

## ORIGINAL ARTICLE

# Connectivity Neurofeedback Training Can Differentially Change Functional Connectivity and Cognitive Performance

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## Abstract

Advances in functional magnetic resonance imaging have made it possible to provide real-time feedback on brain activity. Neurofeedback has been applied to therapeutic interventions for psychiatric disorders. Since many studies have shown that most psychiatric disorders exhibit abnormal brain networks, a novel experimental paradigm named connectivity neurofeedback, which can directly modulate a brain network, has emerged as a promising approach to treat psychiatric disorders. Here, we investigated the hypothesis that connectivity neurofeedback can induce the aimed direction of change in functional connectivity, and the differential change in cognitive performance according to the direction of change in connectivity. We selected the connectivity between the left primary motor cortex and the left lateral parietal cortex as the target. Subjects were divided into 2 groups, in which only the direction of change (an increase or a decrease in correlation) in the experimentally manipulated connectivity differed between the groups. As a result, subjects successfully induced the expected connectivity changes in either of the 2 directions. Furthermore, cognitive performance significantly and differentially changed from preneurofeedback to postneurofeedback training between the 2 groups. These findings indicate that connectivity neurofeedback can induce the aimed direction of change in connectivity and also a differential change in cognitive performance.

**Key words:** cognitive function, connectivity neurofeedback, functional connectivity, functional magnetic resonance imaging (fMRI)

## Introduction

Since real-time functional magnetic resonance imaging (fMRI) has become available in various research fields (Cox et al. 1995;

deCharms 2008), fMRI-based neurofeedback training has been widely used for a variety of purposes, from investigating neural mechanisms (Shibata et al. 2011, 2016; Amano et al. 2016) to

therapeutic interventions for psychiatric disorders (Enriquez-Geppert et al. 2013; Niv 2013; Sulzer et al. 2013; Stoeckel et al. 2014). Currently, there are 3 types of neurofeedback training based on the differences in feedback information. The first, univariate neurofeedback, uses the average blood oxygen level-dependent (BOLD) signal within a specific brain region of interest to increase or decrease the average activity in that region (deCharms et al. 2005). The second, decoded neurofeedback, uses the multivoxel activity pattern in a region to induce a specific piece of information in that region, such as orientation, color, and facial preference (LaConte et al. 2007; Shibata et al. 2011, 2016; deBettencourt et al. 2015; Amano et al. 2016). The third, connectivity neurofeedback, uses the functional connectivity between regions to modulate connectivity (Koush et al. 2013, 2017; Megumi et al. 2015; Liew et al. 2016).

Numerous studies have reported that psychiatric disorders are related to abnormal brain networks (Brody et al. 2009; Stam 2014; Fornito et al. 2015), while some cognitive functions are associated with brain networks (He et al. 2007; Kelly et al. 2008; Barch et al. 2013; Thompson et al. 2013; Liu et al. 2015). This indicates that using connectivity neurofeedback is a promising approach to therapeutic intervention for psychiatric disorders and to improve cognitive function. To our understanding, there are 2 mainstream sets of studies on connectivity neurofeedback. One uses dynamic causal modeling to modulate the state of a functional network and cognitive performance (Koush et al. 2013, 2017). The other uses Pearson's correlation coefficients of activity time courses between 2 regions (Megumi et al. 2015; Liew et al. 2016). Megumi et al. (2015) identified changes in global networks caused by connectivity neurofeedback. Liew et al. (2016) demonstrated improvement of corticothalamic connectivity in patients with stroke. Furthermore, an integrated method of univariate and connectivity neurofeedback has been proposed (Kim et al. 2015).

However, fewer studies have been conducted on connectivity neurofeedback. In particular, the controllability of connectivity neurofeedback is critical for applications aimed at psychiatric disorders. That is, it is important to examine whether connectivity neurofeedback can induce the aimed direction of change (i.e., an increase or a decrease) in functional connectivity and a change in cognitive performance. Previous studies using Pearson's correlation coefficients tested only the increases in connectivity. Here, we examined the hypothesis that connectivity neurofeedback based on Pearson's correlation (Megumi et al. 2015) can induce the aimed direction of change in functional connectivity by flipping the sign of the neurofeedback signal, and the differential change in cognitive performance according to the induced change in connectivity.

Our connectivity neurofeedback training can control functional connectivity by rewarding spontaneous changes in functional connectivity. Subjects underwent training with intermittent feedback of the temporal correlation (functional connectivity) between BOLD signals in 2 brain regions immediately after each trial. Subjects learned to control connectivity in a trial-and-error manner through training. To investigate our hypothesis, we separated subjects into 2 groups, in which we aimed to increase or decrease the functional connectivity, and compared the resulting changes in cognitive performance from preneurofeedback to postneurofeedback training between the 2 groups.

Because our previous study (Megumi et al. 2015) already successfully increased the connectivity between the left primary motor cortex (IM1), which belongs to the motor/visuospatial network (MVN), and the left lateral parietal (ILP) cortex, which belongs to the default mode network (DMN) (Raichle 2010), we selected this connectivity as the target for neurofeedback

training. Furthermore, we conducted a psychomotor vigilance task (PVT), the Eriksen flanker task (EFT), and the color-word Stroop task (CWST) before and after the neurofeedback training, since previous studies have shown that these 3 tasks are associated with the MVN, the DMN, or both (Kelly et al. 2008; Hinds et al. 2013; Thompson et al. 2013; Liu et al. 2015). In the current study, functional connectivity between IM1 and ILP was normally negative (e.g.,  $r = -0.4$ ). Therefore, we hereafter refer to a change in functional connectivity from  $r = -0.4$  to  $-0.1$ , for instance, as an "increase," while we refer to a change such as that from  $r = -0.4$  to  $-0.7$  as a "decrease."

## Materials and Methods

### Participants

Thirty healthy subjects (4 women; mean age [mean  $\pm$  standard deviation {SD}],  $22.7 \pm 1.7$  years; age range, 20–27 years) participated in the neurofeedback experiment. We randomly assigned subjects to an "increased functional connectivity" group ( $n = 18$ :1 woman; mean age,  $22.6 \pm 1.8$  years; age range, 20–27 years) or a "decreased functional connectivity" group ( $n = 12$ :3 women; mean age,  $22.8 \pm 1.6$  years; age range, 21–26 years). Although there are fewer female participants, there was no significant difference in the male–female ratio between the 2 groups (Fisher's exact test:  $P = 0.27$ ). Twenty-five (13 in the "increased functional connectivity" group and 12 in the "decreased functional connectivity" group) of 30 subjects completed behavioral testing sessions before and after the neurofeedback training. The other 5 subjects did not participate in the behavioral testing sessions. We excluded 1 subject in the "decreased functional connectivity" group from cognitive performance analysis because that subject did not follow the instructions. We also excluded another subject in the "decreased functional connectivity" group from the EFT analysis because that subject misunderstood the instructions. All subjects were right-handed according to the Edinburgh inventory (Oldfield 1971). The Institutional Review Board of Advanced Telecommunications Research Institute International (ATR) approved this study, which was performed in accordance with the tenets of the Declaration of Helsinki. All subjects provided written informed consent.

