brain activity as well as region- or network-specific activity synchronization. Our underlying assumption is that we would observe correlations due to network- or region-specific activity synchronization and not those due to region- or network-irrelevant global brain activity. However, how the omission of the procedure of regressing out the whole brain signal changes the results is interesting. Therefore, in addition to the results of analyses of the abovementioned procedures, we included the results of the analyses where the whole brain signal was not regressed out in individual analyses. Correlation maps were produced by extracting the BOLD time course from seed regions, then computing the correlation coefficient between that time course and the time course from all other brain voxels. For the present study, we examined correlations associated with mPFC and the right dorsolateral prefrontal cortex (DLPFC), key DMN nodes, and the EAS, the latter of which also showed a WMT-related increase in resting-rCBF (see Results). The mPFC seed region was a 6-mm-radius sphere centered on the focus. This peak voxel of mPFC (x, y, $z = -1$, 47, -4) was defined as previously (Fox et al., 2005). The right DLPFC seed region was defined using WFU_PickAtlas (http://fmri.wfubmc.edu/cms/software) and in a similar manner as in the previous study (Song et al., 2008). We defined the right DLPFC seed region by intersecting BA46, the right middle frontal gyrus and GM in WFU_PickAtlas, and then re-sliced the generated regions into the same spatial resolution as the preprocessed fMRI images ($3 \times 3 \times 3$ mm^3).

With individual-level analysis, contrast images representing changes in resting-FC with the seed regions following the 27-day intervention and those before intervention were estimated for each subject after preprocessing. These images were then subjected to the belowmentioned group analysis.

2.7. *Preprocessing of resting-rCBF data*

Maps of raw resting-rCBF and longitudinal relaxivity (R1) of each subject were obtained using a dedicated software running on IDL (Research Systems, Boulder, Colo) (2006a; National Neuroscience Institute, Singapore). The following constants were used in the CBF calculation: T1 of arterial blood, 1.65 sec; inefficiency, 95%; blood–brain partition coefficients for gray and white matters, .98 and .82, respectively. For an explanation of the details and technicalities of rCBF calculation, see Petersen et al. (2006a).

Preprocessing and data analysis were performed using SPM5 implemented in Matlab, except in the belowmentioned segmentation procedure using SPM2. This measure was performed because the T1-weighted images taken using the abovementioned MRPAGE sequence were incompatible with VBM5 and SPM5 preprocessing and resulted in many apparent segmentation errors.

Our original template of the T1-weighted structural image, created from the 63 subjects of our previous study (Takeuchi et al., 2011c), was used in resting-rCBF map preprocessing as follows: (a) The T1-weighted structural image of each subject was spatially normalized to the T1 template in SPM5. (b) The normalized T1-weighted structural image of each subject was then smoothed using a Gaussian kernel of 8-mm FWHM and finally averaged across subjects to make an original template of the T1-weighted structural image.

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We segmented the T1-weighted images into the GM, white matter, and CSF. Then, using segmented gray and white matter images as masks, we removed parts that did not belong to the gray and white matter images from the T1-weighted image and created images consisting of gray and white matter parts (called "gray matter + white matter T1-weighted image").

R1 maps from the pre- and post-scans of each subject (these do not show the skull and skin of the head and retain their alignment with the rCBF maps of each subject) were then coregistered to the gray matter + white matter T1-weighted image from the pre-scan of each subject using the within-subject registration method.

Next, a GM mask image consisting of voxels with values higher than .2 in the GM image was generated. This image was applied to the rCBF maps from the pre- and post-scans to limit the analysis to resting-rCBF in GM areas and to generate a GM rCBF image.

Next, a raw T1-weighted structural image from the pre-scan, which maintained its alignment with the gray matter + white matter T1-weighted image from the pre-scan and GM rCBF maps from the pre- and post-scans, of each subject was normalized to our original template of the T1-weighted structural image.

Using the parameters for this normalizing procedure, GM rCBF maps from the pre- and post-scans of each participant were spatially normalized to create images with $2 \times 2 \times 2\ mm^3$ voxels. The processed normalized GM rCBF maps from the pre- and post-scans were then spatially smoothed using a Gaussian kernel of 9-mm FWHM. Finally, the signal change in (resting-)rCBF between the pre- and post-scan images was computed at each voxel by subtracting the former image from the latter for each participant. The maps representing the GM (resting-)rCBF from the pre-scan and (resting-)rCBF changes from the pre- to post-scan were subjected to the belowmentioned group-level analysis.

2.8. *Preprocessing and analysis of structural data*

Voxel-based morphometry (VBM), which is a method for the *in vivo* study of human brain structures that can detect changes in regional GM caused by training (Driemeyer et al., 2008; Ilg et al., 2008), was used to investigate the effect of WMT on brain structures. Preprocessing of the morphological data was performed using the VBM2 software (Gaser, 2007), an extension of SPM2. To reduce the scanner-specific bias, we used a customized GM anatomical template and prior probability maps of GM and white matter images created from T1-weighted structural images obtained using this scanner in our previous study (Takeuchi et al., 2010d, 2011). Next, the T1-weighted structural images from each subject were segmented into GM and white matter partitions using the abovementioned custom GM and white matter prior probability maps. The resulting images included extracted GM and white matter partitions in the native space. The GM partition was then normalized to the abovementioned custom GM probability map. The normalization parameters determined from this initial step were applied to the native T1-weighted structural image. These normalized T1-weighted structural data were then segmented into GM and white matter partitions. In addition, we performed a volume change correction (modulation) by modulating each voxel with the Jacobian determinants derived from spatial normalization, allowing the determination of regional differences in the absolute amount of GM (Ashburner and Friston, 2000). Subsequently, all images were smoothed by convolving them with an isotropic Gaussian kernel of 12 mm FWHM. Finally, the signal change in rGMV between pre- and post-intervention images was computed at each voxel for each participant. In this computation, we included only voxels that showed GMV values >.10 in both pre- and post-scans to avoid possible partial volume effects around the borders between GM and white matter, as well as between GM and CSF. The resulting maps representing the rGMV change between the pre- and post-MRI experiments (rGMV post − rGMV pre) were then forwarded to the group-level analysis described below.

2.9. *Statistical group-level analysis of imaging and behavioral data*

Behavioral data were analyzed using SPSS 16.0 (SPSS Inc., Chicago, IL). Because the superiority of training was our primary interest, in our behavioral analysis, test–retest changes in the WMT group were compared to those in the control group using one-tailed one-way analyses of covariance (ANCOVAs) with the difference between pre- and post-test measures as dependent variables and pretest scores as independent variables ($p < .05$). The use of one-tailed test is consistent with our previous study as well as those of other laboratories (Klingberg et al., 2005, 2002; Takeuchi et al., 2011b, 2011d). However, two-tailed ANCOVAs were used in behavioral measures in which the definition of "superiority" was unclear, namely for the reverse Stroop interference rate showing age-related decline (Sasaki and Hakoda, 1985) and for an increase in the Stroop interference rate in schizophrenics (Sasaki et al., 1993). The two-tailed ANCOVA was also used to compare group differences in changes in creativity test scores, which are associated with impaired selective attention systems, psychosis, and cognitive disinhibition (Beech and Claridge, 1987; Necka, 1999; Stavridou and Furnham, 1996), as was the case with our previous study of WMT involving mental calculation (Takeuchi et al., 2011d). Creativity seems to be an obvious positive trait; however, a vast amount of literature shows that creativity is associated with psychopathologies and impaired selective attention systems [(for the full discussion of this matter, see Takeuchi et al., 2011c)]. These choices were not arbitrary and were consistent with those reported in our previous study of WMT (Takeuchi et al., 2011d). Two-tailed Student's *t*-tests were applied to investigate group differences in cognitive performance before the intervention, and the results are shown in Table 2.

