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Genetic influences on prefrontal activation during a verbal fluency task in adults: A twin study based on multichannel near-infrared spectroscopy

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ABSTRACT

Near-infrared spectroscopy (NIRS) studies have reported that prefrontal hemodynamic dysfunction during executive function tasks may be a promising biomarker of psychiatric disorders, because its portability and noninvasiveness allow easy measurements in clinical settings. Here, we investigated the degree to which prefrontal NIRS signals are genetically determined. Using a 52-channel NIRS system, we monitored the oxy-hemoglobin (oxy-Hb) signal changes in 38 adult pairs of right-handed monozygotic (MZ) twins and 13 pairs of same-sex right-handed dizygotic (DZ) twins during a letter version of the verbal fluency task. Heritability was estimated based on a classical twin paradigm using structured equation modeling. Significant genetic influences were estimated in the right dorsolateral prefrontal cortex and left frontal pole. The degrees of heritability were 66% and 75% in the variances, respectively. This implies that the prefrontal hemodynamic dysfunction observed during an executive function task measured by NIRS may be an efficient endophenotype for large-scale imaging genetic studies in psychiatric disorders.

Abbreviations: DZ, dizygotic; FIQ, full-scale intelligence quotient; LFT, Letter Fluency Task; MZ, monozygotic; NIRS, near-infrared spectroscopy; rCBV, regional cerebral blood volume; SES, socioeconomic status; SNP, single nucleotide polymorphism; WAIS-R, Wechsler Adult Intelligence Scale-Revised; [oxy-Hb], concentration of oxyhemoglobin; [deoxy-Hb], concentration of deoxyhemoglobin.

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Introduction

It is well known that there is a substantial genetic influence in major psychiatric disorders, such as schizophrenia and bipolar disorder. The identification of endophenotypes in psychiatric disorders is becoming an increasingly pressing matter in the elucidation of their genetic underpinnings (Gottesman and Gould, 2003). An endophenotype is a type of biomarker that is both associated with a specific psychiatric disorder and is genetically influenced. Endophenotypes are thought to link complicated pathways from genotypes to phenotypes.

Event-related potentials, such as P50, P300, and mismatch negativity, have been investigated as candidate endophenotypes of schizophrenia and other psychiatric conditions (Bramon et al., 2004; Hall et al., 2006; Umbricht and Krljes, 2005). Working memory and executive performance are also thought to contribute to the psychopathology of schizophrenia. Abnormal functioning of the dorsolateral prefrontal cortex was found not only in patients with schizophrenia, but also in their unaffected siblings (Callicott et al., 2003). More recently, several functional magnetic resonance imaging (fMRI) studies revealed a genetic contribution to prefrontal blood oxygenation level-dependent (BOLD) signal changes in response to a working memory task (Blokland et al., 2008, 2011; Koten et al., 2009). These findings support the contention that such characteristics in cognitive neuroscience might serve as an endophenotype of schizophrenia.

Multichannel near-infrared spectroscopy (NIRS) is a functional neuroimaging modality that enables the noninvasive detection of the concentrations of oxyhemoglobin ([oxy-Hb]) and deoxyhemoglobin ([deoxy-Hb]), which are assumed to reflect the regional cerebral blood volume (rCBV). NIRS is suitable for clinical application, particularly in psychiatric disorders, because it has a relatively low cost, is easy to set up, the subject can be examined in a natural sitting position, and its measurements are relatively insensitive to motion artifacts (Takizawa et al., 2008).

NIRS studies using the letter version of the verbal fluency task (LFT) as a cognitive activation task have revealed an LFT-related increase in prefrontal [oxy-Hb] among healthy subjects (Herrmann et al., 2003; Kameyama et al., 2004). Frontal-task-related NIRS signals are being vigorously investigated as potential clinically applicable biomarkers. Decreased or abnormal LFT-related brain activation was found among patients with a variety of psychiatric disorders, including schizophrenia (Kubota et al., 2005; Suto et al., 2004; Takizawa et al., 2008) and mood disorders (Herrmann et al., 2004; Kameyama et al., 2006; Matsuo et al., 2000). Individuals with pervasive developmental disorders (Kuwabara et al., 2006) and their unaffected siblings (Kawakubo et al., 2009) also showed a decreased hemodynamic response compared with individuals with typical development.

Several previous studies also reported that polymorphisms in the catechol-*O*-methyltransferase (*COMT*) and sigma-1 receptor genes were associated with the variations in prefrontal hemodynamic response observed among patients with psychiatric disorders, such as schizophrenia (Takizawa et al., 2009a,b) and panic disorder (Tanii et al., 2009). Those results imply that there are genetic influences on prefrontal activation as measured by NIRS.

In the present study, we investigated the heritability of LFT-related prefrontal hemodynamic responses, as measured by NIRS, in healthy twins using a conventional twin study paradigm. To our knowledge, this is the first twin NIRS study to further our understanding of the genetic contribution to the variation in brain function, which might deepen our interpretation of individual differences in brain processing and vulnerability to brain disorders.

Materials and methods

Participants

This study was performed as a part of a large-scale neuroimaging study on healthy twins (Todai-TWIN). Fifty-one same-sex twin pairs who had been reared together (102 participants) were recruited via newspaper advertisements and participated in the study. All participants were right-handed according to the Edinburgh Inventory (Oldfield, 1971) and were native Japanese speakers. Twins were screened for significant medical conditions, traumatic brain injuries with loss of consciousness for more than 5 min, current use of medication that was likely to affect cognition, history of neurological and psychiatric disorders, history of alcohol and illicit drug abuse, and family history of axis I psychiatric disorders in their first-degree relatives. The zygosity of 76 twin pairs (74.5%) was confirmed genetically. To do this, DNA extracted from peripheral leukocytes was genotyped using the Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA). The zygosity of the remaining twin pairs was determined using a questionnaire composed of 3 questions that can be used to diagnose zygosity with more than 90% accuracy (Ooki et al., 1990). These analyses revealed that 38 pairs were monozygotic (MZ) (35 females and 3 males) and 13 pairs were dizygotic (DZ) (12 females and 1 male).

Socioeconomic status (SES) was assessed using the Hollingshead scale (Hollingshead, 1957). Full-scale intelligent quotient (FIQ) was estimated using the short version of the Japanese Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Misawa et al., 1993; Wechsler, 1981). The distance between T3 and T4 along the scalp was measured and considered as the index of head size. The demographic data are summarized in Table 1. All twins were scanned on the same day as their cotwins. The ethics committee of the Faculty of Medicine, the University of Tokyo, approved this study (No. 630-(6) & 2450-(2)).

Activation task

Participants were asked to seat themselves on a chair with their eyes open and to minimize bodily movements throughout the NIRS measurements. The procedure of cognitive activation was the same as that used in previous studies (Nishimura et al., 2009; Takizawa et al., 2008), which included a 30-s pre-task baseline, a 60-s verbal fluency task (letter version), and a 70-s post-task baseline (Fig. 1). Prepared vocal instructions were given to the participants during the procedure. During the pre-task and post-task periods, the subjects were instructed to repeat a train of Japanese vowels (/a/, /i/, /u/, /e/, and /o/). This was intended to prevent task-unrelated contemplation and to record brain activities due to vocalization. During the verbal fluency task period, participants were instructed to generate as many Japanese words beginning with a given syllable as possible. The initial syllables were changed every 20 s during the 60-s task period, to reduce wordless time. The responses generated by the participants were assessed, and the number of correct words generated was defined as a measure of task performance.

NIRS measurements

Relative changes in [oxy-Hb] and [deoxy-Hb] were measured using 695 nm and 830 nm wavelengths of near-infrared light. The 52-channel NIRS machine (ETG-4000, Hitachi Medical Co.) used in this study included 16 emitter probes and 15 detector probes that were fixed alternately with thermoplastic 3 × 11 shells, which constitutes 52 adjacent emitter–detector probe pairs separated by 3.0 cm (henceforth termed "channel (Ch)"). These probes were placed over a subject's bilateral prefrontal regions so that the lowest 11 probes were located along the Fp1–Fp2 line according to the international 10–20 system in electroencephalography. This arrangement of the probes can measure [oxy-Hb] and [deoxy-Hb] from bilateral prefrontal and superior temporal cortical surface regions (Fig. 2). For the purpose of estimating the cortical localization of each channel, the use of the virtual registration method (Tsuzuki et al., 2012; Tzourio-Mazoyer et al., 2002) enabled the probabilistic registration of NIRS data onto the Montreal Neurological Institute (MNI) coordinate space without measurement of probe positions or the use of MRI (Fig. 2). The placement of the emitter and detector probes on the scalp at a distance of 3.0 cm from each other enables the detection of [Hb]

Table 1
Demographic data of participants and influences of the genetic component and common environmental component on the variance observed for each item. The first 2 columns show the mean (± SD) of each item. The *r* values and 95% confidence intervals of the correlation between monozygotic and dizygotic cotwins for each item are shown in the third and the fourth columns. The right-most 3 columns show the estimated percentage of influence by additive genetic (A), common environmental (C), and unique environmental (E) components on the variance observed for each item from the most efficient model. Only genetic influence onto FIQ reached significance ($p = 0.004$, asterisk). Note that the homoscedasticity requirement was unsatisfied as for task performance. MZ, monozygotic twins; DZ, dizygotic twins; SES, socioeconomic status.

	Means (SD)		Correlations (95% CIs)		A, C, and E (%) estimates		
	MZ (N = 76)	DZ (N = 26)	MZ	DZ	A	C	E
Age (years)	35.3 (10.7)	30.6 (8.5)					
SES	2.3 (0.7)	2.0 (0.6)	0.49 (0.20, 0.70)	0.07 (−0.50, 0.60)	46	0	54
Education (years)	14.6 (1.8)	15.3 (1.5)	0.56 (0.29, 0.75)	0.43 (−0.16, 0.79)	0	53	47
Task performance	14.5 (4.0)	13.7 (3.3)	0.51 (0.23, 0.72)	−0.24 (−0.70, 0.36)	(46)	(0)	(54)
FIQ	105 (12)	100 (15)	0.69 (0.47, 0.83)	0.15 (−0.44, 0.65)	72*	0	28
T3–T4 interval (cm)	29.1 (1.3)	28.7 (1.1)	0.77 (0.60, 0.88)	0.29 (−0.31, 0.73)	74	0	26

changes on the surface of the cerebral cortex beneath the probes (Hock et al., 1997; Okada and Delpy, 2003; Toronov et al., 2001).

The sampling rate of the data was 0.1 s. During the analysis of task-related relative [Hb] changes, a first-order correction was performed to exclude task-unrelated changes. The mean value recorded across the last 10 s of the pre-task period and the mean value recorded across the last 10 s of the post-task period were defined as the pre- and post-task baselines, respectively: a linear fitting was applied to the data between the two baselines. Subsequently, a moving averaging with a 5-s window was performed to remove short-term motion artifacts. Because moving averaging cannot smooth all the artifacts, the automatic algorithm described in the Supplementary Material of another of our articles (Takizawa et al., 2008) was used to reject quantitatively artifact-contaminated data for each channel. The average number of rejected data in each channel was 9.0 (range, 1–32).

Statistical analyses

The assignment of the twins as number 1 or 2 was performed according to the birth order, if available, indicated in their maternal and child health handbooks (the official birth record in Japan) (92%). When birth order was unavailable, the assignment was performed according to their declaration.

As cognitive-task-related [oxy-Hb] changes are correlated more strongly with blood oxygenation level-dependent signal measured by fMRI than are [deoxy-Hb] changes (Strangman et al., 2002), we focused on [oxy-Hb] here. Because the verbal fluency task draws on a blocked design paradigm, mean [oxy-Hb] changes during the task period were calculated for each channel compared with the pre- and post-task baselines; this mean [oxy-Hb] change was considered as the task-related brain activation (Fig. 1).