### Neurofeedback Training

#### Brain Imaging and Region of Interest Definition

MR images were obtained using a 3-T Siemens MAGNETOM Verio scanner (Kyoto, Japan). BOLD signals were measured using an echo planar imaging (EPI) sequence (repetition time [TR], 2000 ms; echo time [TE], 26 ms; flip angle,  $80^\circ$ ). The entire brain was covered in 33 axial slices (3.5 mm of thickness, no gap), voxel size was  $3 \times 3 \times 3.5$  mm, and field of view was  $192 \times 192$  mm. T1-weighted structural images were acquired with a resolution of  $1 \times 1 \times 1$  mm. T2-weighted structural images were acquired with a resolution of  $0.75 \times 0.75 \times 3.5$  mm.

Following our previous study (Megumi et al. 2015), we selected the IM1, which is included in the MVN, and the ILP, which is included in the DMN, as the 2 target region of interests (ROIs) for calculating a feedback score in the connectivity neurofeedback training. IM1 was defined as Brodmann area 4 according to the anatomical map given in PickAtlas (<http://fmri.wfubmc.edu/software/PickAtlas>) (Lancaster et al. 1997; Maldjian et al. 2003). ILP was defined as a sphere with a 7.5-mm radius centered at  $(x, y, z) = (-45, -67, 36)$  in the Montreal Neurological Institute standard brain coordinates (MNI; Montreal, QC) according to a previous study of brain networks (Fox et al. 2005). We adopted the spherical ROI for ILP because we could

not find an anatomical definition of LP as a part of DMN in the literature. By contrast, we adopted the anatomical ROI for IM1 because (1) it is well defined in anatomical maps including PickAtlas and (2) the spherical ROI centered at M1 may include the somatosensory cortex.

Because these ROIs were defined in the standard brain, we identified corresponding voxels in the functional images of each individual subject's brain using a deformation module in SPM8 (Wellcome Trust Center for Neuroimaging, London, UK; [www.fil.ion.ucl.ac.uk/spm/](http://www.fil.ion.ucl.ac.uk/spm/)). We obtained several volumes of functional images for this purpose at the beginning of the experiment on each day and used the identified voxels as ROIs for calculating scores in the subsequent training blocks. Furthermore, the position and orientation of scan images on every training day were carefully matched those on the first day.

### Neurofeedback Training Procedure

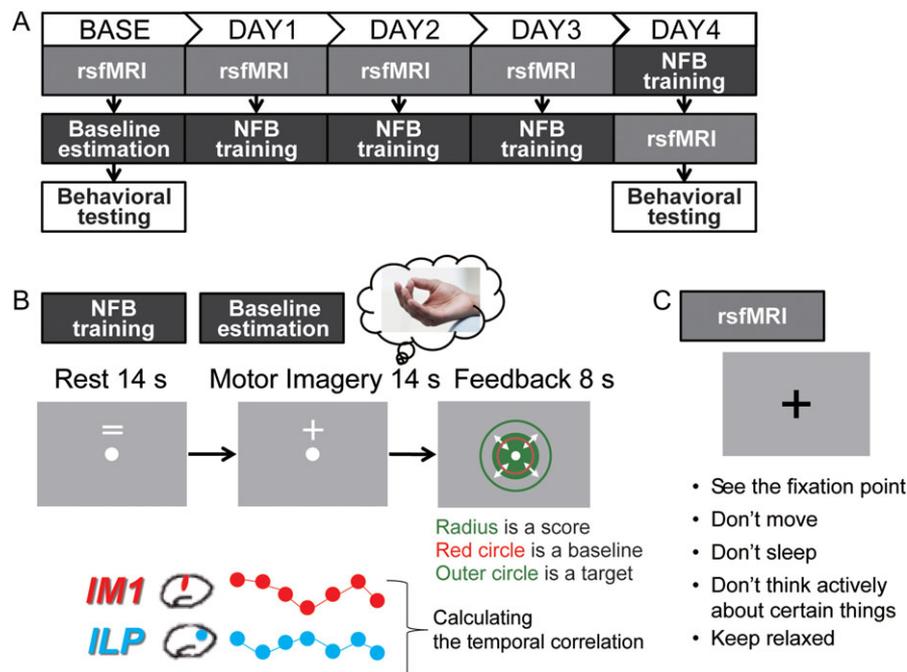
Subjects received neurofeedback training to increase or decrease functional connectivity between the 2 target ROIs. Each subject received training for 4 days (Fig. 1A: DAY1–DAY4). On each training day, subjects performed 6 blocks, each of which was composed of 10 trials. Prior to training, subjects underwent 4 baseline-estimation blocks to measure a subject-specific baseline correlation between the 2 target ROIs (Fig. 1A: BASE). The baseline-estimation block was identical to the neurofeedback training block, except that the score was randomly determined (see Online Calculation of Feedback Score).

Our training procedure in each trial followed our previous study (Megumi et al. 2015). A trial in each block began with a rest period of 14 s, during which the “=” cue was presented on the screen (Fig. 1B: Rest). When the cue changed to “+,” subjects

performed the tapping motor imagery task for 14 s (Fig. 1B: Motor Imagery). Subjects were instructed to imagine tapping their thumbs with their fingers randomly as fast as possible. Furthermore, they were asked to produce kinesthetic imagery, rather than attempt visual imagery, and to not overtly move their hands during the task. If no task instruction was provided, cognitive states during learning were expected to differ largely among subjects, thereby making data analysis difficult. Therefore, we administered a motor imagery task to the subjects to constraint the subjects' cognitive states. After the motor imagery period, a feedback score calculated by the online MRI system (see Online Calculation of Feedback Score) was presented on the screen as a green disc (Fig. 1B: Feedback). Subjects were instructed that the disc becomes bigger as they improve at producing tapping imagery; however, the disc size actually corresponded to the score determined by the temporal correlation (functional connectivity) between BOLD signals in IM1 and ILP (see Online Calculation of Feedback Score). Subjects were instructed to make the disc size as large as possible so that it would become larger than the red circle (a baseline) and reach the outer green circle (a target). The calculations of the baseline and target are described in section Online Calculation of Feedback Score. Subjects were informed that additional monetary reward (up to JPY 3000) would be paid in proportion to their total score, and they received this at the end of the experiments on each day.