In group-level imaging analysis, we tested for groupwise differences in changes in resting-FC with each seed region, resting-rCBF and rGMV across the entire brain.

In the analyses of resting-FC, we performed voxel-wise ANCOVAs with the difference of each measure between pre- and post-scans at each voxel as dependent variables, and the value for the pre-scan at each voxel, the total amount of movement during the scan (which was calculated above), and change of the total amount of movement during the scan from pre-scan to post-scan as independent variables. In the analysis

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Table 2 – The average of all subjects' highest performances in trained WM tasks among the first and last three training sessions.

	First three sessions (*N*)	Last three sessions (*N*)
Visuospatial WM task	8.85 ± .78	11.44 ± 1.88
Auditory backward operation span task	8.95 ± 1.44	15.33 ± 3.78
Dual WM task	7.64 ± .81	10.10 ± 1.55
Dual *N*-back task	2.77 ± .73	4.92 ± 1.19

Note that the number of training sessions differed for each subject, and therefore, it is difficult to show the average progressive improvement of performance as the number of sessions increased.

of resting-rCBF, we performed voxel-wise ANCOVAs with the difference of each measure between pre- and post-scans at each voxel as dependent variables and the value for the pre-scan at each voxel as independent variables. This analysis was performed using Biological Parametrical Mapping (Casanova et al., 2007) implemented in SPM5 and images representing changes in resting-FC/resting-rCBF and resting-FC/resting-rCBF in the pre-scan. In the analyses of resting-FC and resting-rCBF, regions with significance were inferred using cluster-level statistics (Friston et al., 1996). In this procedure, the null hypothesis was rejected for clusters with a large spatial extent. Cluster size distribution can be determined by parametric methods based on the Gaussian random field theory, which accounts for image volume, smoothness, and the cluster-defining threshold. In cluster level, inference is drawn from the cluster size, i.e., the probability that any cluster larger than the critical cluster size is controlled. Only clusters with $p < .05$, after correction for multiple comparisons at cluster size with a voxel-level cluster-determining threshold of $p < .005$ uncorrected, were considered statistically significant in this analysis. However, for regions with the *a priori* hypothesis, the threshold of $p < .1$ (instead of .05, so note this is not lenient at all), after correction for multiple comparisons at cluster size with a voxel-level cluster- determining threshold of $p < .005$ uncorrected, was applied. Using liberal thresholds on areas with a strong *a priori* hypothesis has been performed elsewhere (e.g., Pochon et al., 2002) The precuneus and posterior cingulate gyrus were set as regions with the *a priori* hypothesis in the analysis of resting-FC with mPFC because resting-FC between mPFC and the region around the posterior cingulate gyrus and precuneus is correlated with the performance of the WM task (Hampson et al., 2006).

In the analyses of rGMV, we used the factorial design option in SPM5. In the imaging analysis, the effects of the interventions, estimated by comparing changes in pre- to post-measures as described above, were compared between the groups at each voxel with total GMV in the pre-measurement, pretest scores of measures of general intelligence (RAPM), and two measures of WM (digit span task and visuospatial WM task), as covariates. The differences between the WMT group and the control group were investigated. This analysis corresponds to the ANCOVA. In the analysis of rGMV, applying VBM5 (Gaser, 2007), the level of statistical significance was set at $p < .05$, corrected at the non-isotropic adjusted cluster level (Hayasaka et al., 2004) with an underlying voxel-level of $p < .05$ corrected for false discovery rate (Genovese et al., 2002). Non-isotropic adjusted cluster size tests can and should be applied when cluster size tests are applied to data known to be non-stationary (i.e., not uniformly smooth), such as VBM data (Hayasaka et al., 2004). The abovementioned analysis using biological parametric mapping (BPM) was not applied to the rGMV analysis, as to our knowledge, BPM does not use the non-isotropic adjusted cluster size.

Finally, using simple regression analyses, correlations between improvements in performance on WMT tasks and the amount of neural change in significant areas identified in the abovementioned ANCOVAs were investigated. For this analysis, performance on WMT tasks for each participant in the first and last three sessions was calculated as follows: (highest level of the visuospatial WM task achieved in the first or last three completed sessions) + (highest level of the auditory backward operation span task achieved in the first or last three completed sessions) + 2 × (highest level of the dual WM task achieved in the first or last three completed sessions) + 2 × (highest level of the dual *N*-back task achieved in the first or last three completed sessions). Performance on the dual WM task and dual *N*-back task were multiplied by two because when the level of the dual *N*-back task was increased by one the number of stimuli to be remembered was increased by two. An increase in performance on WMT tasks for each participant from the first three sessions to the last three sessions was regarded as an improvement in performance on WMT tasks. The rationale for this calculation using the first/last three sessions (see also the Results of training data subsection of Results) was to obtain stable results since subjects have to perform four tasks in one session and there simply is not sufficient time to ensure stable performance in one session. We did not perform correlation analysis using the amount of WMT as a covariate because in our study, the amount of WMT varied little among subjects (see Results for details). The mean changes in the clusters identified as significant in the abovementioned ANCOVAs were calculated for all the significant clusters in the analyses of rGMV, resting-rCBF, and resting-FC for each subject. For all clusters identified as significant by ANCOVA simple regression analyses were performed to determine the improvement in performance on WMT tasks and the neural changes calculated as described above. In addition, we performed the same procedure using improvements in RAPM instead of improvements in performance on WMT tasks.

3. Results

3.1. *Results of training data*

Subjects in the WMT group completed 25.87 sessions (SD, 2.18) on an average and at least 17 sessions during the 27-day intervention period. In all four WMT tasks, the highest performance achieved during the last three training sessions by each participant was significantly increased compared with the highest performance achieved during the first three training sessions (paired *t*-test, $p < .001$, Table 2).

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3.2. The effect of WMT on behavioral measures

The WMT group showed significantly greater pre- to post-test increases in the performance of tasks used to determine behavioral measures, such as a digit span task ($p < .001$, one-tailed) and a visuospatial WM task ($p < .001$, one-tailed), than the no-intervention (control) group. Improvements in the performance of non-trained WM tasks, including ones with stimuli modalities different from those of the trained tasks, following WMT were consistent with previous studies (Klingberg, 2010; Takeuchi et al., 2010b). In WMT tasks, auditory, and not visual, verbal stimuli were used, whereas in the WM test of the outcome measure, the opposite was the case.

Furthermore, compared with the control group, the WMT group showed significantly greater pre- to post-test increases in performance on (RAPM; $p = .019$, one-tailed) and significantly greater pre- to post-test decreases in Stroop interference ($p = .007$, one-tailed) (Table 3 presents results for all psychological measures).