First, we calculated the means and variances of demographic variables and task-related brain activations in each channel for 4 groups (MZ twin1, MZ twin2, DZ twin1, and DZ twin2). The equality of means and homoscedasticity across groups, which is the presupposition of genetic modeling, was tested using one-way analysis of variance (ANOVA) and Levene's test. Subsequently, we calculated the correlations of observed data among MZ pairs and DZ pairs to compare the similarity between MZ cotwins with that between DZ cotwins.

Genetic modeling was performed according to classical structured equation modeling in twin studies (Neale and Cardon, 1992). An observed phenotypic value P is decomposed into a linear sum of an underlying additive genetic component (A), a genetic dominance component (D), a common environmental component (C), and a unique environmental component (E). It is known that the contributions of common environmental and genetic dominance cannot be estimated at the same time when data from twins who were reared apart are unavailable (Neale and Cardon, 1992). Therefore, we decided to adopt models containing A, C, and E components (ACE model). This may underestimate, but not overestimate, the overall genetic contribution, which is the sum of additive and dominant genetic components.

Assuming all variables are scaled as deviations from zero, we obtain:

$$P = aA + cC + eE.$$

Such decomposition is possible using structured equation modeling with the following assumptions: the observed correlation between data

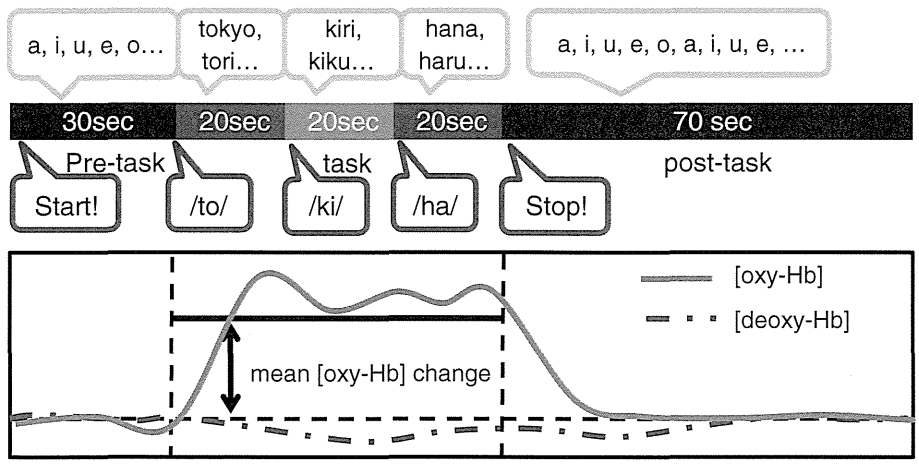

Fig. 1. Design of the Letter Fluency Task (LFT). The LFT is a Japanese version of the verbal fluency task, which includes a 30-s pre-task baseline, a 60-s verbal fluency task, and a 70-s post-task baseline. In the pre- and post-task baseline periods, the subjects were instructed to repeat Japanese vowels (/a/, /i/, /u/, /e/, and /o/) aloud. During the task period, they were instructed to generate as many Japanese words beginning with a designated syllable as possible. LFT-related [oxy-Hb] changes were calculated as the mean relative [oxy-Hb] change during the 60 s of LFT task period compared with the [oxy-Hb] at the pre- and post-task baselines.

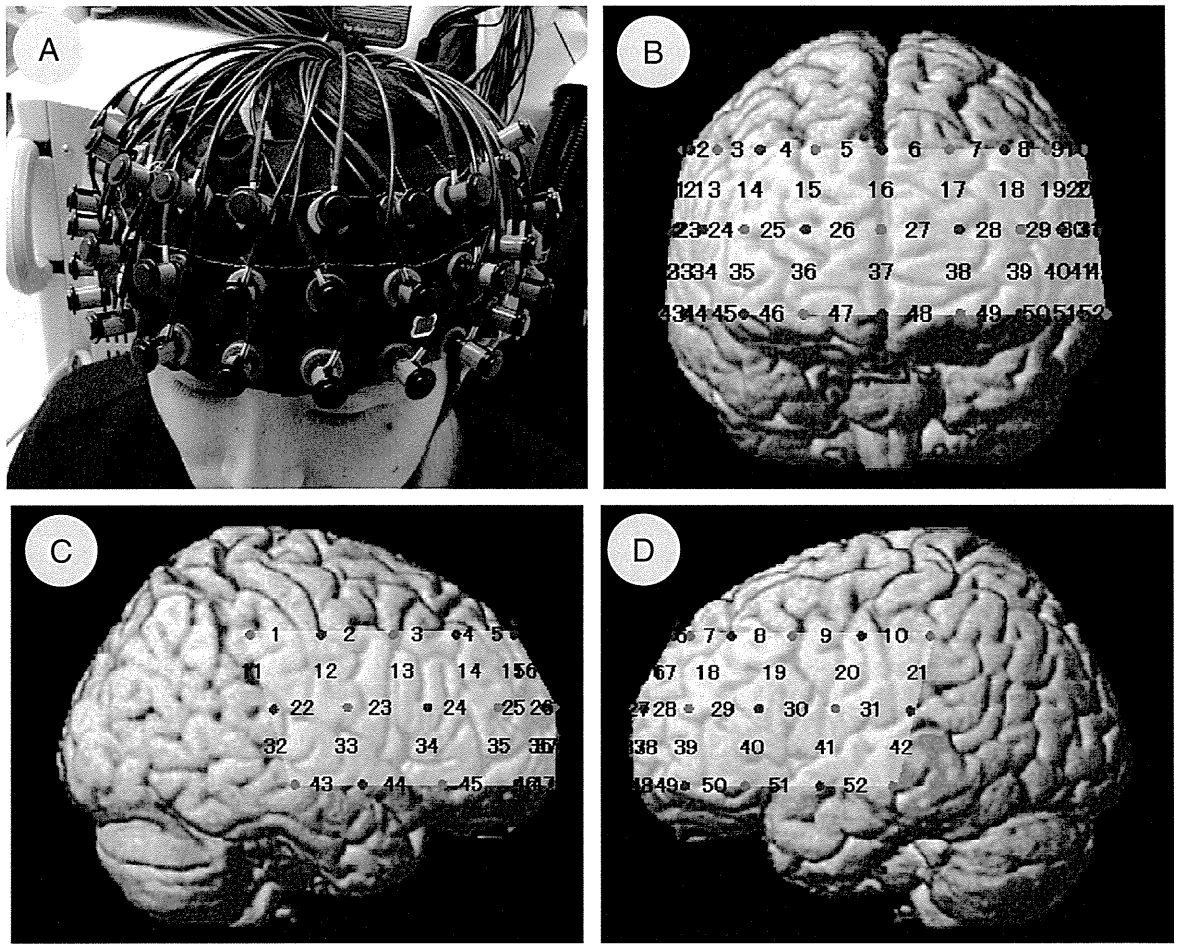

Fig. 2. Probe setting and measurement points of 52-channel near-infrared spectroscopy (NIRS). (A) Placement of the NIRS system over a subject's bilateral frontal regions. (B–D) The 52 measuring points (channels) of the NIRS system are superimposed on a 3D-reconstructed cerebral cortical surface from the Montreal Neurological Institute (MNI) average MRI image (B, frontal view; C, right view; D, left view). Channels are numbered from 1 to 52 from the top-right to bottom-left positions. The red and blue spots represent the placement of emitter and detector probes, respectively.

from twin pairs is caused by their shared genes and/or their common environment. The correlations of common environmental components between cotwins, including socioeconomic status, the rearing style of their parents, and living environment during childhood were fixed as 1 in both MZ and DZ pairs, as such factors are fully shared by twin pairs as long as they are reared together. The correlation of additive genetic factors between cotwins was 1 for MZ pairs, as they share 100% of their genes, and 0.5 for DZ twin pairs, as they share, on average, 50% of their genes. Unique environmental factors are defined as being uncorrelated between cotwins. Subsequently, genetic influences were estimated in cases in which MZ twins were significantly more similar with each other than were DZ twins, because such differences are unexplainable based only on common environmental factors. The path diagram of the ACE model is shown in Fig. 3.

The variances of the latent variables A, C, and E are standardized as unity, and the path coefficients a, c, and e are free parameters, to be estimated. Let Var(X) stand for the variance of X and Cov(X, Y) stand for the covariance between X and Y. In addition, let P_{MZ1}, P_{MZ2}, P_{DZ1}, and P_{DZ2} be the observed phenotypic measures in MZ twin1, MZ twin2, DZ twin1, and DZ twin2. The following equations are derived from the path diagram:

$$\mathrm{Var}(P_{MZ1}) = \mathrm{Var}(P_{MZ2}) = \mathrm{Var}(P_{DZ1}) = \mathrm{Var}(P_{DZ2}) = a^2+c^2+e^2,$$

$$\mathrm{Cov}(P_{MZ1}, P_{MZ2}) = a^2+c^2, \text{ and}$$

$$\mathrm{Cov}(P_{DZ1}, P_{DZ2}) = 0.5a^2+c^2.$$

Using the equations provided above, parameters a, c, and e are estimated by fitting them to observed variances and covariances of phenotypic values by using a maximum likelihood method. The significance of genetic effects was tested by assessing whether dropping factor A from the ACE model (i.e., assuming $a = 0$, called CE model) resulted in a significant increase in the goodness-of-fit chi-squared value. The significance of common environmental effects was tested similarly by dropping factor C from the ACE model (i.e., assuming $c = 0$, called AE model). The efficiency of the full ACE model relative to that of its submodels (i.e., AE model, CE model, and E model, where $a = c = 0$ is assumed) was compared according to the Akaike information criterion (AIC) (Akaike, 1973), and parameters that were calculated from the most efficient model were considered to be the final estimates. Heritability is defined as the rate of the variance of genetic origin among the total variance in observed phenotypic values. Thus, we obtain:

$$\text{heritability} = \frac{a^2}{a^2 + c^2 + e^2}.$$

Similarly, the rates of the contribution from common environmental and unique environmental components are defined as c^2 and e^2 divided by $a^2 + c^2 + e^2$, respectively. The observed phenotypic measures of the ACE model included (1) demographic data (years of education, FIQ,

SES, T3–T4 interval, and task performance) and (2) task-related brain activation in each channel.

In addition, to further corroborate the genetic contribution, we performed 3 confirmatory analyses using the data from the channels from which a significant genetic influence was estimated. First, there were disproportionately more female participants than male participants in this study. Hemodynamic activation in response to cognitive tasks has been reported previously as being greater in males than in females (Kameyama et al., 2004). Therefore, we recalculated the genetic contribution to the brain activation in those channels using data that excluded male participants. Second, to exclude potential confounding factors, we controlled for possible effects of age, years of education, FIQ, SES, head size, and task performance on brain activation in female participants by including their data as covariates. Stepwise multiple linear regressions were performed with a probability of F for conservative entries and removal criteria of 0.05 and 0.10, respectively. In addition, genetic influences were estimated as for the residual values after removing the effect from those potential confounding factors. Third, to rule out the possibility that the results were affected by the existence of outliers, we performed outlier detection using a generalized Mahalanobis distance (Mahalanobis, 1936) and re-estimated genetic contribution using female data that excluded outliers.

For those channels in which genetic influences were confirmed using the procedure described above, we calculated the correlation of the [oxy-Hb] signals at each sampling point during the 60-s task period (601 points at 0.1-s intervals) between monozygotic and dizygotic cotwins. A genetic modeling analysis was applied to estimate the time course of genetic influences on the [oxy-Hb] change during the task period. The statistical analyses were performed using the software package SPSS Amos, Ver. 20.0 (IBM Corp.).