### Online Calculation of Feedback Score

We used in-house MATLAB software (Mathworks Inc.), including realignment modules of SPM8, for online processing. This software ran on a connected computer and accessed data files



**Figure 1.** Neurofeedback training procedures. (A) Neurofeedback (NFB) training schedule. Experiments lasted for 5 days, including baseline estimation of temporal correlation between BOLD signals in the target regions (BASE). Resting-state activity (rsfMRI) was measured daily before NFB training from BASE to DAY3. On DAY4, rsfMRI was measured after NFB training. Subjects performed a cognitive task on BASE and DAY 4. (B) Timeline and displays for subjects in a training trial. After a rest period while the “=” cue was presented on the screen, subjects were instructed to imagine finger tapping during the motor imagery period while the “+” cue was presented. A solid green disc was presented to the subjects in the feedback period: the disc size was proportional to the correlation between BOLD signals in 2 target regions (left primary motor area [IM1] and left lateral parietal region [ILP]) during the motor imagery period. (C) Display for subjects in rsfMRI. Subjects were instructed to keep looking at a fixation point at the screen center, to keep still, to stay awake, and to not think about specific things.

in the MRI system. Each volume of the functional image was realigned in real time to the first volume obtained on each day.

Seven volumes were obtained during the motor imagery period in each trial, but the first volume was discarded and 1 volume from the feedback period was added as compensation for hemodynamic delay. One may argue that a one-volume shift (2 s) is not enough to fully compensate for the hemodynamic delay (4–8 s); however, we followed 2 previous neurofeedback studies (Bray et al. 2007; Megumi et al. 2015) that used a 2-s shift and succeeded in changing brain activity. We followed these studies to minimize the delay of feedback to participants.

BOLD signal time courses were extracted from the LM1 and LLP ROIs (averaged across voxels) in these volumes. To remove several sources of spurious artifacts in BOLD signals, we conducted an online linear regression, including (1) 6 motion parameters, in addition to averaged signals over (2) gray matter, (3) white matter, and (4) cerebrospinal fluid (Fox et al. 2005). To completely remove global signals that may be related to instrumental, motion-related, and physiological fluctuations (Caballero-Gaudes and Reynolds 2016), we included signals averaged over the gray matter in the regression model. However, this may have removed neuronal signals in the ROIs if their activity strongly affected the average signal. In our post hoc analysis, we calculated signals averaged over the gray matter excluding the ROIs, but they were similar to those including the ROIs (temporal correlation:  $r > 0.999$ ), suggesting that the activity of the ROI unlikely affected the average signal.

We estimated coefficients for these parameters from the preceding 180 volumes (a moving window), which corresponded to 1 neurofeedback block, and regressed out the signals correlated with the parameters from a newly acquired volume. To maintain a constant number of moving volumes (180), we used the volumes acquired in the preceding block for the online regression in the early part of each block. Because there was no preceding block for the first block in the neurofeedback training, we conducted the 6-min resting condition block just before the training, which was not included in the offline analysis. Furthermore, to remove low-frequency trends from BOLD signals, a high-pass temporal filter (cutoff frequency of 0.0075 Hz) was applied to the time courses within each block (using only volumes from the same block).

Using the time courses after the noise reduction, the feedback score of the  $i$ th trial was calculated as

$$\text{Score}_i = \frac{50(\text{Correlation}_i + \text{Correlation}_{\text{Target}} - 2\text{Correlation}_{\text{Base}})}{\text{Correlation}_{\text{Target}} - \text{Correlation}_{\text{Base}}}$$

$$0 \leq \text{Score}_i \leq 100 \quad (1)$$

Here,  $\text{Correlation}_i$  represents the correlation between the BOLD signals averaged in each of the 2 ROIs. We developed this score for an intuitive feedback to participants: their baseline performance corresponded to 50 while their better performance was rewarded by the increase in the score from 50. Specifically,  $\text{Correlation}_{\text{Base}}$  was the median correlation in the baseline-estimation block (40 trials) on the first day (BASE). SD was also calculated in the baseline-estimation block.  $\text{Correlation}_{\text{Target}}$  was determined to restrict the appearance of a score of 100 to one time per block on average:  $\text{Correlation}_{\text{Target}}$  was ( $\text{Correlation}_{\text{Base}} + 1.28 \text{ SD}$ ) in the “increased functional connectivity” group and ( $\text{Correlation}_{\text{Base}} - 1.28 \text{ SD}$ ) in the “decreased functional connectivity” group. Therefore, if  $\text{Correlation}_i$  is equal to  $\text{Correlation}_{\text{Base}}$ , the score is 50 in both groups. If  $\text{Correlation}_i$  increases in the

“increased functional connectivity” group (or decreases in the “decreased functional connectivity” group) from  $\text{Correlation}_{\text{Base}}$  to  $\text{Correlation}_{\text{Target}}$ , the score rises from 50 to 100. If  $\text{Correlation}_i$  decreases in the “increased functional connectivity” group from  $\text{Correlation}_{\text{Base}}$  to  $\text{Correlation}_{\text{Base}} - 1.28 \text{ SD}$  (or if  $\text{Correlation}_i$  increases in the “decreased functional connectivity” group from  $\text{Correlation}_{\text{Base}}$  to  $\text{Correlation}_{\text{Base}} + 1.28 \text{ SD}$ ), the score decreases from 50 to 0. Any score below 0 or above 100 was maintained at 0 or 100, respectively. The score was calculated immediately after the acquisition of the first volume in the feedback period (2 s). Preprocessing and score calculation were completed within 2 s. Thus, subjects received the score within 4 s after the end of the imagery periods.

To prevent learning in the baseline-estimation block, we gave the subjects a pseudorandom score, which was generated from a normal distribution having a mean of 50 and SD of 30.3. The SD was determined to restrict the appearance of a score of 0 or 100 to one time per block. At the beginning of the first training day (DAY1), we told subjects that the feedback score had been randomly determined on the previous day (BASE).

#### Change in Score during Training

We investigated the daily changes in score during the neurofeedback training. In total, each subject had 280 scores (BASE = 40 scores, DAY1–DAY4 =  $60 \times 4$  scores). To investigate the daily changes in the score, we applied a mixed-effects model based on linear regression (Aarts et al. 2014) to the scores adopting training day (a continuous value) as a fixed effect and subject as a random effect. We used a maximum likelihood method for estimation of coefficients as implemented in the *lme4* package (<https://github.com/lme4/lme4>) of R version 3.2.1 (<https://www.r-project.org>). We calculated P-values using the *lmeTest* package of R.

#### Change in Functional Connectivity during Training

We investigated the daily changes in functional connectivity between LM1 and LLP during the neurofeedback training in our offline analysis. The fMRI data were preprocessed with SPM8 on MATLAB. Preprocessing steps included slice-timing correction, realignment, coregistration, segmentation of T1-weighted structural image, normalization into MNI space, and spatial smoothing with an isotropic Gaussian kernel of 8 mm full width at half maximum. BOLD signal time courses were extracted from the 2 ROIs (averaged across voxels). Sources of spurious variance were removed as described in section Online Calculation of Feedback Score. Then, we calculated the functional connectivity as Fisher’s z-transformed Pearson’s correlation coefficients between the BOLD signals in the 2 ROIs using 7 volumes during the motor imagery period in each trial, in the same fashion as the online calculation of the feedback score, that is, the first volume was discarded and 1 volume from the feedback period was added as compensation for hemodynamic delay. In total, each subject had 280 functional connectivities (BASE = 40 connectivities, DAY1–DAY4 =  $60 \times 4$  connectivities).