Finally, compared with the control group, the WMT group did not show significantly greater pre- to post-measure changes in the estimate of the total amount of movement during the resting-state fMRI sessions (Table 3).

3.3. The effect of WMT on resting-FC with mPFC

We next compared changes in resting-FC with mPFC (Fig. 1) in the WMT and control groups. mPFC was chosen as a seed region for DMN because (a) this is one of the key nodes of DMN and (b) a previous study showed that resting-FC between this region and PCC was correlated with WM performance (this region was also used as the seed region in that study), (Hampson et al., 2006).

When the images of resting-FC that regressed out the whole brain signal were used, the analysis revealed a statistically significant WMT-related increase from pre- to post-measures in resting-FC between mPFC and the precuneus ($x, y, z = 0, -60, 33$, $t = 3.80$, $p = .010$, corrected for multiple comparisons at the cluster level with a cluster-determining threshold of $p < .005$, uncorrected; Fig. 1a). These results indicate that WMT increases the resting-FC between the two central DMN nodes, namely mPFC and the precuneus. A statistically significant WMT-related decrease from pre- to post-measures was also observed in resting-FC between mPFC and a region in the right posterior parietal cortex (the anatomical cluster that includes the inferior and superior parietal lobules) ($x, y, z = 51, -42, 54$, $t = 4.10$, $p = .001$, corrected for multiple comparisons at the cluster level with

Table 3 – Pre- and post-test scores for psychological measures (mean ± S.E.M).

	WMT		Control (no-intervention)		Planned contrast	p value[a]	p value[b]
	Pre	Post	Pre	Post			
Non-verbal reasoning							
RAPM (score)	28.7 ± .5	31.9 ± .4	29.1 ± .9	31.2 ± .9	WMT > control	.019	.654
BOMAT (score)	8.03 ± .36	9.26 ± .32	7.72 ± .57	9.72 ± .54	WMT > control	.848	.646
WM							
Digit span (score)	36.4 ± 1.3	47.1 ± 1.7	35.6 ± 1.4	36.7 ± 1.6	WMT > control	<.001	.702
Visuospatial WM (score)	28.7 ± 1.3	34.4 ± .7	27.9 ± 1.0	30.2 ± .9	WMT > control	<.001	.466
Intelligence test with speeded tasks							
Tanaka B-type intelligence test	115.1 ± 1.5	123.9 ± 1.6	112.4 ± 2.1	120.3 ± 2.8	WMT > control	.258	.274
Stroop							
Reverse Stroop interference (%)	14.4 ± 1.6	17.3 ± 1.5	20.7 ± 2.3	17.6 ± 2.1	Two-tailed	.387	.031
Stroop interference (%)	8.7 ± 1.3	6.1 ± 1.1	8.7 ± 1.9	11.6 ± 2.4	WMT < control	.007	.995
Arithmetic							
Simple arithmetic (items)	33.3 ± .9	34.7 ± .9	31.8 ± 1.2	33.0 ± 1.4	WMT > control	.362	.334
Complex arithmetic (items)	7.10 ± .53	7.77 ± .55	6.72 ± .55	7.25 ± .72	WMT > control	.693	.663
Complex mathematic							
Numerical factor in Kyodai SX test	11.0 ± .3	12.2 ± .3	11.3 ± .5	12.3 ± .5	WMT > control	.480	.576
Creativity							
SA creativity test (total grade)	25.7 ± .9	26.8 ± .8	28.8 ± 1.3	27.3 ± 1.2	Two-tailed	.313	.054
Movement							
Movement during resting-state fMRI scans (mm)	12.4 ± .3	12.2 ± .3	12.4 ± .7	13.3 ± .9	WMT < control	.066	.967

All tasks were untrained tasks. The visuospatial WM task used as an outcome measure is similar to one of the training tasks because it is also a computerized visuospatial WM task. The digit span task is a visual verbal WM task, and visual verbal WM components were not included in the training tasks.

a One-way ANCOVAs with test–retest differences in psychological measures as dependent variables and pretest scores of the psychological measures as covariates.

b Two-tailed Student's t-tests were used to compare group differences in cognitive performance before training.

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a cluster-determining threshold of $p < .005$, uncorrected; Fig. 2a). This parietal region belongs to the areas activated by WM task as shown in Fig. 2c. Substantial WMT-related decreases from pre- to post-measures were also observed in resting-FC between mPFC and regions in the bilateral LPFCs (including the inferior and middle frontal gyri) (>15 voxels, with a cluster-determining threshold of $p < .005$, uncorrected). For consistency of the results when the right DLPFC was taken as a seed region of interest (ROI), see the subsection of "The effect of WMT on resting-FC with the right DLPFC" below.

When the images of resting-FC in which the whole brain signal was not regressed out were used, a statistically significant WMT-related increase from pre- to post-measures in resting-FC between mPFC and the precuneus ($x, y, z = 0, -60, 33$, $t = 3.92$, $p = .015$, corrected for multiple comparisons at the cluster level with a cluster-determining threshold of $p < .005$, uncorrected; Fig. 1b) was observed. However, no statistically significant or substantial tendency toward a WMT-related decrease from pre- to post-measures in resting-FC between mPFC and a region in the right posterior parietal cortex (0 voxels, with a cluster-determining threshold of $p < .005$, uncorrected) was observed. There were no other significant results.

3.4. *The effect of WMT on resting-FC with the right DLPFC*

Next, we compared changes in resting-FC with the right DLPFC. The right DLPFC is chosen as the seed ROI for EAS because the DLPFC is the key node of the EAS and as described below the right DLPFC showed resting-rCBF changes following WMT.

When the images of resting-FC in which the whole brain signal was regressed out were used, this analysis revealed a statistically significant WMT-related decrease from pre- to post-measures in resting-FC between the right DLPFC and the anatomical cluster that included mPFC and the ventral anterior cingulate gyrus ($x, y, z = -3, 36, -3$, $t = 4.54$, $p = .004$, corrected for multiple comparisons at the cluster level with a cluster-determining threshold of $p < .005$, uncorrected, Fig. 3a). Together with the findings from the analysis of resting-FC with mPFC, these results showed that WMT decreases resting-FC between mPFC and nodes of the EAS. No region showed statistically significant WMT-related increases in resting-FC with the right DLPFC. However, there was a tendency toward a WMT-related increase in resting-FC between the right DLPFC and the left posterior parietal cortex (15 voxels, with a cluster-determining threshold of $p < .005$, uncorrected).

When the images of resting-FC in which the whole brain signal was not regressed out were used, the same analysis did not reveal a statistically significant or substantial tendency toward any WMT-related decrease from pre- to post-measures in resting-FC between the right DLPFC and an anatomical cluster that included mPFC and the ventral anterior cingulate gyrus (0 voxels, with a cluster-determining threshold of $p < .005$, uncorrected; Fig. 3b). There were no other significant results. However, there was a tendency toward a WMT-related increase in resting-FC between the right DLPFC and the left posterior parietal cortex (62 voxels, with a cluster-determining threshold of $p < .005$, uncorrected).