Results

Demographic data

No significant differences in the mean values of all demographic variables (age at measurement, years of education, FIQ, SES, head size as measured by the T3–T4 interval, and task performance) were found across the 4 groups (MZ twin1, MZ twin2, DZ twin1, and DZ twin2). Homoscedasticity was maintained in all demographic variables, with the exception of task performance ($p = 0.03$). Greater correlations were found between MZ cotwins than between DZ cotwins regarding FIQ, SES, T3–T4 interval, and task performance. In structured equation modeling, genetic influences on those demographic variables were estimated. However, dropping the genetic factor from the ACE model resulted in a significant increase in the goodness-of-fit chi-squared value only for FIQ ($p = 0.003$). Because moderate correlations were found both in MZ and DZ cotwins, the insignificant ($p = 0.29$) influence of the common environmental component was estimated based on years of education.

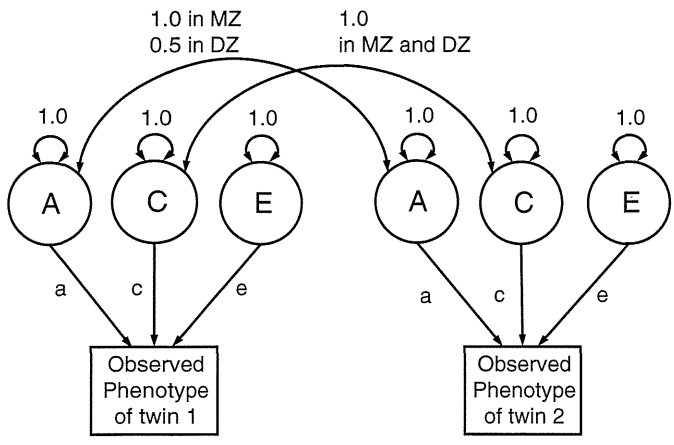

Fig. 3. Path diagram of the ACE model. According to the convention, circles represent latent variables, rectangles represent observed variables, two-way arrows represent variances and covariances, and one-way arrows represent causal influences. MZ, monozygotic twins; DZ, dizygotic twins; A, additive genetic factor; C, common environmental factor; E, unique environmental factor.

NIRS results

Grand average waveforms of [oxy-Hb] during cognitive tasks were similar between the groups (Fig. 4). No significant differences in the mean values for all 52 channels across the 4 groups were found regarding task-related brain activation. Homoscedasticity in task-related activation across groups was achieved in most channels, with the exception of Ch9 ($p = 0.04$) and Ch30 ($p = 0.01$).

As illustrated in Fig. 5, a moderate-to-strong correlation of task-related brain activation was found between MZ cotwins in channels covering the bilateral frontal poles and the right dorsolateral prefrontal cortex. Correlations reached significance for Ch1, 10, 12, 22, 24–26, 28, 33–37, 39, 40, 42, 44–50, and 52 (median p value is 0.007). Moreover, the observed correlations between DZ cotwins fluctuated because of smaller sample size. Although the correlations were weaker between DZ cotwins in most prefrontal regions, they were stronger than the correlations between MZ cotwins in some channels, including those corresponding to the ventrolateral PFC (cf. Broca's area) and right superior temporal gyrus, among which only Ch11, 20, and 22 reached significance (the median p value was 0.03). The variance observed in task-related brain activation was best explained by models containing additive genetic components (i.e., the ACE model or the AE model) in 18 channels (Ch 1, 8, 14, 16, 21, 24, 27, 33, 34, 37, 39, 41, 42, 44, 47, 48, 49, and 50). As shown in Fig. 6, the estimated heritability in those channels ranged from 23 to 75% (mean, 43%). Among those channels, significant genetic influences were found in 2 channels corresponding to the right dorsolateral prefrontal cortex (Ch24, $p = 0.02$) and the left frontal pole (Ch48, $p < 0.01$), respectively. Genetic contribution to the observed variance in task-related brain activation was estimated as being 66% and 75% of the variances calculated for these 2 channels, respectively. None of the channels were significantly influenced by common environmental components.

We performed additional confirmatory analyses. First, the significant genetic contribution observed in the 2 channels remained almost unchanged when data from male participants were excluded (62% of the variance in Ch24, $p = 0.03$; 75% of the variance in Ch48, $p < 0.01$). Second, a stepwise multiple regression analysis revealed that 5.4% and 5.8% of total variance in brain activation in Ch 24 and Ch 48 were explained by age ($\beta = -0.23$, $p = 0.03$ for Ch24; $\beta = -0.24$, $p = 0.03$ for Ch48). After removing the effect of age, the significant genetic contributions were retained (59% of the variance in Ch24, $p = 0.04$; 73% of the variance in Ch48, $p < 0.01$). Other demographic factors did not exhibit significant correlations with brain activation in those channels. Third, a Mahalanobis outlier analysis detected 1 significant outlier in MZ twin pairs in each channel. The estimation of correlations using data that excluded outliers revealed that genetic contributions were essentially unchanged and remained significant (68% of the variance in Ch24, $p < 0.01$; 66% of the variance in Ch48, $p = 0.01$).

The [oxy-Hb] signals in each sampling point during the 60-s task period at 0.1-s intervals in Ch24 and Ch48 were analyzed further. The time course of correlation coefficients of [oxy-Hb] signals during the task period in Ch24 and 48 between monozygotic and dizygotic twin pairs is shown in Fig. 7. Throughout the task period, the correlation was greater among MZ twins compared with DZ twins. A genetic modeling analysis revealed that genetic factors accounted for 40–80% of the total variance in the [oxy-Hb] signals during most of the task period. Regarding Ch48, which recorded the strongest genetic influence, the time course of the estimated heritability of [oxy-Hb] signals during the task period is illustrated in Fig. 8.

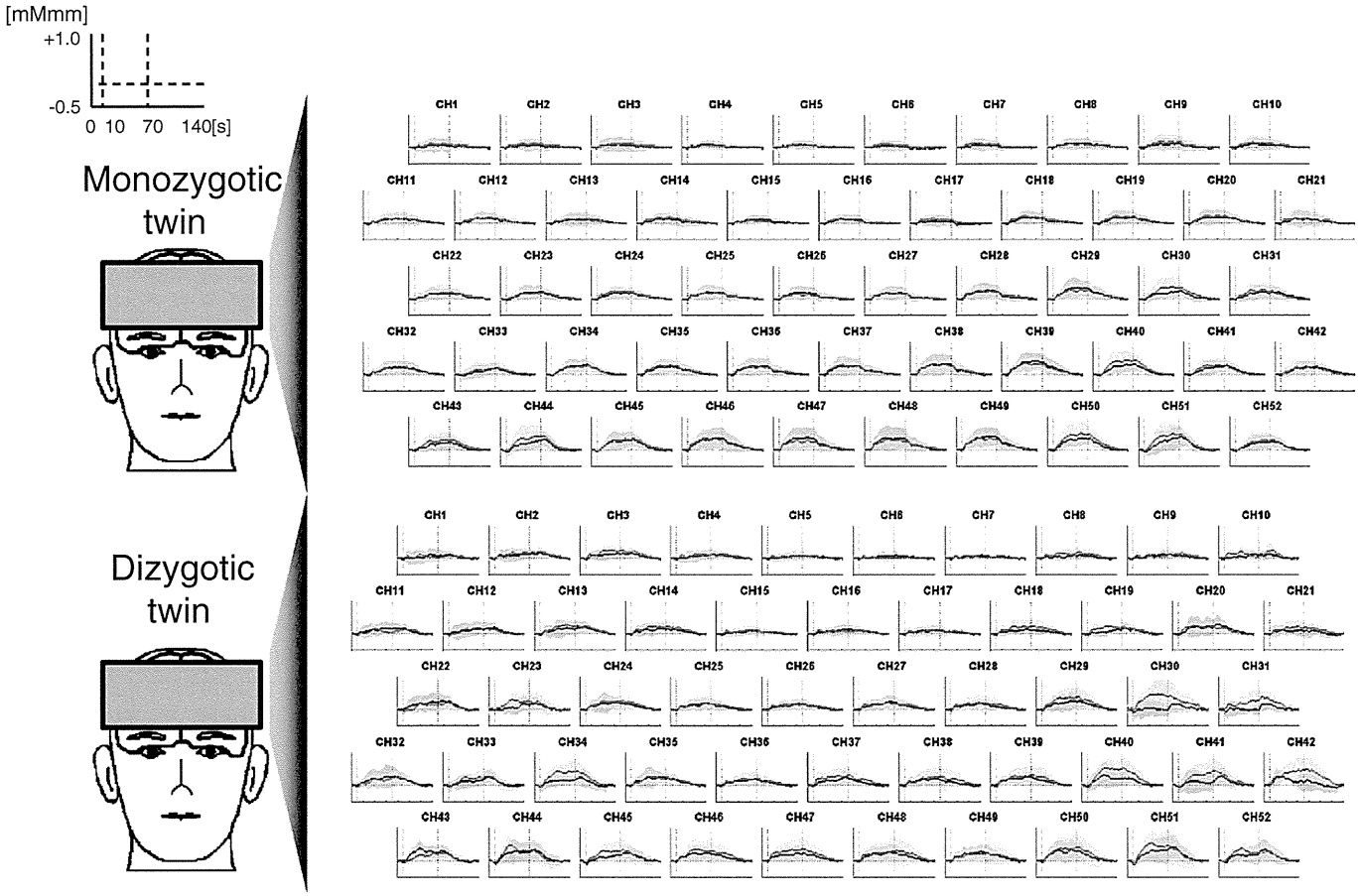

Fig. 4. Grand average waveforms in monozygotic and dizygotic twins. [oxy-Hb] changes during cognitive activation are presented as grand average waveforms with their standard deviation in 52 channels in twin1 (blue lines with light blue bands) and twin2 (red lines with pink bands).

Fig. 5. Correlation of letter fluency test (LFT)-related brain activation between monozygotic twin pairs and dizygotic twin pairs.

Discussion

This is the first twin NIRS study to investigate the heritability of prefrontal activation during a verbal fluency task. A significant genetic influence on prefrontal NIRS signals was detected even after controlling for potential confounding factors. In particular, the frontopolar and dorsolateral prefrontal subregions were more influenced by genetic factors. The "imaging genetics" approach has been used to identify susceptibility genes for heterogeneous psychiatric disorders (Meyer-Lindenberg, 2012). For this purpose, the sample size of the data should be very large. As NIRS is portable and its measurement is easier compared with other neuroimaging modalities, such as MRI, NIRS may serve as an efficient endophenotype for large-scale imaging genetic studies in psychiatric disorders.

Heritability of LFT performance and task-related brain activation

The LFT is a version of the verbal fluency task in which participants generate as many words beginning with given phonological cues as possible. The LFT is supposed to recruit executive function, including self-initiated retrieval of words from long-term memory storage, working memory capacity to keep track of the aforementioned items, and inhibition of the habitual behavior of treating words according to their meaning (Henry and Crawford, 2004; Perret, 1974). Earlier neuropsychological studies have shown that about 50% of individual variance in the number of words generated during this task is accounted for by genetic factors (Bratko, 1996; Vandenberg, 1962), which is comparable to the estimated heritability of LFT task performance (46% of the variance) calculated in this study.

The LFT is thought to reflect prefrontal function, particularly that of the dorsolateral prefrontal cortex (DLPFC), as assessed in lesion studies (Henry and Crawford, 2004) and in functional imaging studies (Frith et al., 1991; Phelps et al., 1997). Previous multichannel NIRS studies have replicated the results of brain activation measured based on the [oxy-Hb] increase detected in a broad prefrontal area, including both the right and left DLPFC and the frontal pole (Brodmann area 10) (Herrmann et al., 2006; Kameyama et al., 2004; Takizawa et al., 2008).