To compare the daily changes in functional connectivity between groups, we applied a mixed-effects model to the functional connectivities including training day (a continuous value), group, and the interaction between group and day as fixed effects, and subject as a random effect. Further, to investigate whether the changes of functional connectivity were induced in the aimed direction in each group, we applied the mixed-effects model, as a post hoc analysis of the effect of training day on functional connectivity, separately to each group.

## Resting-State Functional MRI

To investigate the daily changes in resting-state functional connectivity between the target ROIs (IM1 and LLP) as well as in connectivity between the network-level ROIs (DMN and MVN), we measured resting-state fMRI (rsfMRI) every day (Fig. 1A). The rsfMRIs were measured before the neurofeedback training except for the last day (after the neurofeedback on DAY4).

### Brain Imaging and Calculation of the Resting-State Functional Connectivity

During the rsfMRI measurements, subjects were instructed to keep looking at a fixation point at the center of a screen, to keep still, to stay awake, and to not think about specific things. MRI scans were obtained using a 3-T Siemens MAGNETOM Verio scanner. BOLD signals were measured using an EPI sequence (time, 10 min; TR, 2500 ms; TE, 30 ms; flip angle, 80°). The entire brain was covered in 40 axial slices (3.5 mm of thickness, no gap), voxel size was 3.3 × 3.3 × 3.5 mm, and field of view was 212 × 212 mm.

The rsfMRI data were preprocessed with SPM8 on MATLAB. The first 4 volumes were discarded to allow for T1 equilibration. Preprocessing steps included those listed in section Change in Functional Connectivity during Training. BOLD signal time courses were extracted from the 4 ROIs (IM1, LLP, MVN, and DMN) and averaged across voxels in each ROI. To determine network-level ROIs (DMN and MVN), we applied a spatial independent component analysis (Calhoun et al. 2001) to rsfMRI data from 66 subjects (12 women; mean age, 23.2 ± 2.3; age range, 20–31 years). We visually inspected MVN and DMN ROIs based on the following criteria: MVN includes bilateral primary motor cortex and supplementary motor area (Biswal et al. 1995), while DMN includes the medial prefrontal cortex, medial parietal cortex, and lateral parietal cortex (Raichle 2010). These network ROIs correspond to ICN8 (MVN) and ICN13 (DMN) as shown in Figure 2 of a previous study (Laird et al. 2011). To remove several sources of spurious variance, linear regression was performed, including (1) 6 motion parameters in addition to averaged signals over (2) whole brain, (3) white matter, and (4) cerebrospinal fluid. A temporal band-pass filter of 0.009–0.08 Hz was applied to the time series to restrict the analysis to low-frequency fluctuations that characterize rsfMRI

BOLD activity (Fox et al. 2005). Furthermore, to reduce spurious changes in functional connectivity by head motion, we calculated frame-wise displacement (FD) and removed volumes with FD > 0.5 mm, as proposed by the original article on scrubbing (Power et al. 2012). FD represents head motion between 2 consecutive volumes as a scalar quantity (summation of absolute displacements in translation and rotation). According to the above threshold, 3.8% (almost 9 volumes) ± 7.0% (1 SD) volumes were removed per 10 min of rsfMRI session (240 volumes). Then, we computed the resting-state functional connectivity as Fisher's z-transformed Pearson correlation coefficients between the preprocessed BOLD signals in 2 target ROIs (IM1 and LLP) and in 2 network-level ROIs (MVN and DMN).

### Change in Resting-State Functional Connectivity

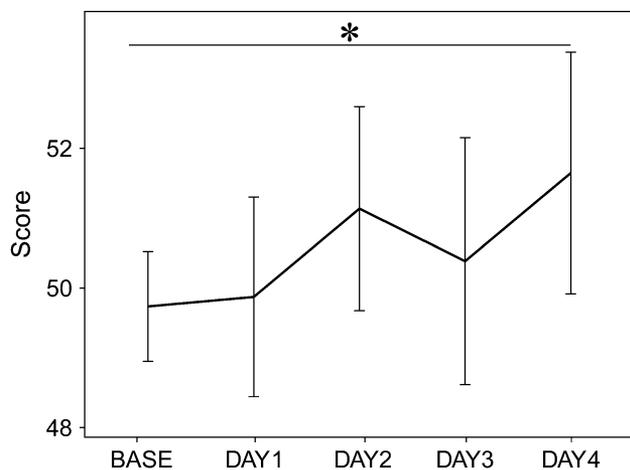
To statistically evaluate the daily changes in resting-state functional connectivity and to compare the changes between groups, we applied a mixed-effects model to the resting-state functional connectivities. We included group, training day, and the interaction between group and day as fixed effects and subject as a random effect. Further, as a post hoc analysis of the effect of day on the resting-state functional connectivities in each group, we applied a mixed-effects model, as a post hoc analysis of the effect of day on resting-state functional connectivity, separately to each group.

## Cognitive Tasks

To investigate the effect of the neurofeedback training on cognitive performance, subjects carried out a PVT, EFT, and CWST outside the MRI using a personal computer and keyboard before and after the entire neurofeedback training. Here, we know the direct relationship between cognitive performance and strength of the functional connectivity of MVN and DMN only in the vigilance task, in which the more the functional connectivity decreases the faster subjects react to a target and vice versa.

### Cognitive Task Procedures

Task procedures in the current study followed those in the previous studies.



**Figure 2.** Change in score during neurofeedback training. Score averaged across subjects ( $n = 30$ ) as a function of training day (error bars: standard error). A mixed-effects model identified a significant main effect of training day ( $t = 1.73$ ,  $P = 0.044$  [one-side]). \* $P < 0.05$ .

1. **PVT:** PVT is a task that measures the ability to sustain attentional focus. Subjects pressed a key in response to a stimulus that occasionally appeared on a screen. Subjects fixated on a centrally presented white cross on a gray background. When the cross was changed to black, subjects pressed the left arrow key with their right index finger as quickly as possible. Then, the cross changed to white again. If subjects failed to respond within 9 s, the cross automatically returned to white. Subjects performed 4 blocks (about 5 min × 4), each block containing 5 trials. The intertrial interval (ITI) varied from 10 to 90 s. We measured reaction time as the time from the change of the cross color to the key press. If reaction time was over 2 SD from average in each subject, the trial was excluded from further analysis. This definition of reaction time and exclusion criterion was also used in the following tasks.
2. **EFT:** EFT is a response inhibition test that measures the ability to suppress inappropriate responses in a particular context. Subjects fixated on a centrally presented black cross on a gray background. When 5 arrows (arrow direction was right or left) appeared on screen, subjects pressed the right

or left arrow key as quickly as possible, which corresponded to the direction of the central arrow in the array of 5 arrows. Subjects used their right index or middle finger to press the right or left arrow key, respectively. The task included incongruent and congruent conditions. Under the congruent condition, 5 arrows pointed in the same direction (e.g., <<<<<), whereas under the incongruent condition, the central arrow pointed to the opposite direction from the others (e.g., <<><<). If subjects failed to respond within 3 s, the arrows disappeared. Subjects performed 2 blocks (6 min  $\times$  2), each block containing 24 trials for each condition, presented in a pseudorandom order. ITI was 4.5 s. We calculated the reaction time for each condition.