To sum up the results of the effect of WMT on resting-FC with mPFC and those of the effect of WMT on resting-FC with mPFC, regardless of the removal of the whole brain signal in individual analyses, WMT increased resting-FC between mPFC and the precuneus (within network resting-FC). However, when the whole brain signal was not regressed out in individual analyses, a WMT-related decrease in resting-FC between mPFC and the nodes of EAS (WMT-related increase in

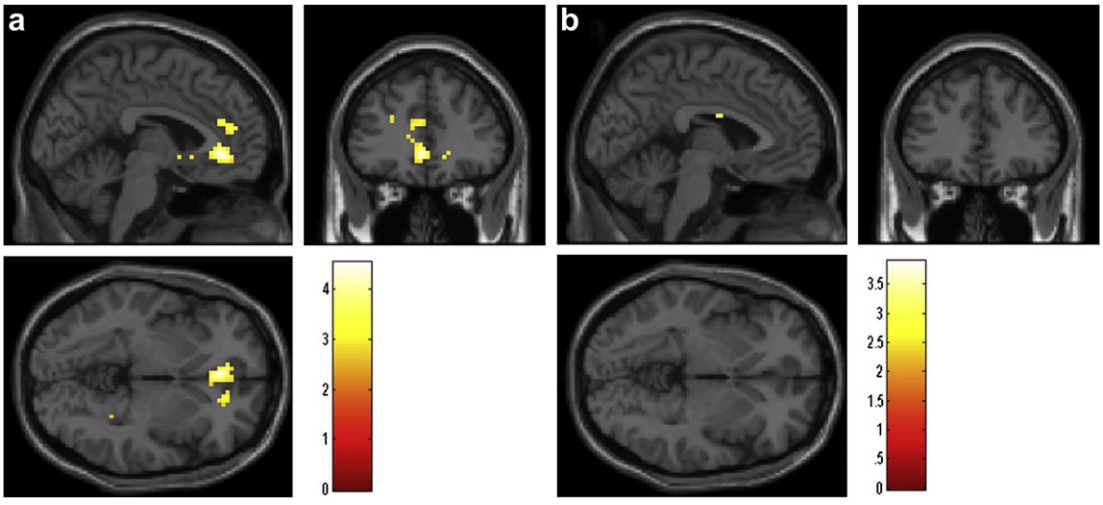

Fig. 3 – (a) WMT-related changes in resting-FC. Decrease in resting-FC with right DLPFC in the WMT group compared with the control group. Results are shown with a threshold of $p < .005$, uncorrected. Compared with no-intervention, WMT resulted in a decrease in resting-FC between right DLPFC and mPFC consistent with the tendency of the results seen in Fig. 2. These are the resting-FC results when the time course of the whole brain signal was regressed out in individual analyses. (b) These are the resting-FC results when the time course of the whole brain signal was not regressed out in individual analyses. Everything else is presented in the same manner as for (a). The significant results and tendencies seen in (a) were not seen in this analysis.

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anticorrelations between the nodes of EAS and the node of DMN), which was observed when the whole brain signal was regressed out in individual analyses, was not detected.

3.5. The effect of WMT on resting-rCBF

Next, we compared changes in resting-rCBF in the WMT and control groups. This analysis revealed a statistically significant WMT-related increase from pre- to post-measures in resting-rCBF in the right LPFC (including the right inferior and middle frontal gyri) (x, y, z = 34, 26, 26, $t = 3.51$, $p = .031$, corrected for multiple comparisons at the cluster level with a cluster-determining threshold of $p < .005$, uncorrected; Fig. 4). No region showed statistically significant WMT-related decreases in resting-rCBF.

3.6. The effect of WMT on rGMV

Next, we compared changes in rGMV in the WMT and control groups. This analysis revealed a statistically significant WMT-related increase from pre- to post-measures in rGMV in an extensive anatomical cluster that included the bilateral DLPFC, the left ventral LPFC, the bilateral lateral rostral PFCs, mPFC, the anterior cingulate cortex, the left perisylvian area, the superior parts of the bilateral parietal cortices, an anatomical cluster around the left caudate, the right cerebellum, and an anatomical cluster that included regions in the left middle and inferior temporal gyri ($p < .05$, corrected for multiple comparisons at the non-isotropic adjusted cluster level with an underlying voxel-level of $p < .05$ corrected for a false discovery rate; Fig. 5a and b, Table 4). No region showed statistically significant WMT-related decreases in rGMV.

3.7. Regression analysis

Simple regression analyses that tested correlations between improvements in performance on WMT tasks and the amount of neural changes in the significant clusters identified in the abovementioned ANCOVAs were also performed. The results revealed no significant correlations in any of the clusters. There was, however, a somewhat insignificant tendency toward a positive correlation between improvements in performance on WM tasks and the amount of rGMV changes in significant clusters in the frontal area ($r = .18$, $p = .28$), left temporal area ($r = .20$, $p = .23$), right parietal area ($r = .22$, $p = .18$), left parietal area ($r = .25$, $p = .11$), and left caudate ($r = .26$, $p = .10$). No significant correlation was observed between the amount of resting-CBF changes in the significant clusters identified in resting-CBF analysis and the amount of resting-FC changes in any of the three significant clusters identified in resting-FC analyses ($p > .05$). This may be due to a lack of effective training-related variance (see Results of training data) since the amount of training was controlled.

Next, simple regression analyses that tested for correlations between improvements in RAPM performance and the amount of neural change in significant clusters identified in the abovementioned ANCOVAs were performed. No significant correlations were detected for any of the clusters. Regarding resting-state measures and RAPM, among all the subjects analyzed in this study, resting-FC between mPFC and the right posterior parietal cortex before the intervention was significantly and negatively correlated with performance on RAPM before the intervention ($r = -.508$, $t = -4.53$, $p = 2.89 \times 10^{-5}$). This pattern (a negative correlation between a psychometric intelligence measure and resting-FC between nodes of DMN and EAS) is congruent with that observed in a previous report (Song et al., 2008). However, there were no other significant results, and given the countless number of regression tests which were not planned or designed before the experiment, the result may have to be interpreted somewhat cautiously.

There could be a number of reasons for the observed lack of correlation between training-related RAPM changes and neural changes. One possible reason is a lack of statistical power because we could only analyze data from subjects in the WMT group. On the other hand, large individual differences in performance changes were observed in the control group, indicating that much of the variance in performance changes in the WMT group may not have been related to WMT, either. In addition, this experiment was not designed to perform this type of correlation analysis. While the amount of training is correlated with training-related neural changes (Takeuchi et al., 2010a), in this study, we strictly controlled the

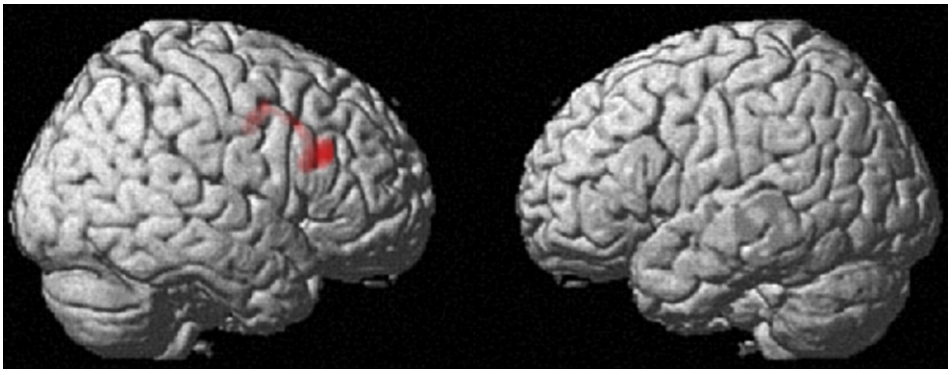

Fig. 4 – Increase in resting-rCBF in the WMT group compared with the control group ($p < .05$, corrected for multiple comparisons at cluster size with an underlying voxel-level of $p < .005$, uncorrected). Compared with no-intervention, WMT resulted in an increase in resting-rCBF in the right LPFC. The scale cannot be presented due to the limitation of the software. The density of the red color represents both *T* values and depth of the voxels.