The results of the present study suggest that a substantial portion of LFT-related [oxy-Hb] changes in the frontal pole and the right dorsolateral prefrontal cortex are genetically determined. Time course analysis indicated sustained genetic influence on the [oxy-Hb] changes throughout the task period. The genetic influence on brain activation might not be mediated by task performance because most NIRS reports, including the present study, found no significant correlation between task performance and the brain activation in those channels (Herrmann et al., 2003; Kono et al., 2007; Pu et al., 2008). In contrast, the *r* values of the correlation of brain activation between MZ twins in the ventrolateral PFC were not high. This suggests that the brain activation in that area is substantially influenced by environmental (i.e., nongenetic) factors. Further investigations of the environmental contributions to these brain activations are needed.

Two fMRI studies reported the genetic contribution of BOLD signal changes using a working memory task. The first group used a digit memory task with arithmetic distraction in 10 families with male MZ twins and an additional non-twin brother, and genetic influences were implicated in regions including the inferior frontal gyrus and the anterior cingulate cortex (Koten et al., 2009). The second group used an N-back task in 319 healthy MZ and DZ twins and found that 40–65% of the

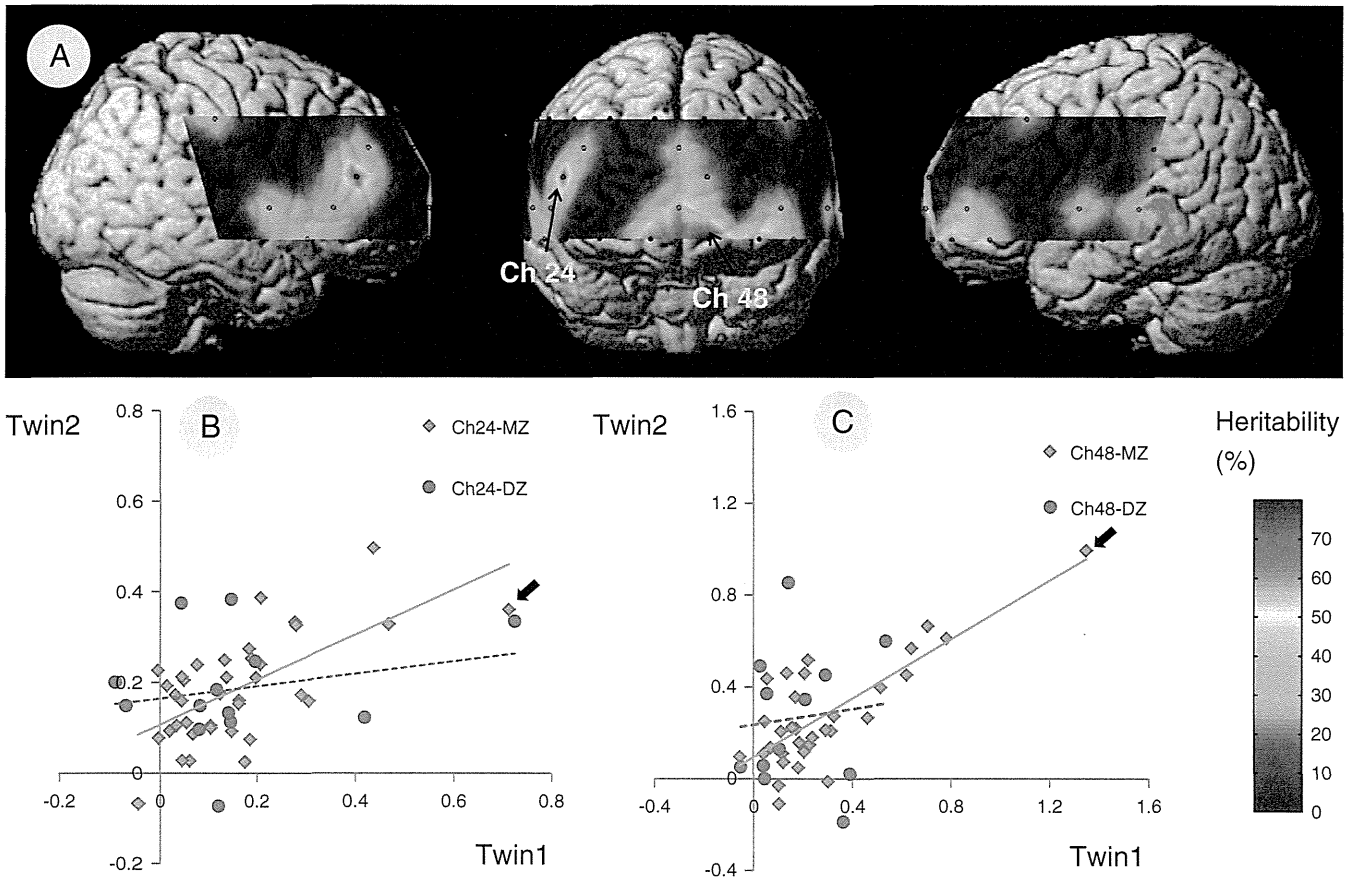

Fig. 6. Estimated genetic influences on LFT-related mean [oxy-Hb] change during the LFT. (A) Topographical map of estimated heritability. Channel 24 (right DLPFC) and Ch 48 (left frontal pole) were significantly influenced by genetic factors ($p < 0.05$). (B) and (C) Scatter plot of LFT-related mean [oxy-Hb] change in each twin pair in Ch 24 and Ch 48. Blue squares represent monozygotic (MZ) data and red circles represent dizygotic (DZ) data. Blue and red lines are linear regression lines of MZ and DZ data, respectively. Black arrows designate twin pairs that were judged as outliers in the Mahalanobis outlier analysis.

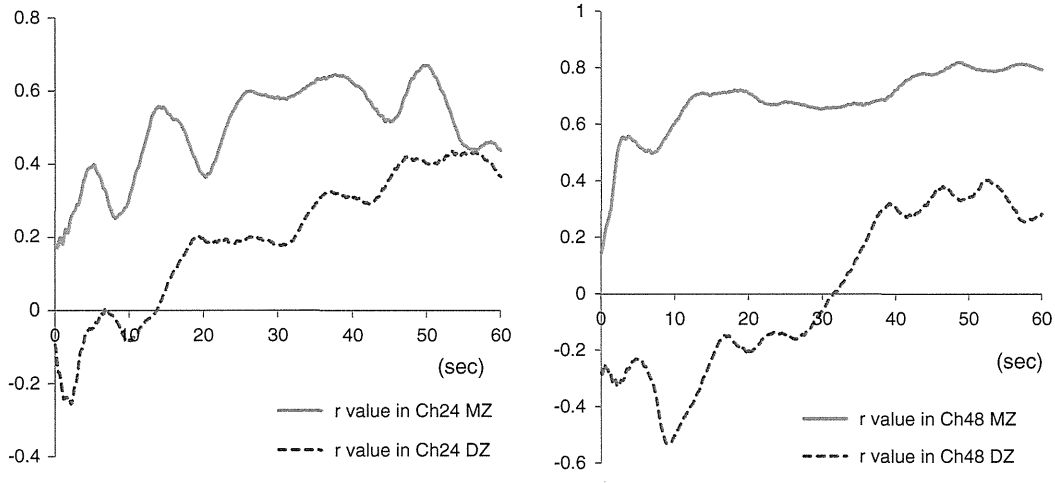

Fig. 7. Time course of the *r* values of correlation coefficients of [oxy-Hb] signals during the 60-s task period in Ch24 and Ch48 between monozygotic and dizygotic twin pairs. Blue solid lines and red dotted lines represent *r* values in monozygotic and dizygotic twins, respectively.

variance in the task-related BOLD signals was accounted by genetic factors in the regions including the bilateral inferior, middle, and superior frontal gyri (Blokland et al., 2008, 2011). The results of the present study further support the contention that the prefrontal brain activities underlying executive function are partially heritable.

Prefrontal NIRS signals activated by LFT as a candidate endophenotype of psychiatric disorders

Verbal fluency impairment is associated with various psychiatric disorders, including schizophrenia (Bokat and Goldberg, 2003), bipolar disorder (Martinez-Aran et al., 2004), and unipolar depression (Henry and Crawford, 2005). Altered brain function has been detected in several groups of patients with schizophrenia during an LFT; however, the findings of regions are inconsistent. In comparison with healthy controls, patients with schizophrenia have been reported to display reduced brain activation in the left DLPFC (Curtis et al., 1998), loss of deactivation in the superior temporal cortex (Frith et al., 1995), deactivation of the precuneus (Spence et al., 2000), and loss of left frontal dominance (Weiss et al., 2004). This might be due to the small size of the samples included in each study. NIRS studies consistently reported reduced or abnormal prefrontal brain activation in the bilateral DLPFC and in the frontal pole in patients with depression and schizophrenia compared with healthy controls (Suto et al., 2004; Takizawa et al., 2008), even if their task performances were matched. These neurophysiological disturbances might be the underlying neural basis of the executive-function impairment observed in psychiatric disorders.

In addition, it has been reported that executive performance as assessed by LFT is associated with two genetic polymorphisms that are known to increase the risk of developing schizophrenia. One such example is the Val^{158}Met polymorphism located in the *COMT* gene. Carriers of the Val allele (higher enzymatic activity) compared with carriers of the Met/Met genotype (low enzymatic activity) reportedly exhibit low performance in various executive and visuospatial tasks, including the LFT (de Frias et al., 2005). Another example is the A allele of the SNP4 polymorphism located in the metabotropic glutamate receptor-modulating synaptic glutamate gene, which is a potential candidate gene for schizophrenia and is associated with lower performance in the LFT (Egan et al., 2004).

Based on those evidences and on the heritability of brain activation during an LFT observed in this study, the prefrontal hemodynamic abnormality reported among patients with schizophrenia and mood disorder in previous NIRS studies can be considered as a candidate endophenotype for those disorders. However, caution should be taken, because the variance among the healthy population and differences between psychiatric patients and healthy controls might not necessarily have the same etiology. To confirm that the differences in neural correlates during an LFT observed between healthy controls and psychiatric patients are also genetically determined, evidence from family studies of individuals with psychiatric disorders and their unaffected siblings should be gathered.

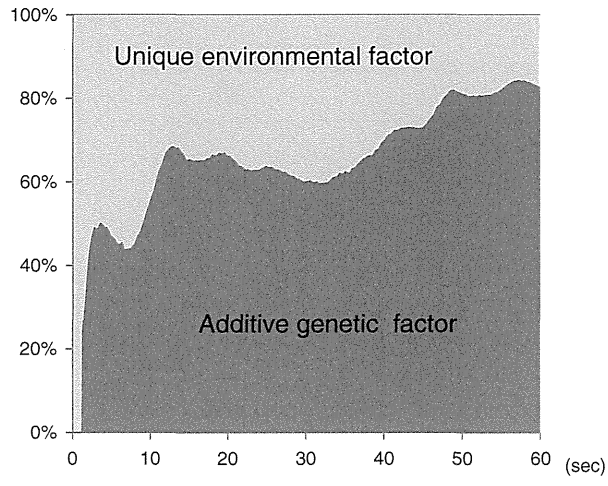

Fig. 8. Time course of the estimated heritability of the [oxy-Hb] signals during the task period in Ch48.

Limitations

First, before definite implications can be drawn from this study, the present results should be confirmed in a future twin NIRS study using a larger and more balanced sample. In addition, the number of female participants was disproportionately larger than that of male participants. Although the genetic influences on brain activation observed in the whole sample were reproduced using data that excluded male participants, we should be careful when extending the results of the present study to the estimation of the heritability of brain activation in male participants.