3. CWST: CWST is also a response inhibition test, and it measures the ability to suppress inappropriate responses in a particular context. Subjects fixated on a centrally presented black cross on a gray background. According to the pretask cue (“+” or “-”) presented before presenting stimuli, subjects pressed the key that corresponded to the meaning or color of the presented stimulus with their right index, middle or ring finger as quickly as possible. The stimulus was word (red, blue, or yellow, in Japanese) with color (red, blue, or yellow). For example, if the pretask cue was “+,” subjects pressed the key corresponding to the meaning of the word; if the pretask cue was “-,” subjects pressed the key corresponding to the color of the word. The task included incongruent and congruent conditions. Under the congruent condition, the color of the word was the same as the meaning of the word, whereas under the incongruent condition, the color of the word was different from the meaning of the word. If subjects failed to respond within 3 s, the stimulus disappeared. Subjects performed 2 blocks (6 min  $\times$  2), each block containing 24 trials for each condition presented in a pseudorandom order. ITI was again 4.5 s. We calculated the reaction time for each condition.

### Change in Cognitive Performance

To compare the changes in the reaction time of each task from preneurofeedback to postneurofeedback training between the 2 groups, we applied a mixed-effects model to the all reaction times separately for each task. We included group, day (pre-neurofeedback and postneurofeedback training), and the interaction between group and day as fixed effects and subject as a random effect. Further, as a post hoc analysis of the effect of day on reaction times in each group, we applied a mixed-effects model including day as a fixed effect and subject as a random effect separately to each group in each task.

## Results

### Change in Score

Figure 2 shows the change in score during the neurofeedback training, averaged across the blocks and subjects as a function of training day. Here, the score increases when the connectivity changes in the aimed direction for each subject group Equation (1). We applied a mixed-effects model to the scores and examined whether a regression coefficient for the day was greater than zero. As a result, we found a significantly positive effect of day on the score (DAY:  $t = 1.70$ ,  $P = 0.044$  [one-side]). This result indicates that subjects increased their score during the neurofeedback training.

### Change in Functional Connectivity during Training

Figure 3 shows the change in functional connectivity between IM1 and LLP during the neurofeedback training, averaged across the blocks and subjects as a function of training day in each group. To compare the daily changes in functional connectivity between groups, we applied a mixed-effects model to the functional connectivity. As a result, we found significant effects for the day and the interaction between group and day (DAY:  $t = -3.22$ ,  $P = 0.0012$ ; DAY  $\times$  Group:  $t = 3.86$ ,  $P = 0.00011$ ) but not for the group (Group:  $t = -0.627$ ,  $P = 0.53$ ). This suggests that the change in functional connectivity across days was different between the groups.

Since we defined the LLP ROI as a sphere with a 7.5-mm radius, one may argue that the size was so large it could include confounding noise. In our post hoc analysis, we reduced the radius from 7.5 to 4.0 mm, and recalculated the connectivity between the ROIs. We still found significant effects for the day and the interaction between group and day (DAY:  $t = -3.34$ ,  $P = 0.00082$ ; DAY  $\times$  Group:  $t = 3.82$ ,  $P = 0.00013$ ), but not for the group (Group:  $t = -0.834$ ,  $P = 0.40$ ).

Further, to investigate whether the changes in functional connectivity were induced in the aimed direction during the training in each group, we applied a mixed-effects model separately for each group. We found a significant effect of training day in both groups (“increased functional connectivity” group:  $t = 2.17$ ,  $P = 0.029$ ; “decreased functional connectivity” group:  $t = -3.18$ ,  $P = 0.0014$ ). Mean functional connectivity increased from  $-0.12 \pm 0.029$  at BASE to  $-0.061 \pm 0.046$  on DAY4 in the “increased functional connectivity” group and decreased from  $-0.11 \pm 0.034$  at BASE to  $-0.22 \pm 0.044$  on DAY4 in the “decreased functional connectivity” group. These results indicate that functional connectivity between IM1 and LLP during the training changed from preneurofeedback to postneurofeedback training in the aimed direction in each group, that is, the functional connectivity increased in the “increased functional connectivity” group and decreased in the “decreased functional connectivity” group.

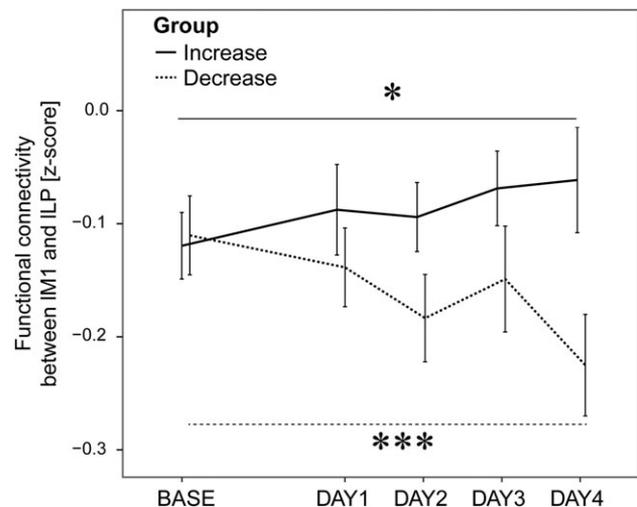


Figure 3. Change in functional connectivity between the left primary motor area (IM1) and the left lateral parietal region (LLP) during neurofeedback training. Functional connectivity averaged across subjects in each group (solid line: increased functional connectivity group,  $n = 18$ ; broken line: decreased functional connectivity group,  $n = 12$ ) as a function of training day (error bars: standard error). A mixed-effects model identified a significant interaction between group and day ( $t = 3.86$ ,  $P = 0.0014$ ). \*\*\* $P < 0.005$ , \* $P < 0.05$  according to post hoc analysis of the main effect of day for each group.