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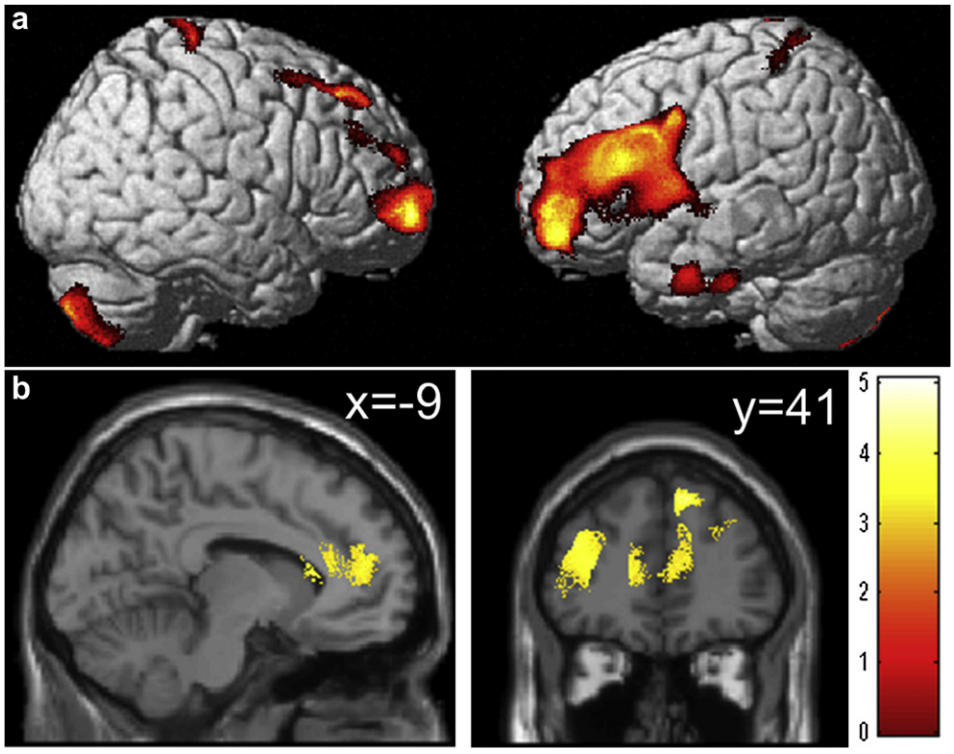

Fig. 5 – Increase in rGMV in the WMT group compared with the control group ($p < .05$, corrected for multiple comparisons at the non-isotropic adjusted cluster level with an underlying voxel-level of $p < .05$, corrected for false discovery rate). (a) Compared with no-intervention, WMT resulted in an increase in rGMV in the bilateral extensive lateral prefrontal areas, left perisylvian area, bilateral parietal regions, left middle/temporal gyrus, and right cerebellum. (b) Compared with no-intervention, WMT also resulted in an increase in rGMV in the bilateral anterior cingulate cortex and left caudate. The intensity of the color represents the *T* values in Figures (a) and (b). However, the scaling is different in the two figures, and the same intensity does not represent the same *T* value in the two figures. This cannot be altered because of a limitation of the software.

amount of training in the WMT group. Therefore, little variance was observed among subjects in this respect. To perform these types of correlation analyses successfully, we should not have controlled the amount of training so strictly. Furthermore, all subjects in the WMT group underwent prolonged and intense training for 1 month, whereas the control group received no such training. However, the significance of group differences in RAPM was only marginal. This means that the ratio of meaningful variance in the WMT group to meaningless variance in the control group was low.

4. Discussion

The present study revealed the effect of WMT on resting-FC, resting-rCBF and rGMV. WMT (a) increases resting-FC between mPFC and the precuneus, (b) decreases resting-FC between mPFC and the right posterior parietal cortex/right LPFC, and (c) increases resting-rCBF in the right LPFC. However, training-related changes in resting-FC between the nodes of the different networks (b) were only observed when

Table 4 – WMT-related rGMV changes when compared with the control group.

Area		MNI Coordinates			T score	Corrected *p* value (cluster)
		x	y	z		
Middle frontal gyrus(B)/Superior frontal gyrus(B)/Inferior frontal rygus(L)/Anterior cingulate cortex (B)/Medial frontal gyrus/Superior temporal gyrus(L)/Rolandic operculum(L)	B	−37	19	35	5.06	<.001
Superior parietal lobule/Postcentral gyrus	L	−21	−49	75	4.46	<.001
Middle temporal gyrus/Inferior temporal gyrus/Middle temporal pole	L	−59	0	−32	4.31	<.001
Extra-nuclear/Caudate	L	−9	22	10	4.17	<.001
Precentral gyrus/Postcentral gyrus/Paracentral lobule	R	16	−30	73	4.05	<.001
Cerebellum	R	18	−78	−37	3.97	<.001

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the whole brain signal was regressed out in individual analyses. They were not observed when the whole brain signal was not regressed out in individual analyses. On the other hand, training-related changes in resting-FC within the network (a) were not affected by whether the whole brain signal was regressed out in individual analyses. Furthermore, in this study WMT led to increase of rGMV in the extensive regions in the bilateral LPFCs, the mPFC and the anterior cingulate cortex as well as the bilateral parietal cortices, the left perisylvian area, the right cerebellum and left middle and inferior temporal gyrus.

The lower resting-FC among the DMN nodes, higher resting-FC between the DMN nodes and the node of the EAS (reduced anticorrelations between the two networks), and lower resting-rCBF in PFC are all associated with conditions with reduced or lowered WMC. Thus, WMT-related changes in these resting-FC and resting-rCBF may underlie or reflect an increase in WMC following WMT and may provide insights into the application of this training for many conditions with reduced WMC. A lower resting-FC among the DMN nodes is associated with lower individual WMC (Hampson et al., 2006) and generally [for comments on discrepancies among studies, see (Broyd et al., 2009)] with many conditions with reduced WMC, which include Alzheimer's disease, schizophrenia, autism spectrum disorder, attention deficit/hyperactivity disorder, and aging (Baddeley, 2003; Broyd et al., 2009; Goldman-Rakic, 1994; Sambataro et al., 2010; Steele et al., 2007; Westerberg et al., 2004; Wingfield et al., 1988). Albeit less frequently, reduced anticorrelations between the two networks are observed in schizophrenia, autism spectrum disorder, attention deficit/hyperactivity disorder, and aging (Broyd et al., 2009; Sambataro et al., 2010; Whitfield-Gabrieli et al., 2009). Finally, reduced rCBF in PFC has been reported in schizophrenia (Weinberger et al., 1986), Alzheimer's disease (Johnson et al., 2005), and normal aging (Martin et al., 1991). Thus, these observed WMT-related changes in resting-FC and resting-rCBF may underlie the increase in WMC and amelioration of certain disorders with reduced WMC, such as attention deficit/hyperactivity disorder, following WMT (Klingberg, 2010) and may provide insights into the application of this training for many other conditions with reduced WMC.