Second, the brain areas that are activated by the LFT have been shown to be highly heritable in twin studies investigating brain structure (Schmitt et al., 2007; Thompson et al., 2001). This imposes special consideration for twin studies employing the NIRS system. Our NIRS system calculated the product of hemoglobin concentration changes and the differential pathlength factor (DPF) (ΔC^*L) as a solution to the simultaneous equations based on the modified Beer–Lambert law (Yamashita

et al., 1996). However, DPF might be influenced by the regional brain volume and the morphology of the areas through which the near-infrared light passes. Therefore, part of the estimated genetic influences on the task-related [oxy-Hb] changes in the prefrontal area was confounded by the heritability of the brain volume and morphology of that area. Although head size (as measured by the T3–T4 interval) was not correlated with task-related brain activation, this result is insufficient to rule out the possibility stated above. Therefore, the technology used for the real-time measurement of the estimated DPF at each channel, to determine the proportion of changes in hemoglobin concentration that are unaffected by DPF, should be evaluated as a separate issue in future NIRS studies.

Third, one recent study indicated that the major part of [Oyx-Hb] signals from forehead probes of NIRS are explained by skin blood flow change (Takahashi et al., 2011). This may imply that the correlation of LFT-related [oxy-Hb] change in the left frontal pole between cotwins might mainly be due to their similarity in task-related skin blood flow fluctuations. If that is the case, the estimated heritability in the left frontal polar brain activation should be reinterpreted as the heritability of the skin blood flow change of the forehead. In contrast, the correlation of brain activations between MZ twin pairs was stronger in the right hemisphere than in the left hemisphere. As a result, genetic influence was estimated in a channel corresponding to the right dorsolateral prefrontal area. Such laterality and inhomogeneous distribution are difficult to explain solely by the effect of skin blood flow. These findings suggest that most NIRS [oxy-Hb] signals cannot be exclusively explained by skin blood flow.

Conclusions

The results of the present study suggest that hemodynamic activation in the prefrontal cortex during an executive task, as measured by functional NIRS, is a genetically influenced trait. These findings indicate that prefrontal NIRS signals induced by cognitive activation may be a promising endophenotype for large-scale imaging genetic studies aimed at disentangling the genetic background of heterogeneous psychiatric disorders.

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Contributors

RT, YK, and KK were involved in the conception, design, and management of the study. ES, RT, YN, and KK analyzed and interpreted the data and wrote the first draft of the paper. RT, YN, KM, MK, SE, SY, HI, YT, KT, MS, HY, TK, and KK conducted data acquisition. The other contributors revised the first draft critically for important intellectual content. All contributors have approved the final version of the manuscript.

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Conflict of interest

Regarding all financial and material support for the present study, Dr. Kasai has a potential conflict of interest (see below for details). All other authors have no relevant conflicts of interest.

Beginning July 31, 2003 and continuing through to present, the University of Tokyo and the Hitachi Group (Advanced Research Laboratory, Hitachi Ltd. and The Research and Developmental Center, Hitachi Medical Corporation) have had an official contract for a collaborative study on the clinical application of near-infrared spectroscopy (NIRS) in psychiatric disorders, which has been approved by the Research Promotion Office, University of Tokyo Hospital. The principal investigator of this study is Kiyoto Kasai. For this study, the Hitachi Medical Corporation provided a project grant (JPY 300,000 per year).

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Language-specific cortical activation patterns for verbal fluency tasks in Japanese as assessed by multichannel functional near-infrared spectroscopy

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ABSTRACT

In Japan, verbal fluency tasks are commonly utilized as a standard paradigm for neuropsychological testing of cognitive and linguistic abilities. The Japanese "letter fluency task" is a mora/letter fluency task based on the phonological and orthographical characteristics of the Japanese language. Whether there are similar activation patterns across languages or a Japanese-specific mora/letter fluency pattern is not certain. We investigated the neural correlates of overt mora/letter and category fluency tasks in healthy Japanese. The category fluency task activated the bilateral fronto-temporal language-related regions with left-superior lateralization, while the mora/letter fluency task led to wider activation including the inferior parietal regions (left and right supramarginal gyrus). Specific bilateral supramarginal activation during the mora/letter fluency task in Japanese was distinct from that of similar letter fluency tasks in syllable-alphabet-based languages: this might be due to the requirement of additional phonological processing and working memory, or due to increased cognitive load in general.

1. Introduction

Verbal fluency tasks have long served as a standard paradigm for neuropsychological testing of cognitive and linguistic abilities (e.g., Lotsof, 1953; Rogers, 1953), and have gained scientific as well as clinical importance in psychiatry, neurology, and nuerosurgery (e.g., Frith et al., 1995; Monsch et al., 1992; Watanabe et al., 1998). In performing verbal fluency tasks, subjects are requested to generate words, in general vocally, according to a given rule. Typically, words are generated within a particular semantic category (e.g., fruits) to form a category fluency task, or with a particular letter (e.g., E-words) to form a letter fluency task. These are often referred to as semantic and phonological fluency tasks, respectively (Lezak, Howieson, Bigler, & Tranel, 2012). An extensive meta-analysis of lesion studies revealed a differential association between verbal fluency tasks and brain regions: deficits in category fluency tasks are associated with temporal lobes, including Wernick language areas, while those in letter fluency with frontal lobes, including Broca's area as well as temporal lobes (Henry & Crawford, 2004).

There appear to be two major uses of verbal fluency tasks. First, reflecting the functional association between the verbal fluency tasks and language-related brain regions suggested by the lesion studies, the tasks are commonly used for diagnosing expressive aphasia, and have proven to be valuable tools for detecting hemispheric language dominance before neurosurgery (c.f. Watanabe et al., 1998). Second, they have been adopted to examine executive control processes in a variety of neurological and psychiatric disorders including traumatic brain injury (Henry & Crawford, 2004), depression (Wolfe, Granholm, Butters, Saunders, & Janowsky, 1987), Alzheimer's disease (Monsch et al., 1992), schizophrenia (Phillips, James, Crow, & Collinson, 2004; Saykin et al., 1991), attention deficit/hyperactivity disorder (Geurts, Verte, Oosterlaan, Roeyers, & Sergeant, 2004), and autism (Turner, 1999).

Reflecting the clinical and functional importance of verbal fluency tasks, considerable interest has been taken in elucidating the neural mechanism underlying these tasks using neuroimaging methods (Birn et al., 2010). In typical neuropsychological procedures requiring free recall of words, subjects are requested to

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overtly produce as many words as possibile within a limited time period. The tasks are performed face-to-face with experimenters to faciliate natural speech in a natural environment. This allows experimenters to monitor the task performance through direct observation and to record behavioral performance data (Birn, Cox, & Bandettini, 2004). However, typical neuroimaging environments, especially that of fMRI, do not provide an ideal environment for performing verbal fluency tasks. Moreover, fMRI measurements are often purturbed by task-related facial and orolingual motion resulting from overt speech. To reduce task-related motion artifacts, fMRI studies generally utilize covert word generation (e.g., Gurd et al., 2002; Hirshorn & Thompson-Schill, 2006; Perani et al., 2003). Alternatively, overt, single-word production paced by experimenters may be adopted (e.g., Abrahams et al., 2003; Phelps, Hyder, Blamire, & Shulman, 1997). However, covert word fluency tasks fail to provide behavioral indices, and make it difficult to interpret and validate experimental results, especially when patients are being studied (Billingsley et al., 2004; Birn et al., 2010). In addition, covert tasks may not serve as simple substitutes for their overt counterparts due to their reduced cognitive loads. Experimenter-paced single-word production tasks also pose a similar problem: albeit providing the behavioral correlate, the single-word production tasks are qualitatively different from the word fluency tasks performed within a limited time period, and may produce lower cognitive loads (Abrahams et al., 2003; Basho, Palmer, Rubio, Wulfeck, & Muller, 2007).

Conversely, fNIRS measurement is relatively immune to these problems due to its less straining experimental setting, and thus it possesses substantial potential for applications such as linguistic functional monitoring (reviewed in Dieler, Tupak, and Fallgatter (2012)). In particular, fNIRS enjoys a low susceptibility to movement-related noise that is often entailed in overt verbal fluency tasks. This feature has been most quantitatively demonstrated by Schecklmann, Ehlis, Plichta, and Fallgatter (2010) in their simultaneous fNIRS and EMG measurements over the fronto-temporal areas during an overt word fluency task, revealing fluency-related activation and no systematic association between fNIRS and EMG signals. Indeed, fNIRS has been implemented in letter and category fluency tasks: typically these studies demonstrate bilateral activations in the inferior frontal gyri (IFG), middle frontal gyri (MFG), and, more roughly, in the fronto-temporal regions (Ehlis, Herrmann, Plichta, & Fallgatter, 2007; Herrmann, Walter, Ehlis, & Fallgatter, 2006; Kakimoto et al., 2009; Reif et al., 2011; Richter, Herrmann, Ehlis, Plichta, & Fallgatter, 2007; Schecklmann et al., 2007, 2008, 2010; Suto, Fukuda, Ito, Uehara, & Mikuni, 2004) with activation being left-pronounced and higher in the phonological than in the semantic conditions (Ehlis et al., 2007; Schecklmann et al., 2008). In addition, verbal fluency tasks have been used to explore cortical activation patterns specific to psychiatric patients compared to normal, healthy control subjects (e.g., Herrmann, Ehlis, & Fallgatter, 2003; Matsuo, Kato, Fukuda, & Kato, 2000; Suto et al., 2004; Watanabe et al., 1998).

These bodies of literature have presented accumulating evidence that fNIRS is a promising imaging modality for functional assessment during verbal fluency tasks. This further suggests the importance of describing prototypical cortical activation patterns for verbal fluency tasks in order to establish canonical referential data for future clinical diagnoses. Reflecting this notion, a systematic study employing multichannel fNIRS to cover bilateral language-related cortical regions has reported on a comparison of the cortical activation pattern between letter and category fluency tasks. A German group employing 50 subjects revealed oxy-Hb increase in the fronto-temporal cortices with a general left-lateralization in both tasks. This lateralization was more pronounced in the semantic task (Tupak et al., 2012). This general tendency was also confirmed in the older adults, but with bilaterally reduced activation in the inferior frontal junction (IFJ) and increased activation in the middle-frontal and supramarginal gyri (Heinzel et al., 2013). In addition, a Canadian group, setting IFG as the region of interest, reported bilateral activation during category and letter fluency tasks in younger and older adults with reduced activation in the latter (Kahlaoui et al., 2012).

Although prototypical activation patterns for verbal fluency tasks are being elucidated, we now start to face a fundamental question of linguistic studies: generalizability and specificity in languages. It has been demonstrated that even the same reading tasks performed in different languages could recruit different cortical regions. For example, Italian is orthographically more consistent than English: in a PET study during word and non-word reading tasks, Italian readers exhibited greater activation than English readers in the superior temporal regions, involved in phoneme processing (Paulesu et al., 2000). Differences in the orthographical and phonological structures in languages might also affect cortical activation patterns during word fluency tasks.

Phonologically speaking, while English, German, and Italian are all syllable-based languages, Japanese is mora-based. Scriptically, English, German, and Italian are alphabetical, while Japanese is moraic. The syllable is an important phonological unit in syllable-based languages. It is formed around a vowel (V), and a V can take up a few proceeding and subsequent consonants (C). The most common form of syllable in English is CVC. On the other hand, the principal suprasegmental unit in the Japanese language is the mora (Mattys & Melhorn, 2005; Otake, Hatano, Cutler, & Mehler, 1993). A mora is generally composed of a single V or combination of CV, and generally corresponds to a single Japanese phonetic character (McQueen, Otake, & Cutler, 2001; Otake et al., 1993). Accordingly, morae tend to have fewer Cs and Vs than do syllables. In other words, the number of phonetic segments in a word is larger in morae than in syllables. For example, the English word "system" (sys·tem) consists of two segments, while its Japanese version consists of four segments (si·su·te·mu). Conversely, the English word "system" includes six letters, while its Japanese version includes four morae.