## Change in Resting-State Functional Connectivity

To compare the daily changes in resting-state functional connectivity between the 2 groups, we applied a mixed-effects model to the resting-state functional connectivities between IM1 and LLP and to the connectivity between MVN and DMN. We did not find any significant effect in any connectivity (for IM1-LLP, DAY:  $t = 0.41$ ,  $P = 0.67$ ; Group:  $t = 1.28$ ,  $P = 0.20$ ; DAY  $\times$  Group:  $t = -0.78$ ,  $P = 0.43$ ; for MVN-DMN, DAY:  $t = 1.20$ ,  $P = 0.23$ ; Group:  $t = -0.11$ ,  $P = 0.91$ ; DAY  $\times$  Group:  $t = 0.89$ ,  $P = 0.37$ ). Further, to investigate the change in connectivity across days in each group, we applied a mixed-effects model separately for each group. Consequently, we found a significant effect of training day in the “increased functional connectivity” group for the connectivity between MVN and DMN (“increased functional connectivity” group:  $t = 2.93$ ,  $P = 0.0045$ ; “decreased functional connectivity” group:  $t = 1.18$ ,  $P = 0.24$ ), but not for the connectivity between IM1 and LLP (“increased functional connectivity” group:  $t = -0.70$ ,  $P = 0.48$ ; “decreased functional connectivity” group:  $t = 0.43$ ,  $P = 0.66$ ). Specifically, functional connectivity between MVN and DMN increased from  $-0.26 \pm 0.058$  at BASE to  $-0.13 \pm 0.064$  on DAY4 in the “increased functional connectivity” group. Therefore, the direction of change in functional connectivity between MVN and DMN in the “increased functional connectivity” group was consistent with our aimed direction, the connectivity change in the neurofeedback sessions (Fig. 3), and that in a previous study (Megumi et al. 2015).

## Change in Cognitive Performance

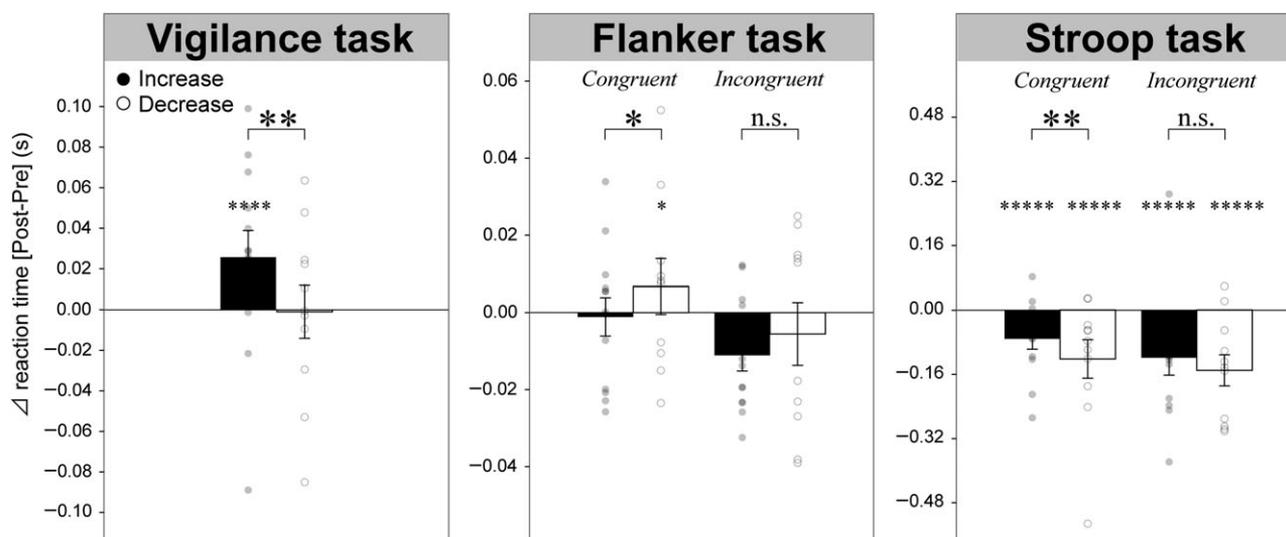
Figure 4 shows the changes in reaction time from the preneurofeedback to postneurofeedback training stage averaged across subjects in each group. Note that there was no significant difference in reaction time or error rate between the 2 groups for the preneurofeedback training stage (Supplementary Table 1). Owing to the absence of significant differences in task

performance before the training, we show only the changes in reaction time in Figure 4. However, the following statistical analyses were applied to raw reaction-time data without subtraction or averaging. To compare the changes in reaction time from the preneurofeedback to postneurofeedback training stage between the 2 groups, we applied a mixed-effects model to the reaction times in each task. As a result, the interaction effect between group and day was significant in PVT, EFT congruent, and CWST congruent (PVT:  $t = -2.72$ ,  $P = 0.0065$ ; EFT congruent:  $t = 2.41$ ,  $P = 0.016$ ; CWST congruent:  $t = -2.67$ ,  $P = 0.0075$ ), but not in EFT incongruent or CWST incongruent (EFT incongruent:  $t = 1.18$ ,  $P = 0.23$ ; CWST incongruent:  $t = -0.50$ ,  $P = 0.61$ ). These significant interactions suggest that the changes in reaction time from the preneurofeedback to postneurofeedback training stage were different between the groups.

Further, we applied a mixed-effects model to the reaction times separately for each group in PVT, EFT congruent, and CWST congruent. The main effect of training day was significant in the “increased functional connectivity” group in PVT and CWST congruent (PVT:  $t = -3.85$ ,  $P = 0.00013$ ; EFT congruent:  $t = 0.58$ ,  $P = 0.56$ ; CWST congruent:  $t = 6.93$ ,  $P < 0.0001$ ) and in the “decreased functional connectivity” group in EFT congruent and CWST congruent (PVT:  $t = 0.12$ ,  $P = 0.90$ ; EFT congruent:  $t = -2.52$ ,  $P = 0.011$ ; CWST congruent:  $t = 8.53$ ,  $P < 0.0001$ ). These results indicate that the change in the reaction time from the preneurofeedback to postneurofeedback training stage could be identified in the “increased functional connectivity” group in PVT and CWST congruent and in the “decreased functional connectivity” group in EFT congruent and CWST congruent. Although some of these main effects of day might have been affected by repetition of the same task, the interaction effects between the groups could not be explained by such repetition.