Resting-FC among the DMN nodes, anticorrelations between DMN and the EAS, and lower resting-rCBF in LPFC may be specifically associated with WMC or conditions with reduced WMC in their respective ways. Resting-FC between mPFC and the precuneus and FC between the two regions during a WM task are correlated with individual WM performance (Hampson et al., 2006). Thus, although the precise mechanism remains unknown, the integrated DMN has been suggested to facilitate the performance of demanding cognitive tasks (Hampson et al., 2006). Regarding anticorrelations between DMN and the EAS, deactivation of DMN during cognitive tasks is considered to reflect reallocation of cognitive resources from the network active during rest (DMN) to the network actively involved in the task (EAS) (McKiernan et al., 2003). Thus, increased anticorrelations between the two networks may reflect conditions in which cognitive resources are easily reallocated from one network to the other, and these conditions may help one successfully perform cognitive tasks using rich cognitive resources. Finally, because many cognitive and neural mechanisms may underlie differences in resting-rCBF, the precise mechanisms by which resting-rCBF is associated with conditions with reduced WMC cannot be revealed from this study. However, one possible mechanism is that increases in blood vessels in the relevant region may lead to increased resting-rCBF in the region and improved performance in demanding tasks by allowing a rich supply of rCBF. The other possible mechanism is that increased neuronal structures in the relevant regions facilitate cognitive performance and increase metabolic demand, which may lead to increased resting-rCBF. By utilizing these mechanisms, resting-FC among the DMN nodes, anticorrelations between DMN and the EAS, and lower resting-rCBF in LPFC may be associated with WMC or conditions with reduced WMC.

We speculate that WMT-related increases in resting-FC between the key DMN nodes and a decrease in resting-FC between mPFC and nodes of the EAS (LPFC and the posterior parietal cortex) may be mediated by a primary change in the function and structure of LPFC induced by WMT. WMT leads to changes in the function, structure, and neurochemistry of the EAS (Takeuchi et al., 2010b). However, in this study, changes were observed in resting-FC within the network and between networks involving DMN. These changes might be caused by the primary change in LPFC because LPFC of the EAS has been suggested to be the region that suppresses the activity of DMN when it is activated (Greicius et al., 2003). Following WMT, LPFC whose functions are augmented through training may increasingly regulate DMN activation. These changes in the regulation of DMN activation through LPFC may lead to increases in anticorrelations between the EAS and DMN and increases in resting-FC within DMN.

The present results complement a series of recent findings that have shown experience-dependent change in resting-FC. In previous studies, motor learning (Albert et al., 2009), motor training (Taubert et al., 2011), and visual perceptual learning (Lewis et al., 2009) were shown to induce changes in resting-FC within networks thought to be involved with tasks or resting-FC between those networks and other networks. Our results are congruent with these findings because EAS plays a key role in WM and DMN may also be actively involved in WM (Hampson et al., 2006; Owen et al., 2005). We showed here for the first time that WMT, which is associated with improvements in general cognitive abilities (Jaeggi et al., 2008; Klingberg et al., 2005), can induce a change in resting-FC between two intrinsic networks, DMN and EAS, both of which are associated with general cognitive abilities (Hampson et al., 2006; Song et al., 2008).

WMT-related decreases in resting-FC between the nodes of the different networks (increased anticorrelations between nodes of EAS and DMN) were not observed when the whole brain signal was not regressed out in individual analyses of resting-FC; this could be due to the effects of changes in aspects of global brain activity. As described in the Methods, regressing out the whole brain signal in individual resting-FC has been criticized as "artifactually" creating anticorrelations (Murphy et al., 2009) although a recent study suggested that anticorrelations observed in resting-state connectivity are not an artifact introduced by global signal regression and might have biological origins (Chai et al., 2012). The controversy is

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ongoing. In this study, we cannot enter into in-depth discussions related to this controversy because we did not measure respiratory- or physiologically related signals and therefore cannot regress out these signals. Moreover, since this controversy is new, we do not know much about the meaning of the different results obtained when the whole brain signal was regressed out in individual analyses and when it was not. However, a global signal correlation was observed partly because the brain was globally activated (Schölvinck et al., 2010). Thus, when these global brain activities are not regressed out, FC between two brain regions shows correlated activity because of global brain activity as well as because of region- or network-specific activity synchronization, but when these global brain activities are regressed out, FC between the two brain regions shows correlated activity because of network-specific activity synchronization. In this case, the significant WMT-related results in resting-FC analyses in which the whole brain signal was regressed out can be explained by WMT-related changes in region- or network-specific activity synchronization. The difference in results relating to WMT-related changes in resting-FC when the whole brain signal is regressed out and when it is not would be due to some aspect of WMT-related change in global brain activity. On the other hand, if WMT simply increased the effects of global brain activity on resting-FC uniformly, then the WMT-related results of resting-FC when the whole brain signal was not regressed out would show larger WMT-related increases in resting-FC between mPFC and the precuneus; however, no such difference was observed. One possible reason for the differences in the abovementioned results is that after WMT, the whole brain signal conforms to EAS activity (but not DMN activity). In other words, the contribution of EAS activity to the whole brain signal might increase after WMT. If this happens, the removal of the whole brain signal would not affect the training-related results regarding resting-FC between mPFC and the precuneus (within DMN resting-FC). On the other hand, in this case, since DMN activity must always contribute to (correlate with) global brain activity, when the whole brain signal was not regressed out, it seems after WMT, the relationship between EAS and DMN activities must shift toward an increase in resting-FC between these networks although this shift can be explained by an increase in the correlation between EAS activity and global brain activity alone. Consistent with this notion, when the whole brain signal was not regressed out, the tendency toward a WMT-related increase in resting-FC with the right DLPFC was observed in a substantially larger area and across a multiple bilateral brain areas compared with when the whole brain signal was regressed out (Fig. 6a). In fact, in the former analysis, the largest cluster spread across multiple bilateral areas was significant when the cluster size test using a cluster-determining threshold of $p < .05$ uncorrected was used ($p = 2.50 \times 10^{-6}$, corrected at the cluster level under the cluster-determining threshold of $p < .05$, uncorrected). However, no such difference was observed in the case of the analyses of WMT-related increases in resting-FC with mPFC (Fig. 6b). Thus, WMT might lead to a weak increase in the resting-FC between the nodes of EAS and multiple areas.

WMT may increase resting-rCBF in the right LPFC through changes in the capillary and increases in metabolic demand. Experience-dependent neural plasticity involves increases in the width and density of the capillary (Borowsky and Collins, 1989; Sirevaag and Greenough, 1987). It also involves increases in the number and volume of glia (Diamond et al., 1966) and mitochondria, respectively (Sirevaag and Greenough, 1987), which leads to increases in metabolic demand. WMT may also increase resting-rCBF through these changes in the capillary and increases in metabolic demand.