Given these orthographical and phonological differences between alphabet-syllable-based and moraic languages, letter fluency tasks in these languages cannot be considered fully compatible, while category fluency tasks are. In letter fluency tasks, usually a target letter is given as a minimum orthographical unit. In alphabet-syllable-based languages, it is a certain alphabetical letter phonologically representing the first C or V, while in moraic languages, the unit is a certain moraic letter that has a one-to-one correspondence with a particular mora sound. In English, a target letter is selected from 26 letters, whereas in Japanese, the target letter can be selected from among up to 67 characters. Taken together, what is referred to as "letters" in alphabet-syllable-based and moraic languages have different orthographical and phonological characteristics. Therefore, we hereafter designate a Japanese letter fluency task as a "mora/letter fluency task".

Indeed, a behavioral psychiatric study suggested that verbal fluency tasks in Japanese may be functionally different from those in alphabet-syllable-based languages. In patients with schizophrenia speaking alphabet-syllable-based languages, category fluency is more disturbed than phonological fluency. However, Japanese-speaking schizophrenic patients tend to exhibit similar impairment for both verbal fluency tasks (Sumiyoshi et al., 2004). This observation suggests that cortical functions recruited for verbal fluency tasks may be different between moraic languages (e.g., Japanese) and alphabet-syllable-based languages (e.g., English, German, and Italian). If this is the case, canonical activation patterns for verbal fluency tasks obtained from alphabet-syllable-based language speakers may not be appropriate for Japanese speakers.

Thus, it is necessary to explore prototypical cortical activation patterns for verbal fluency tasks in Japanese to establish canonical referential data for future clinical diagnoses for Japanese patients. However, we should note here that many reports have employed Japanese mora/letter fluency tasks to explore cortical activation patterns specific to psychiatric patients compared to normal, healthy control subjects (e.g., Herrmann et al., 2003; Matsuo et al., 2000; Suto et al., 2004; Watanabe et al., 1998). They have adopted letter fluency tasks as tools for diagnosing psychiatric patients, and their main foci have been on the prefrontal functions, but not on fronto-temporal language-related regions. Most of them have solely used mora/letter fluency tasks. The only exception comparing cortical activations for letter and category fluency adopted two-channel fNIRS, and failed to report a holistic view of the activation patterns (Ikezawa et al., 2009).

Therefore, our primary goal was to investigate the neural correlates of verbal fluency during overt mora/letter and category fluency tasks in Japanese. We examined whether there are similar activation patterns across languages or Japanese-specific activation patterns for mora/letter and category fluency tasks. In so doing, we aimed to present a prototypical cortical activation patterns for Japanese subjects performing verbal fluency tasks.

2. Methods

2.1. Participants

Twenty-eight right-handed, healthy volunteers (21 males, 7 females, average age = 32.9 years, SD = 10.7, range: 22–59 years) participated in the study. Handedness was assessed by means of the Edinburgh Inventory (Oldfield, 1971). All were native Japanese speakers. Written informed consent was given by all participants and the study was approved by the Jichi Medical University ethics committee.

2.2. Verbal fluency tasks

Category and mora/letter fluency tasks were applied in Japanese. Each task was performed in five blocks. In the category fluency task, subjects had to overtly generate examples for the following five categories: fruits, vegetables, animals, sports, and vehicles. In the mora/letter fluency task the participants were required to generate nouns starting with the following morae: /a/, /i/, /u/, /o/, /ka/, /ki/, /ku/, or /sa/. These morae were selected using the following procedure. According to the corpus by Amano and Kondo (1999), we selected relatively common Japanese words with auditory and visual word familiarity scores above 4.0 (on a scale of 1–7). We examined the frequency of the first mora appearance among these words, and selected the top 25%, resulting in 19 morae. After removing the dull-sounded mora, /ga/, we randomly selected eight of the remaining 18 morae for the clinical routine. Of these, five morae were presented in a pseudorandom order among the subjects. Subjects were instructed to not use proper names, a grammatical variation of the previous word, or to repeat previous responses. Participants were asked to vocalize as softly as possible to reduce movement-related artifacts but loudly enough to convey their response to the experimenter. The task paradigm was a periodic block design with 5 alternating conditions of rest (30 s) and experimental task (category or mora/letter fluency, 20 s).

2.3. fNIRS instruments

We used the multichannel fNIRS optical topography system ETG-4000 (Hitachi Medical Corporation, Kashiwa, Japan), using two wavelengths of near-infrared light (695 and 830 nm). We analyzed the optical data based on the modified Beer–Lambert Law (Cope et al., 1988) as previously described (Maki et al., 1995). This method allowed us to calculate signals reflecting the oxygenated hemoglobin (oxy-Hb), deoxygenated hemoglobin (deoxy-Hb), and total hemoglobin (total-Hb) concentration changes, calculated in arbitrary units (millimolar–millimeter) (Maki et al., 1995). The sampling rate was set at 100 ms.

2.4. fNIRS probe placement

We used two 3 × 5 multichannel probe holders, each consisting of eight illuminating and seven detecting probes arranged alternately at an inter-probe distance of 3 cm, resulting in 22 channels (CH) per hemisphere (Fig. 2a). The specific setting was as previously described (Moriai-Izawa et al., 2012). We set the fNIRS probes to cover the lateral prefrontal cortices in reference to previous studies (Ehlis et al., 2007; Herrmann et al., 2006; Kakimoto et al., 2009; Richter et al., 2007; Schecklmann et al., 2007, 2008, 2010; Suto et al., 2004). After the fNIRS measurement, positions of illuminators and detectors were subjected to probabilistic registration of fNIRS channel positions to MNI space (Jurcak, Tsuzuki, & Dan, 2007; Okamoto et al., 2004; Okamoto & Dan, 2005; Singh, Okamoto, Dan, Jurcak, & Dan, 2005; Tsuzuki et al., 2007, 2012) with reference to the macroanatomical brain atlas LBPA40 (Shattuck et al., 2008).

2.5. Analyses of fNIRS data

For the first level analyses, individual timeline data for the oxy-Hb and deoxy-Hb signals of each channel were analyzed using the General Linear Model (GLM) with regression to a hemodynamic response function (HRF) as modified for fNIRS by Schroeter, Zysset, and von Cramon (2004) and Plichta, Heinzel, Ehlis, Pauli, and Fallgatter (2007). This approach is compatible with typical fMRI analyses.

First, data containing bad measurements, mostly due to insufficient probe contact, were excluded. Since the fNIRS instrument does not record any signal for such channels, all data for a channel were excluded if 10% or more of the timeline data was unmeasured. Properly measured individual timeline data for the oxy-Hb and deoxy-Hb signals of each channel were preprocessed by the Wavelet minimum description length (Wavelet-MDL) detrending to remove global trends due to breathing, cardiac movement, vasomotion and other experimental errors (Jang et al., 2009) and by temporal smoothing with convolution of the canonical HRF to the individual timeline data (Friston et al., 2000). Changes in the autocorrelation structure of the timeline data due to temporal smoothing were adjusted using the pre-coloring method (Worsley & Friston, 1995), which has also been shown to be adequate for fNIRS analyses (Ye, Tak, Jang, Jung, & Jang, 2009). The pre-coloring method adjusted the degree of freedom.

In this study, adaptive methods were used to find the optimal HRF for temporal analysis of fNIRS data (Sano et al., 2012). The GLM with regression to temporally variable HRFs was applied to fNIRS data during category and mora/letter fluency tasks. The following HRF $h(\tau_p, t)$ was used according to Friston et al. (1998).

$$h(\tau_p, t) = \frac{t^{\tau_p} e^{-t}}{(\tau_p)!} - \frac{t^{\tau_p+\tau_d} e^{-t}}{A(\tau_p+\tau_d)!}$$

where τ_p stands for the first peak delay and was set as a variable changing from 6 to 56 s to yield variable HRF. In typical fMRI studies, τ_p is usually fixed to 6 s. τ_d is the second peak delay and was set to 10 s as in typical fMRI studies. A is the amplitude ratio between the first and second peaks and was set to 6 as in typical fMRI studies. Basis functions $f(\tau_p, t)$ were generated by convolving variable HRF $h(\tau_p, t)$ with a boxcar function $N(t)$.

$$f(\tau_p, t) = h(\tau_p, t) * N(t)$$

In addition, the temporal and dispersion derivatives of the canonical HRF were included to adjust the onset and dispersion of the model functions to the individual's hemodynamic response. A bias component was also included.

Fig. 1 illustrates the inter-subject, inter-channel grand average waveforms of oxy-Hb and deoxy-Hb signal changes, overlaid with canonical hemodynamic response functions. For oxy-Hb, a peak delay of 10 s was adopted for category and mora/letter fluency tasks. Deoxy-Hb exhibited somewhat complicated behavior with decrease during task period and subsequent concentration increase during the resting phase, which elongated into the next task period, causing apparent increase of deoxy-Hb interlocked with the task period. Since further examination of deoxy-Hb would require technical details in signal processing issues beyond the range of the current study, we limited our analysis to stereotyped oxy-Hb signal, and will report deoxy-Hb results in a technical context elsewhere (in preparation).

The β-values (response amplitudes) and t-values of the oxy-Hb and deoxy-Hb signals were estimated for the variable HRFs predictor. These values were calculated using a least-squares-model fitting procedure maximizing model-to-data fitting (Bullmore, Brammer, et al., 1996; Bullmore, Rabe-Hesketh, et al., 1996). To examine the effects of τ_p, average t-values over 44 channels and 28 participants were calculated for all τ_p values tested. The τ_p values that yielded the maximum average t-value were adopted.

For the second-level group analyses, obtained beta-values served as contrasts, which were subjected to random effects group analyses using one-sample t-tests against zero. A p-value of less than 0.05 was considered significant. Family-wise errors due to multichannel measurement were subjected to Bonferroni correction.

The first and second level analyses were performed using in-house Matlab toolboxes that are available through our website (http://www.jichi.ac.jp/brainlab/tools.html).

3. Results

3.1. Behavioral data

The subjects produced on average (±standard deviation) 56.6 (±7.5) words in the category task and 37.3 (±6.0) words in the mora/letter fluency task. This corresponds to 34.0 (±4.5) and 22.4 (±3.6) words/min for the category and mora/letter fluency tasks, respectively. The subjects generated significantly more words during the category fluency task ($t = 11.587$, $p < 0.001$).