## Discussion

Using the connectivity neurofeedback training method, we experimentally manipulated the functional connectivity



**Figure 4.** Changes in cognitive performance from preneurofeedback to postneurofeedback training. All panels show changes in reaction time of the increased ( $n = 13$ ) and decreased ( $n = 11$ ) functional connectivity groups. Each marker (black or white circle) represents 1 subject; each bar represents each group's average (error bars: standard error). A mixed-effects model identified a significant interaction between group and training day (Vigilance:  $t = -2.72$ ,  $P = 0.0065$ ; Flanker congruent:  $t = 2.41$ ,  $P = 0.016$ ; Stroop congruent:  $t = -2.67$ ,  $P = 0.0075$ ). Among these tasks, in which interaction effects were significant, the main effect of day was significant in the increased functional connectivity group in Vigilance and Stroop congruent (Vigilance:  $t = -3.85$ ,  $P = 0.00013$ ; Stroop congruent:  $t = 6.93$ ,  $P < 0.0001$ ) and in the decreased functional connectivity group in Flanker congruent and Stroop congruent (Flanker congruent:  $t = -2.52$ ,  $P = 0.011$ ; Stroop congruent:  $t = 8.53$ ,  $P < 0.0001$ ). \*\*\*\* $P < 0.0001$ , \*\*\*\* $P < 0.0005$ , \*\* $P < 0.01$ , \* $P < 0.05$ , n.s.: not significant.

between IM1 and ILP and examined the change in performance from the preneurofeedback to postneurofeedback training stage. The functional connectivity in each group indeed changed in the aimed direction during the training (Fig. 3). Furthermore, we identified significant change in some cognitive performances between the groups (Fig. 4). These findings indicate that connectivity neurofeedback can induce the aimed direction of change in functional connectivity as well as induce a differential change in cognitive performance.

### Directions of Change in Reaction Times Dependent on the Tasks

We found significant change in some cognitive performances, but the directions of change in reaction time were different for each task. For example, the reaction times of the vigilance task increased in the “increased functional connectivity” group but the reaction times of the flanker task (*congruent*) increased in the “decreased functional connectivity” group.

Regarding the EFT, a previous study (Kelly et al. 2008) on brain-behavior relationships investigated the coefficient of variation (CV; standard deviation divided by mean) as an index of task performance. Thus, we could not predict the direction of change in reaction time for EFT. However, Kelly et al. (2008) showed that the functional connectivity between MVN and DMN is positively correlated with CV. Our additional analysis indicated that the directions of change in CV were consistent with those in the previous study, that is, CV increased from preneurofeedback to postneurofeedback training in the “increased functional connectivity” group and decreased in the “decreased functional connectivity” group, although their interaction effect did not reach a statistically significant level (Supplementary Text 1).

Regarding the CWST, a previous study (Liu et al. 2015) on brain-behavior relationships investigated the Stroop effect (mean reaction time of incongruent condition – mean reaction time of congruent condition) as an index of task performance. However, because they investigated the relationship between the regional homogeneity (ReHo) and Stroop effect, we also could not predict the direction of change in reaction time. Our additional analysis indicated that the change in Stroop effect was not significant (Supplementary Text 1). Furthermore, from Figure 4, we can easily assume that there are considerable learning effects on the reaction time in both groups, since the Stroop task may be more difficult than the other 2 tasks. However, even if there were learning effects, the significant interaction between group and day suggests that the reaction times were significantly and differentially changed from preneurofeedback to postneurofeedback training between the 2 groups. However, we could not conclude that neurofeedback training influenced the reaction time itself or the learning effect in the Stroop task (*congruent*).

Unlike the other 2 tasks, previous studies found a concrete relationship between reaction time in the vigilance task and functional connectivity. Thompson et al. (2013) divided their subjects into 2 groups according to fast or slow reaction time for PVT. They reported that the fast reaction time group showed more greatly decreased negative resting-state functional connectivity between MVN and DMN than did the slow reaction-time group. This finding is consistent with our results. Hinds et al. (2013) examined fMRI activities of MVN and DMN during PVT. Their subjects could rapidly respond to a stimulus when activity in a part of MVN (the supplementary motor area) increased and activity in DMN decreased. It is assumed that

DMN is more active than MVN when subjects are waiting for a stimulus in PVT, whereas MVN becomes more active than DMN when subjects respond to the stimulus. Based on these studies, as well as our own, one could hypothesize that subjects who have a more negative resting-state functional connectivity could more rapidly enhance MVN activity and suppress DMN activity, which leads to a shorter reaction time. However, further evidence is needed to verify this hypothesis.

### Difference in Behaviors During Training between Subject Groups

In the neurofeedback experiment, only the rewarded direction of change in functional connectivity was flipped during the neurofeedback training between the “increased functional connectivity” and “decreased functional connectivity” groups. Nevertheless, the changes in the reaction time of some tasks from the preneurofeedback to postneurofeedback training stage differed between the 2 groups (Fig. 4). This suggests that the change in functional connectivity influences the change in cognitive performance. However, factors other than the rewarded direction, which cannot be experimentally controlled, may have differed between the 2 groups and caused the difference in the change in cognitive performance. We examined these factors as follows.

#### Total Score during Training

The score was calculated according to the equivalent formula for the 2 groups (see Online Calculation of Feedback Score). The resulting total score may have differed between the groups, and this difference may have caused a difference in their motivation during training and thus a change in cognitive performance. Therefore, we compared the total score over the training in the increase group with the score in the decrease group (Supplementary Text 2). The total score averaged across subjects was  $14\,379 \pm 809$  (mean  $\pm$  95% confidence interval [CI]) for the “increased functional connectivity” group and  $13\,860 \pm 1448$  for the “decreased functional connectivity” group. We used a 2-sample t-test to compare the total score but did not obtain a significant difference between the groups ( $t = 0.68$ ,  $P = 0.50$ ).

#### Strategies Adopted by Subjects

We provided identical instructions to both groups, telling them that the disc (score) becomes bigger as subjects improve at producing the tapping imagery during the training. However, the actual strategies that were adopted by the subjects may have differed between the groups through their trial-and-error learning. The difference in strategy may have caused a difference in regional brain activity and thus affected subsequent cognitive performance. We conducted a postexperiment debriefing with 25 of the 30 subjects (13 subjects in the increase group and 12 subjects in the decrease group) and examined the differences in their strategy for the motor imagery (Supplementary Text 3 and Supplementary Table 2). We analyzed the reported strategies in 5 aspects: image category (items: kinesthetic, visual, or both), hand laterality (left, right, or both hands), tapping sequence (fixed or random), imagery with or without manipulated object (e.g., a subject imagined typing on a computer keyboard), and imagery with or without a rhythm (Supplementary Table 3). We counted the numbers of items across subjects and compared these numbers between the groups. We calculated the  $P$  value as the probability that these results would be

obtained if we separated subjects randomly (Supplementary Table 4). As a result, there was no significant difference in the numbers between the groups.

### Difference in the Activity of Target ROIs during Training Between the Groups

Because the aim of the current study is to control the functional connectivity but not to control the averaged activity in a specific ROI, it is important to check the changes in activities in the target regions. We applied a mixed-effects model to averaged activity in each target region in the same manner as our analysis of functional connectivity (see Change in Functional Connectivity during Training and Supplementary Text 4). As a result, we found a significant interaction effect of day on the LLP activation. This result may indicate that subjects altered only the activity in the LLP and that the change in temporal correlation between the target regions is an epiphenomenon. However, this was not the case owing to the following reason. We investigated whether subjects could get the information about the activity in the LLP from the feedback score to calculate the correlation between the feedback score and the activity in LLP (Supplementary Text 5). If there is no correlation between the feedback score and the brain activity in LLP, subjects could not have directly altered the activity in LLP through the training. As a result, we did not find a significant correlation between the feedback score and activity in the LLP (“increased functional connectivity” group:  $r = 0.016$ ,  $P = 0.23$ ; “decreased functional connectivity” group:  $r = -0.01$ ,  $P = 0.54$ ). These results indicate that subjects could not get information about activity in the LLP from the feedback score. Therefore, subjects altered the functional connectivity between the LLP and IM1, and the activity in the LLP might have been collaterally altered.