In our previous study, relatively mild WMT led to an increase in rGMV in regions of EAS in which rGMV decreased after a 1-week intensive program of concentrated WMT, suggesting that training protocols affect whether rGMV increases or decreases in EAS following WMT. In this study, relatively mild WMT (up to 1 h per day for 4 weeks) led to increased rGMV in bilateral DLPFCs, superior parts of the bilateral parietal cortices, and the left perisylvian area. These regions showed a decrease in rGMV after the 1-week intensive concentrated WMT (4-h training per day) in our previous study (Takeuchi et al., 2010b). These findings are congruent with our hypothesis that cognitive training may lead to nonlinear changes in rGMV (an initial increase followed by a decrease) that are affected by training length and intensity (greater intensity leads to more rapid nonlinear change) (Takeuchi et al., 2011b). The findings are also consistent with those of previous studies in which relatively mild training led to increased rGMV (Draganski et al., 2004; Driemeyer et al., 2008) as well as our previous cognitive training study in which a 1-week intensive concentrated WMT (4-h training per day) led mainly to a decrease in rGMV (Takeuchi et al., 2011b).

WMT also led to changes in performance on certain cognitive tests. Our previous review (Takeuchi et al., 2010b) showed that the effects of WMT on some cognitive functions vary between studies, the differences between this study and other studies (Dash et al., 2010; Jaeggi et al., 2008; Takeuchi et al., 2011d) may be explained by difference of subjects' characteristics, training tasks and statistical deviations. Of note, in this study, WMT led to average increase in performance on the creativity test. This is in clear contrast to the result of our previous study of WMT involving mental calculations, which showed a clear training-related decrease in creativity. One possible cause of this discrepancy in WMT tasks could be division of attention. In this study, we included dual WM and dual *N*-back tasks, both of which apparently require divided attention. In our previous study, the main training task was mental multiplication, which may require an extended period of focused attention with no distractions to avoid losing retained information. On the other hand, creativity has been suggested to be associated with widened attention, which allows one to process a lot of information concurrently (Mendelsohn, 1976), but also with impaired selective attention (Necka, 1999). Thus, cognitive training that requires divided attention may improve creativity, while cognitive training that requires focused attention may suppress creativity. WMT has previously been shown to increase performance on non-verbal reasoning tasks (Takeuchi et al., 2010b), but this finding was recently contested (Redick et al., in press). Our study results relating to this aspect are weak and inconclusive, but given the recent controversy, they may be worth noting. First, the Tanaka B-type intelligence test consists of several speeded tasks (although these include

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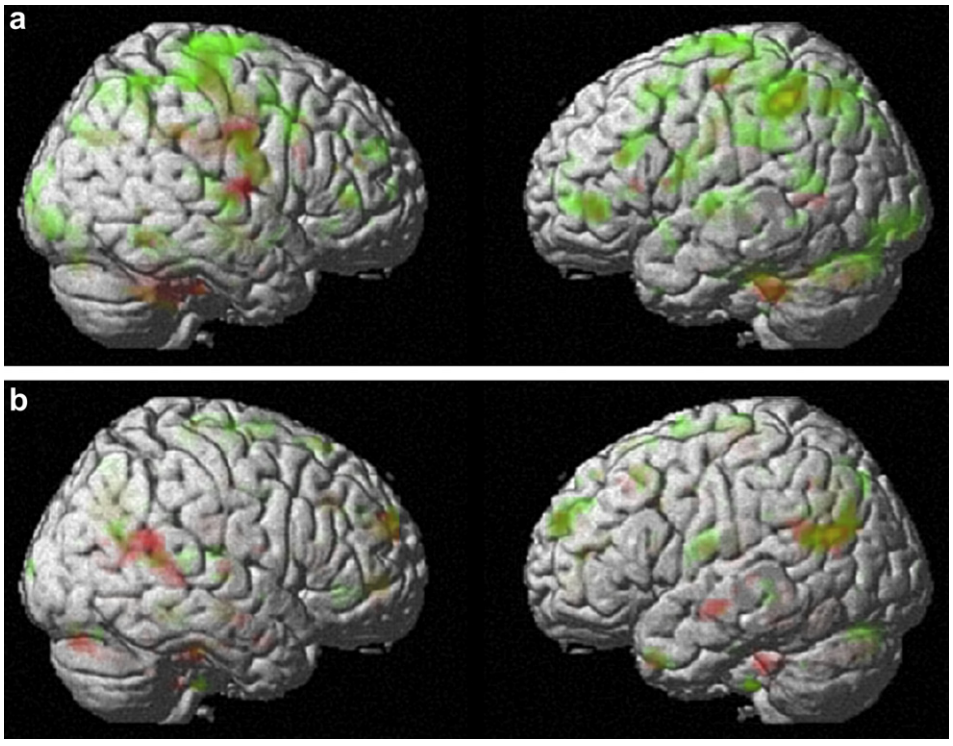

Fig. 6 – Tendencies of WMT-related increases in resting-FC. (a) Increase in resting-FC with right DLPFC in the WMT group compared with the control group. Results are shown with a threshold of $p < .05$, uncorrected. Red areas show resting-FC when the time course of the whole brain signal was regressed out in individual analyses. Green areas show resting-FC when the time course of the whole brain signal was not regressed out in individual analyses. As can be seen, when the whole brain signal was not regressed out, the tendency toward a WMT-related increase in resting-FC with the right DLPFC covered a substantially larger total area and included more brain areas than when the whole brain signal was regressed out. (b) Increase in resting-FC with the mPFC in the WMT group compared with the control group. Results are shown with a threshold of $p < .05$, uncorrected. Red areas show resting-FC when the time course of the whole brain signal was regressed out in individual analyses. Green areas show resting-FC when the time course of the whole brain signal was not regressed out in individual analyses. As can be seen, no substantial difference between red and green areas was observed.

complex speeded tasks) (Takeuchi et al., 2011b), and the nature of this test content differs from that in RAPM and BOMAT. The insignificant results relating to the Tanaka intelligence test observed in this study may be somewhat congruent with the results of our previous review (Takeuchi et al., 2010b), which concluded that WMT may not affect performance on processing speed tasks in normal subjects. WMT led to improved performance on RAPM but not on BOMAT. This finding contrasts with the results of previous studies that reported significant effects of WMT involving dual *N*-back or dual WM tasks on BOMAT (Jaeggi et al., 2008; Takeuchi et al., 2010b). Although the exact cause of this discrepancy is unclear, the performance of subjects on BOMAT in this study was lower than that in the previous study, and this might have made the test unsuitable for assessing performance (Jaeggi et al., 2008). The difference may also be due to lower test–retest reliability due to the unusual manner in which the test was administered, but the low number of subjects in the no-intervention group made it difficult to reliably assess test–retest reliability. On the other hand, although several studies have showed the effects of WMT on Raven matrix tests (Takeuchi et al., 2010b), the effects of WMT on non-verbal reasoning fluid intelligence tasks have recently been contested and there may be a number of reasons for the lack of significant effects on BOMAT or other non-verbal reasoning tasks (Redick et al., in press; Takeuchi et al., 2010b). Our previous study of WMT involving mental calculations (Takeuchi et al., 2011d) involved an active control group, an intervention group, and testers blinded to the group assignments, and this study failed to show any significant effects of training on RAPM although the training led to a marked increase in performance on the letter span task, which has barely any components in common with the training tasks. In the present study, we performed two non-verbal reasoning fluid intelligence tests and only one was weakly significant; the other showed no hint of any training effects. These discrepancies could be due to subject characteristics such as a tendency toward attention deficits as WMT may affect attention (Takeuchi et al., 2010b). In that case, the results may differ based on country because of great variation in the prevalence of attention deficit hyperactivity disorder across countries (Skounti et al., 2007), or even within the same country based on how the subjects were recruited. Thus, the effects of WMT on non-verbal reasoning fluid intelligence tasks remain elusive.