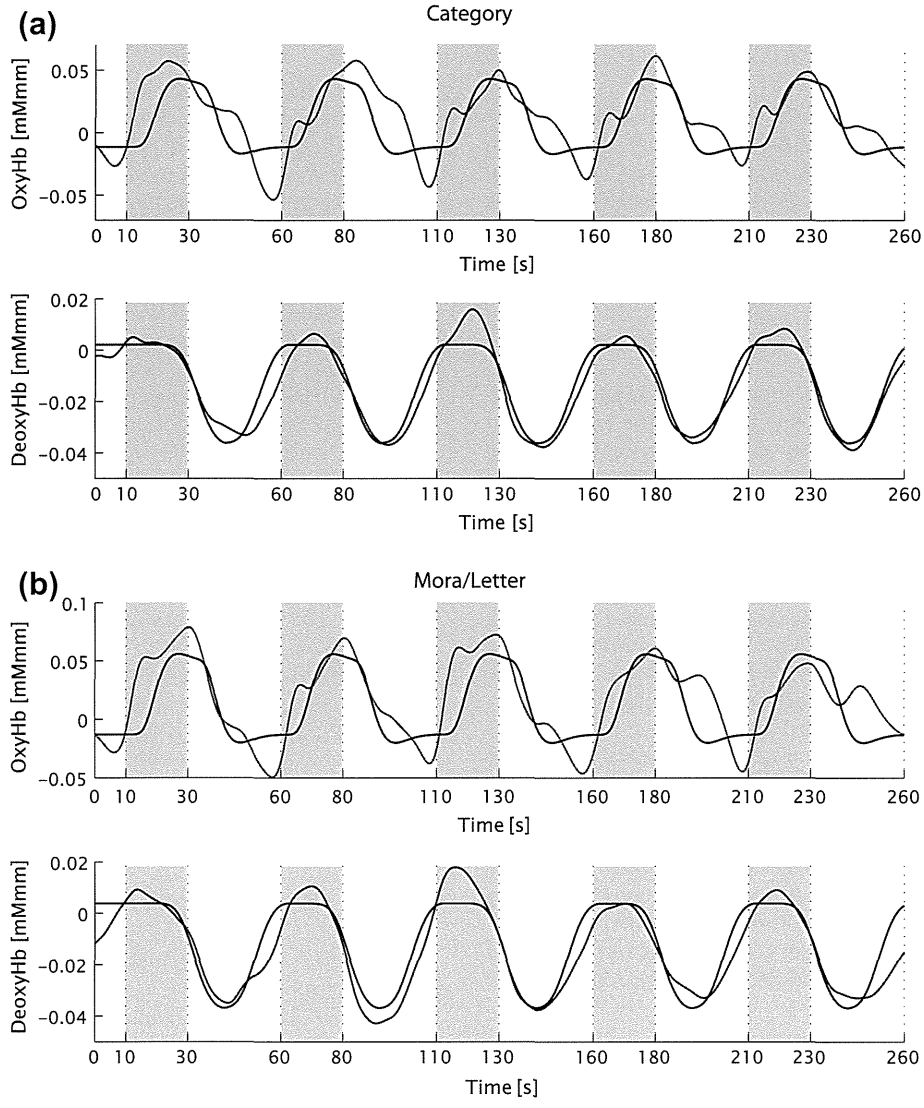

Fig. 1. Regressors used for general linear model (GLM) analyses. The canonical hemodynamic response functions (black lines) are overlaid with inter-subject, inter-channel grand average waveforms of oxy-Hb (red lines) and deoxy-Hb (blue lines) signals for category (a) and mora/letter (b) fluency tasks. Task periods (five blocks) are indicated with green backgrounds. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

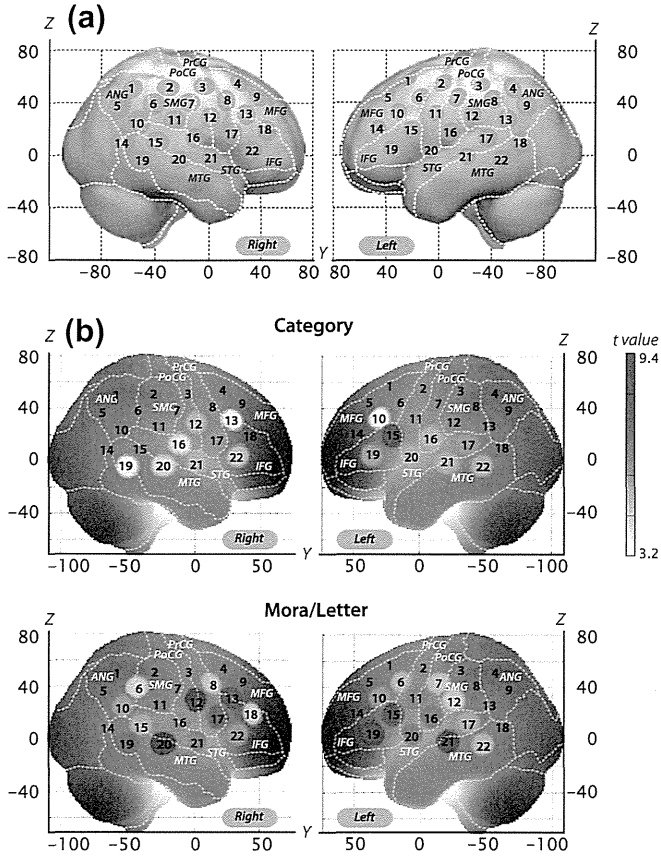

Fig. 2. fNIRS analyses of verbal fluency tasks. (a) Statistically estimated fNIRS channel locations on the brain (centers of green circles) for the group of subjects, and their spatial variability (SDs, radii of the green circles) associated with the estimation are exhibited in MNI space. (b) Results of functional analyses. Differences of cortical activation patterns between baseline and verbal fluency tasks are shown as t-maps of oxy-Hb signal. *t*-Values, colored as per the color bar, are indicated only for the statistically significant channels ($p < 0.05$, Bonferroni-corrected).

3.2. fNIRS data

On-brain estimation of each channel location was performed based on information coded in a macroanatomical brain atlas, LBPA40 (Shattuck et al., 2008). For each channel, we assessed the effect of the two fluency tasks on oxy-Hb using a one-sample *t*-test against zero (one-tail) with Bonferroni correction. Significant oxy-Hb increase was found in 9 channels on the left hemisphere and 8 channels on the right hemisphere in the category fluency task, and 12 channels (left) and 15 channels (right) in the mora/letter fluency task (Fig. 2b). In the category fluency task, significant activations were found in the bilateral inferior frontal gyri (IFG), superior and middle temporal gyri (STG and MTG), and pre- and post-central gyri (PrCG and PoCG), with slight extension to the left middle frontal gyrus (MFG) (Table. 1). In the mora/letter fluency task, in addition to the fronto-temporal cortices activated in the category fluency task, significant activations extended to the middle frontal gyrus (MFG) and supramarginal gyrus (SMG) on the left hemisphere, as well as to the SMG (slightly overlapping with the border of angular gyrus) on the right hemisphere (Fig. 2b and Table. 1).

4. Discussion

4.1. Overall activation patterns for verbal fluency tasks in Japanese

The current study revealed activation patterns for verbal fluency tasks in the moraic language, Japanese. The category fluency tasks activated the bilateral fronto-temporal language-related regions with left-superior lateralization, while the mora/letter fluency tasks led to more widespread activation including inferior parietal regions (left and right SMG). Overall, activation was greater for mora/letter fluency than category fluency tasks. The left-lateralization was more eminent in the category fluency task.

In addition, performance for the mora/letter fluency (22.4 words/min) and category fluency (34.0 words/min) tasks in the current study were comparable to those typical for normal, healthy adult subjects reported formerly: 22.2 words/min for the mora/letter fluency and 32.7 words/min for category fluency tasks (Sumiyoshi et al., 2004). Thus, the activation patterns observed in the current study are expected to be typical cortical representations of mora/letter fluency and category fluency tasks performed by normal, healthy Japanese adult subjects.

The observed activation patterns resembled those of the former report by Tupak et al. employing similar word fluency tasks in a syllable-alphabet-based language, German (Tupak et al., 2012). They reported similar fronto-temporal activation patterns for the letter and category fluency tasks, with the former showing left-superior lateralization, and the latter more bilateral activation. However, they did not observe parietal activation in the letter fluency task.

In addition to this, twelve more imaging studies have explored letter and category fluency tasks to our knowledge (5 fMRI (Birn et al., 2010; Heim, Eickhoff, & Amunts, 2008; Kircher, Nagels, Kirner-Veselinovic, & Krach, 2011; Paulesu et al., 1997; Perani et al., 2003), 1 structural MRI (Grogan, Green, Ali, Crinion, & Price, 2009), 2 PET (Gourovitch et al., 2000; Mummery, Patterson, Hodges, & Wise, 1996), 1 MEG (Billingsley et al., 2004), and 4 fNIRS (Ehlis et al., 2007; Heinzel et al., 2013; Kahlaoui et al., 2012; Tupak et al., 2012). To summarize, among the regions covered in the current study, these studies reported activations in the following regions. Category fluency tasks recruited the left inferior frontal gyrus (IFG; BA44, 45, 46), MFG (BA9), post-central gyrus (PoCG; BA3), precentral gyrus (PrCG; BA6), superior temporal gyrus (STG; BA22), middle temporal gyrus (MTG; BA21), right IFG (BA45, 46), MTG (BA21), and STG (BA22). Letter fluency tasks recruited the left IFG (BA44, 45), dorsolateral prefrontal cortex (DLPFC; BA6, 9, 10; including MFG and superior frontal gyrus), PrCG (BA6), PoCG (BA3, 4), STG (BA22, 42), MTG (BA21), and right IFG (BA45, 46). Moreover, an extensive comparative meta-analysis by Indefrey and Levelt (2004) indicated reliably activated areas for word fluency including the left MFG, IFG, PrCG, STG, MTG, right MFG, STG, and MTG. These fronto-temporal activation patterns are mostly consistent with our results, which also demonstrated inferior parietal activations for the mora/letter fluency task in our study.

4.2. SMG activation in mora/letter fluency task

Among the 23 word-fluency reports analyzed in the meta-analysis (Indefrey & Levelt, 2004), only one reported left SMG activation, and no report was found for the right inferior parietal region. A meta-analysis of over 82 word-production studies have also reported only two occurrences of right-SMG activation out of 64 studies and five of left-SMG activation out of 67, and thus concluded that the SMG is not reliably activated for word production. Only Heinzel et al. (2013) found a meaningful SMG activation during a letter fluency task, reporting that it was smaller than that of other regions and that it correlated with age.

The SMG activation in the Japanese mora/letter fluency task, which seemingly contradicted activation patterns typically observed in letter fluency tasks in other languages, may reflect a unique task characteristic and its underlying cortical functions. It has rarely been indicated, but "letter fluency tasks" are heavily depen-

Table 1
Spatial profiles of the channels screened for involvement with category and mora/letter fluency tasks.

Ch	MNI coordinates *x*, *y*, *z* (SD)	Macroanatomy	Prob	Brodmann area	Prob
Left					
6	−50, 14, 43 (8)	Middle frontal G	0.75	9 – Dorsolateral prefrontal C	0.39
7	−62, −14, 43 (8)	Post-central G	0.84	1 – Primary somatosensory C	0.39
10	**−50, 30, 32 (7)**	**Middle frontal G**	**0.98**	**45 – Pars triangularis Broca's area**	**0.77**
11	**−62, 2, 31 (7)**	**Precentral G**	**0.68**	**6 – Pre-motor and supplementary motor C**	**0.52**
12	−67, −26, 30 (8)	Supramarginal G	0.99	2 – Primary somatosensory C	0.98
15	**−59, 20, 19 (7)**	**Inferior frontal G**	**0.95**	**44 – Pars opercularis, part of Broca's area**	**0.68**
16	**−66, −9, 18 (7)**	**Post-central G**	**0.95**	**43 – Subcentral area**	**0.61**
17	**−68, −37, 13 (8)**	**Superior temporal G**	**0.99**	**22 – Superior temporal G**	**0.96**
19	**−54, 35, 5 (6)**	**Inferior frontal G**	**1.00**	**45 – Pars triangularis Broca's area**	**1.00**
20	**−61, 6, 5 (9)**	**Precentral G**	**0.58**	**48 – Retrosubicular area**	**0.72**
21	**−69, −22, −1 (7)**	**Middle temporal G**	**0.79**	**21 – Middle temporal G**	**0.83**
22	**−67, −47, −3 (8)**	**Middle temporal G**	**0.94**	**21 – Middle temporal G**	**0.52**
Right					
6	65, −41, 39 (10)	Supramarginal G	0.60	40 – Supramarginal G part of Wernicke's area	0.96
8	54, 12, 41 (10)	Precentral G	0.62	6 – Pre-motor and supplementary motor C	0.39
10	64, −51, 25 (11)	Angular G	0.64	22 – Superior temporal G	0.63
11	69, −26, 27 (10)	Supramarginal G	0.82	2 – Primary somatosensory C	0.68
12	**65, −0, 29 (9)**	**Post-central G**	**0.64**	**43 – Subcentral area**	**0.56**
13	**54, 28, 30 (9)**	**Inferior frontal G**	**0.55**	**45 – Pars triangularis Broca's area**	**0.67**
14	60, −62, 10 (12)	Middle temporal G	0.37	37 – Fusiform G	0.74
15	70, −38, 11 (10)	Superior temporal G	0.59	22 – Superior temporal G	1.00
16	**68, −11, 13 (9)**	**Superior temporal G**	**0.51**	**22 – Superior temporal G**	**0.75**
17	**61, 17, 17 (9)**	**Precentral G**	**0.84**	**44 – Pars opercularis, part of Broca's area**	**0.51**
18	50, 44, 18 (9)	Inferior frontal G	0.73	45 – Pars triangularis Broca's area	0.75
19	**68, −48, −4 (9)**	**Middle temporal G**	**0.93**	**37 – Fusiform G**	**0.45**
20	**71, −23, −3 (8)**	**Middle temporal G**	**0.80**	**21 – Middle temporal G**	**0.91**
21	**64, 1, −1 (10)**	**Superior temporal G**	**0.88**	**48 – Retrosubicular area**	**0.49**
22	**57, 33, 4 (8)**	**Inferior frontal G**	**1.00**	**45 – Pars triangularis Broca's area**	**0.97**