### Change in Resting-State Functional Connectivity

Our previous study (Megumi et al. 2015) showed the significant increase in the resting-state functional connectivity between the target ROIs (IM1 and LLP) from preneurofeedback to post-neurofeedback training. However, our current study failed to observe a significant change in the resting-state functional connectivity between the target ROIs. A possible reason is that the effect of neurofeedback training may have been smaller than in our previous study. In fact, our previous study showed an increase of about 0.2 in correlation between the 2 target ROIs during the training in comparison to about 0.1 in our current study. This change in correlation between ROIs might have been insufficient for generalization of the training effect from the training to rest periods.

By contrast, at the network level, we found a significant increase in resting-state functional connectivity between MVN and DMN from preneurofeedback to postneurofeedback training despite the smaller effect of neurofeedback training than that in our previous study. A possible reason is the difference in the number of voxels between ROI and network analyses: network-level ROIs have more voxels (about 5000 voxels) than target ROIs (IM1 and LLP: about 100 voxels). Correlation calculated from signal time courses averaged over the larger number of voxels is more reliable than that from smaller number of voxels in most cases. This may have helped us find a significant increase in resting-state functional connectivity between the 2 network-level ROIs. However, we did not observe a significant decrease in the resting-state functional connectivity between MVN and DMN in the “decreased functional

connectivity” group. Because the connectivity is negative between MVN and DMN in nature, further decreasing the negative connectivity may be difficult (e.g., changing correlation from  $r = -0.4$  to  $-0.6$ ) in comparison to increasing it (e.g., from  $r = -0.4$  to  $-0.2$ ). In fact, we confirmed that the distribution of the functional connectivity between DMN and MVN is positively skewed (skewness = 0.68), suggesting that probability of a decrease is less than that of an increase in correlation.

### Effect of the Initial Functional Connectivity on Training

We examined whether the differential changes in functional connectivity and cognitive performances between the 2 groups were induced by the difference in initial functional connectivity (Supplementary Text 6). At group level, we did not observe a significant difference in the initial functional connectivity (IM1-LLP) between the 2 groups ( $t$ -test,  $t = 0.20$ ,  $P = 0.84$ ). Thus, the initial difference unlikely explains the differential changes between the 2 groups. At individual level, we did not find any significant correlations between the initial functional connectivity and the change in functional connectivity and cognitive performances (see Supplementary Text 6 for details). These results indicate that the change in functional connectivity and cognitive performances were not induced by the difference in the initial functional connectivity between the 2 groups.

### Associations Among Change in Functional Connectivity During Training, Change in Resting-State Functional Connectivity, and Change in Cognitive Performance

We examined the associations among (1) the changes in functional connectivity of IM1-LLP during neurofeedback training, (2) the changes in resting-state functional connectivity of MVN-DMN, and (3) the changes in cognitive performance of the 3 tasks, in which the interaction between groups and days yielded significant effects. We analyzed data of the “increased functional connectivity” group, in which a significant change in resting-state connectivity of MVN-DMN was observed. Using linear regression, we conducted a moderation/mediation analysis. This displayed a significant effect of the change in functional connectivity during training on the change in reaction time of CWST *congruent* ( $\beta = -1.41$ ,  $SE = 0.61$ ,  $t = -2.29$ ,  $P = 0.044$ , adjusted  $R^2 = -0.22$ ) (Supplementary Text 7 and Supplementary Figure 1). This result suggests that the change in reaction time of CWST *congruent* was directly affected by changes in the functional connectivity during training rather than by changes in the resting-state functional connectivity. However, our moderation/mediation analysis shed light on only a fraction of many factors related to the connectivity neurofeedback. Further studies are required to verify the robust relationship between cognitive function and functional connectivity.

### Application of Connectivity Neurofeedback Training

Disturbances in regional or brain-wide functional connectivity have been reported for numerous neurological and psychiatric diseases (Fox and Raichle 2007; Broyd et al. 2009; Stam 2014; Fornito et al. 2015). These pathological disturbances have been related to the severity of cognitive dysfunctions in individual patients (He et al. 2007; Hawellek et al. 2011; Yahata et al. 2016). From this perspective, online fMRI neurofeedback (Sulzer et al. 2013) is expected to become a next-generation therapeutic tool (Esmail and Linden 2014; Stoeckel et al. 2014, Decoded Neurofeedback Project within the Strategic Research Program

for Brain Sciences [SRPBS]: <http://www.cns.atr.jp/decnefpro/>). In the future, connectivity neurofeedback training methods may contribute to a remedy for such disturbances and to improvement of impaired cognitive functions by regulating the functional connectivity rather than only the level of regional brain activity, as traditionally implemented by most neuromodulation techniques such as single-ROI-based neurofeedback, transcranial magnetic stimulation, and deep brain stimulation.

Our current study shows that connectivity neurofeedback can not only increase but also decrease functional connectivity. Therefore, connectivity neurofeedback shows potential for future therapeutic interventions against psychiatric and neurological disorders caused by not only hyperconnectivity but also hypoconnectivity. For example, in patients with Alzheimer's disease, functional connectivity is reduced between the right hippocampus and many component regions of the DMN, while connectivity increases between the left hippocampus and the right dorsolateral prefrontal cortex (Broyd et al. 2009). In patients with depression, functional connectivity is increased between the subgenual cingulate cortex and the DMN (Greicius et al. 2007; Broyd et al. 2009). In autism spectrum disorders, connectivity is reduced between the anterior and posterior DMN regions (Broyd et al. 2009).

Furthermore, our study suggests the possibility of developing a technique of neurofeedback manipulation to cancel out the behavioral change induced by previous methods of neurofeedback manipulation. This is important for ensuring safeguards in clinical applications of connectivity neurofeedback.

## Conclusion

In this study, using the connectivity neurofeedback training method, we tested the hypothesis that connectivity neurofeedback can induce the aimed direction of change in functional connectivity and cognitive performance. As a result, subjects could increase or decrease the functional connectivity between 2 brain regions, and cognitive performance was significantly and differentially changed from preneurofeedback to postneurofeedback training between the 2 groups. We did not find a significant difference in behaviors between the groups during the training, except for the rewarded direction of change in functional connectivity between the 2 regions. These findings suggest that connectivity neurofeedback can induce the aimed direction of change in functional connectivity as well as a change in cognitive performance.

## Supplementary Material

Supplementary data is available at *Cerebral Cortex* online.

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## Notes

*Conflict of Interest:* None declared.

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