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One limitation of this study was its complex training protocols (Jaeggi et al., 2008; Tang et al., 2007). These challenges are common in this type of study in which the training is related to WMT (Jaeggi et al., 2008), video games (Green and Bavelier, 2003) or meditation (Tang et al., 2007). These types of studies typically have no strict control groups or any other conditions as those in normal fMRI studies. Although it would be statistically challenging, it would be interesting to disentangle the multiple complex cognitive training protocols (for example, do auditory and visual WMT have different effects?) and investigate the effect of each component of training in the future. Finally, with regard to the control group, unlike our previous study (Takeuchi et al., 2011d), we did not include an active control group because all the available evidence indicate that an active control group does not have any impact in this type of study. It is true that under certain conditions, the components that an active control group tries to control can affect behavioral performance. For example, taking placebo drugs can affect cognitive performance under certain conditions (Oken, 2008; Oken et al., 2008), whereas active control training does not affect cognitive performance, especially in young adults (Takeuchi et al., 2011d). Training in computer use may also involve many learning processes for individuals who are not used to using a computer, which may affect neural mechanisms. However, the use of computerized training tasks in young adults does not have such effects (Takeuchi et al., 2011d). Contact with the experimenter or another individual may be meaningful for older adults who have little contact with others (Bassuk et al., 1999). However, we included young, healthy university students who were willing to take part in the experiment alone, and for these individuals, contact with the experimenter does not have any effect on cognitive performance (Takeuchi et al., 2011d). Besides, the only prolonged social interaction with the experimenters occurred during the training period when the training tasks were explained on the first day, and this period ended after less than 20 min. Furthermore, the additional analysis of the profile of mood states (Yokoyama, 2005) performed on the first and last days of the experiment (Takeuchi et al., 2011a) revealed that subjects in the WMT group did not show significant WMT-related improvements in vigor ($p = .235$, one-tailed ANCOVA), which may well be related to motivation or any WMT-related increase in fatigue ($p = .919$, one-tailed ANCOVA, indicating that, if anything, WMT was associated with reduced fatigue). These results indicate that chronic fatigue induced by cognitive training and increased motivation caused by training or expectancy effects can be ruled out (although subjects may have been specifically motivated during certain measures). We can also say that the subjects were probably not trying to show that they were smarter, determined as per our analysis of the questionnaire responses. For one related measure, we administered a psychological questionnaire relating to critical thinking (Hirayama and Kusumi, 2004). When the score for this questionnaire was analyzed like the other psychological measures, no significant effects of WMT were detected, and the average score after the training period was actually lower in the WMT group. If the subjects were attempting to show that their cognitive performance had improved because of the training, the training would have had a stronger effect on questionnaire measures of cognition as it would be much easier to report that they became smarter than to actually show that they became smarter. We are not trying to say that active control training has been proven to be ineffective in all existing behavioral and imaging measures because this is impossible. Instead, we are saying that there is no scientific evidence indicating that there are effects in what the active control group is trying to control for (fatigue, expectancy effects, computer use, contact with experimenters, etc.) in experiments such as this one. On the other hand, what the active control group is "not" trying to control for does have effects on behavioral and imaging measures in some cases. Video games, learning of substantially new knowledge or skills, and low-level cognitive training in subjects with compromised cognitive abilities have also been used as active control training (Takeuchi et al., 2010b). However, there is evidence indicating that these interventions affect cognitive performance and brain structures (Takeuchi et al., 2010b). Because of such limitations, we cautioned against the too easy use of active control groups in imaging studies in our previous review of WMT (Takeuchi et al., 2010b). The lack of an active control group may be a limitation of this study, but if so, then all intervention studies of cognitive training published to date will have bigger or equivalent limitations, and if we use an active control group in imaging intervention studies, we usually end up having to face other possible limitations depending on the purpose of the study. The inclusion of two (active and passive) control groups may be ideal, but practically, the design would have a lower statistical power (Takeuchi et al., 2010b).

We discussed the likelihood of the effects of factors that active control groups are usually trying to control for above. Nonetheless, it is still a limitation of our study that the experimental group had a much different experience than the control group. For resting-FC, for example, resting-FC within DMN and EAS is associated with a wide range of diseases and cognitive functions (Broyd et al., 2009; Hampson et al., 2006; Song et al., 2008; Takeuchi et al., in press). Thus, although the effects of some motor and other perceptual training appear to be related to resting-FC involving networks that are thought to be involved with such tasks some other interventions may also affect resting-FC of EAS and DMN, as shown in this study. Thus, future studies need to clarify this area.

5. Summary and conclusion

In summary, this is the first study to investigate the effects of WMT on resting-FC and resting-rCBF. The present results show that WMT increases resting-FC between the key DMN nodes, decreases resting-FC between the key DMN node and nodes of the EAS, and increases resting-rCBF in the right LPFC. Lower resting-FC among the DMN nodes, higher resting-FC between DMN and the EAS, and lower resting-rCBF in PFC have been observed in many conditions with reduced WMC. Our results indicated resting-state neural mechanisms which are assumed to reflect brain's intrinsic activities and connectivities are affected by cognitive training. However, the training-related decreases in resting-FC between the key DMN node and the nodes of EAS was only observed when the whole

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brain signal was regressed out in individual analyses, and these changes were not observed when the whole brain signal was not regressed out in individual analyses. Further analyses indicated that these differences may be mediated by a weak but a widespread increase in resting-FC between the nodes of EAS and activity of multiple bilateral areas across the brain. And, the present results may provide insight into the application of WMT for many conditions with reduced WMC. Moreover, the WMT program used in this study was relatively mild and led to an increase in rGMV in regions of EAS, whereas a decrease in rGMV after a 5-day intensive (4-h training per day) WMT program involving mental calculations was observed in similar regions in our previous study. This result is congruent with our hypothesis that training intensity determines whether rGMV increases or decreases after training.

Acknowledgments

We thank Yuki Yamada for operating the MRI scanner, Sarah Michael for checking the English of this manuscript, Haruka Nouchi for conducting the psychological tests, the subjects, and all our other colleagues in IDAC, Tohoku University for their support. This study was supported by JST/RISTEX, JST/CREST and a Grant-in-Aid for Young Scientists (B) (KAKENHI 23700306) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT).

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Please cite this article in press as: Takeuchi H, et al., Effects of working memory training on functional connectivity and cerebral blood flow during rest, Cortex (2012), http://dx.doi.org/10.1016/j.cortex.2012.09.007

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Please cite this article in press as: Takeuchi H, et al., Effects of working memory training on functional connectivity and cerebral blood flow during rest, Cortex (2012), http://dx.doi.org/10.1016/j.cortex.2012.09.007