For MNI coordinates, the most likely coordinate values are presented with standard deviation (SD) in units of mm. Macroanatomical estimation is based on LBPA40 (Shattuck et al., 2008). Brodmann area estimations are based on MRIcro (Rorden & Brett, 2000). Anatomical labels (Macroanatomy and Brodmann) with highest probability are listed for each channel. Channels indicated in bold were activated significantly in both tasks, whereas channels written in plain text were activated only for the mora/letter fluency task. Abbreviations: Ch, channels; Prob, probability; C, cortex; G, gyrus.

dent on the orthography of the languages in which they are being undertaken. Specific associations between graphemes and phonemes can influence how the task is performed. In English, the minimum phonological unit is called a phoneme, which is represented by various graphemes. This one-to-many manner of association between phonemes and graphemes allows English speakers to find various alternatives in a word search (Sumiyoshi et al., 2004).

There are mainly three phonemic-based strategies (Robert et al., 1998; Troyer, Moscovitch, & Winocur, 1997). First, a word search is performed based on the first few letters, such as "click," "clip," and "cliche" (first letters). Second, words with the same consonant but with different vowel sounds are generated, such as "cut," "cat," and "coat" (vowel sounds). Third, words with the same pronunciation but with different spellings are generated, such as "two," "too," and "to" (homonyms).

In Japanese, the minimum phonological unit, called a mora, usually consists of one consonant and a vowel (e.g., S + A = SA) or a single vowel (e.g., A). Each mora is assigned a specific "kana" script in the one-to-one fashion (e.g., /sa/: "さ"). This rigid mora-grapheme association may restrict searching flexibility in the Japanese mora/letter fluency task. Specifically, Japanese speakers have to perform a comprehensive search by enumerating possible combinations of the first and subsequent morae to generate a meaningful word. In a search using first letters, English speakers are allowed flexible choices of consonant and vowel combinations: for example, "frisk" may be searched for via "f", "fr", or "fri". However, a first-letter search in Japanese is limited to a specific mora. Moreover, there are no strategic choices such as word searches using alternate vowel sounds. These orthographical characteristics of the Japanese language make letter fluency tasks phonologically more demanding and difficult to perform.

4.3. Involvement of phonological processing and working memory in mora/letter fluency tasks

The higher phonological demand described above may lead to greater SMG activation as this area is implicated in phonological processing and working memory. For example, an fMRI study with patients with lesions in the left SMG suggested its role in acoustic–phonetic processing (Caplan, Gow, & Makris, 1995). Early fMRI and PET imaging studies enrolling speech perception tasks reported stronger activation of the left SMG in phonological than in semantic tasks suggesting its role in phonological processing (Celsis et al., 1999; Demonet, Price, Wise, & Frackowiak, 1994). Phonological working memory tasks have been reported to evoke coactivation of the left SMG and the triangular part of the left IFG in fMRI studies (Hautzel et al., 2002; Jonides et al., 1998; Rypma, Prabhakaran, Desmond, Glover, & Gabrieli, 1999). A meta-analysis on phonological working memory also confirmed left SMG activation, suggesting its role in the phonological storage area in the phonological loop postulated by Baddeley (1992). A recent study using a rhyming verbal fluency task, in which participants generated words that rhymed with pseudo-word stimuli thus requiring stored phonological information about the rhymed word, reported greater left SMG activation in the rhyming than in the letter and semantic verbal fluency tasks (Kircher et al., 2011).

The association of the SMG with phonological processing and working memory does not seem to be limited to the left hemisphere, and many previous fMRI studies have reported bilateral SMG involvement in phonological decisions as compared to semantic decision tasks (Buchsbaum & D'Esposito, 2009; Devlin, Matthews, & Rushworth, 2003; McDermott, Petersen, Watson, & Ojemann, 2003; Price, Moore, Humphreys, & Wise, 1997). A recent TMS study demonstrated that the accuracy and reaction times of

phonological, but not semantic or perceptual, decisions were disrupted upon TMS functional intervention to the left, right, or bilateral SMG, revealing that the right, as well as the left, SMG contribute to accuracy and efficiency in phonological decisions (Hartwigsen et al., 2010). Interestingly, laterality seems to be related to the balance between the phonological and semantic demand of the task employed. An early fMRI study reported right lateralized bilateral SMG activation during a tone decision task relative to a semantic decision task (Binder et al., 1997). A recent large-scale fNIRS study employing a word repetition task in Japanese elementary school children reported that SMG activation was greater on the right than on the left in a low-frequency word repetition task compared to a higher frequency task in both Japanese and English (Sugiura et al., 2011). While low-frequency words maintained appropriate phonological structures, they were semantically less familiar to the participants. Thus, their repetition is expected to require phonological processing and working memory of semantically less obscure word stimuli.

We gather from these studies that the bilateral SMG activation during the Japanese version of the letter fluency task reflects a phonological working memory load required in the task execution. The mora/letter fluency task in Japanese is usually performed as a Japanese version of the letter fluency task in syllable-alphabet-based languages such as English, German and Italian. However, important functional differences may have been lost in translation. In the moraic letter fluency task in Japanese, after a cue mora is presented (e.g., sa), a typical word search is executed by adding random morae (e.g., sa·shi) to it until a meaningful word is generated (e.g., sa·shi·mi, meaning "sliced raw fish"). This process is basically the generation of phonemes, and thus would require a substantial amount of phonological processing. Moreover, this process entails more generation of nonsense words and low-frequency words than letter fluency tasks in other languages. Nonsense words are to be eliminated after being kept in the phonological working memory, while low-frequency words would require a more extensive lexical search than high-frequency words and demand a heavier phonological working memory load. This greater demand of phonological processing and working memory would lead to SMG activation.

Moreover, letter fluency tasks are thought to involve an orthographic visual strategy as well as a phonemic strategy. In the visual orthographic approach, one generates a visual representation of the letter to search the orthographic lexicon for meaningful words. On the other hand, the auditory phonemic approach leads one to use the sound of the letter to search the phonemic lexicon for meaningful words. Letter fluency tasks (in syllable-alphabet-based languages) may involve one or both strategies to generate potential words (Friedman et al., 1998). Although the moraic letter fluency task involves both strategies, the Japanese task is more oriented toward the phonemic strategy. Such a slant toward phonological processing may be a possible reason for the relatively high right SMG activation.

4.4. Effects of task difficulty in mora/letter fluency tasks

The argument presented above focuses on the qualitative differences in word search strategies for mora/letter and syllable-alphabet-based letter fluency tasks. However, we may also have to consider the quantitative difference: the different activation patterns may arise from differences in task difficulty. When task difficulty is calculated as the ratio of generated words in category versus letter fluency tasks (CF/LF ratio), it is 1.28 for nine studies on syllable-alphabet based languages (Birn et al., 2010; Ehlis et al., 2007; Gourovitch et al., 2000; Grogan et al., 2009; Heim et al., 2008; Kircher et al., 2011; Kubota et al., 2005; Paulesu et al., 1997; Tupak et al., 2012). Among these, category fluency tasks resulted in significantly more words generated than did letter fluency tasks, while four studies reported non-significant differences. On the other hand, the CF/(M/LF) ratio for Japanese tasks is 1.49 among four studies (Ikezawa et al., 2009; Suga, Uetsuki, Takizawa, Araki, & Kasai, 2011; Sumiyoshi et al., 2004, and this study), and significantly more words were generated in category fluency tasks in all of the studies. Thus, when we assume that category fluency tasks are analogous among different languages, mora/letter fluency tasks performed in Japanese are more difficult than typical letter fluency tasks in syllable-alphabet-based languages.

Indeed, Drager et al. (2004) observed right SMG activation with increased task difficulty in a letter fluency task in German, while typical activation patterns in the left hemispheric language-related areas remained constant. They interpreted that the more difficult the word searches, the more attention, working memory, response selection and executive control are required, and thus the right SMG activation may be attributed to fronto-parietal attention and associated executive control systems in the right hemisphere. The right inferior parietal cortex, including the SMG, is believed to contribute to selective attention (Pugh et al., 1996). Indeed, in a language study, its increased activation in response to increased demands on various facets of attentional control has been demonstrated for word-identification tasks (Shaywitz et al., 2001). Hence, we should not neglect the possibility that the right SMG activation reflects greater functional demands on the fronto-cingulate-parietal executive control network associated with execution of difficult-to-perform word searches during moraic letter fluency tasks in Japanese.

An interesting support of this view came from a recent fNIRS study examining cortical activation patterns for letter fluency in older adults (Heinzel et al., 2013). A positive correlation between activation and age was found for the bilateral SMG and MFG. They interpreted that this activation would reflect increased use of the fronto-cingulo-parietal cognitive control network in compensation for aging-related decrease of inferior frontal junction function associated with verbal fluency. Although age-related decline of the language network is not the case in the current study, functional compensation for an overloaded language network by the executive control network may be implemented for moraic letter fluency tasks. In order to distinguish whether SMG activation for moraic letter fluency reflects qualitative or quantitative functional differences, further studies enrolling differential task difficulty and, preferably, cross-cultural functional comparison employing compatible verbal fluency tasks would be necessary.

4.5. Conclusion

In conclusion, the current study provides an initial referential framework for verbal fluency tasks in normal, healthy Japanese, which could contribute to diagnoses of patients with neurological or psychiatric disorders including aphasia and schizophrenia. What is generally described as a "letter fluency task" in Japanese is actually a mora/letter fluency task based on the phonological and orthographical characteristics of the Japanese language. Reflecting this, cortical activation patterns during moraic letter fluency tasks in Japanese were different from those during letter fluency tasks in syllable-alphabet-based languages: in addition to consistent activation in fronto-temporal language areas, there was distinct bilateral SMG recruitment. Mora/letter fluency tasks are more difficult to perform than are letter fluency tasks in syllable-alphabet-based languages. Regardless of whether this is due to the requirement of additional phonological processing and working memory or due to increased cognitive load in general, there would be higher chance of failure to perform a mora/letter fluency task in patients with aphasia or schizophrenia.

Considering cortical functional characteristics of mora/letter fluency tasks, their difficulty may have to be optimized depending on the symptoms of aphasic patients. For example, rather than providing participants with a single mora (e.g., sa), sets of morae (e.g., sa, shi, su, se, so) may be presented as a target, but there is no guarantee that these are functionally compatible verbal fluency tasks. However, neuroimaging assessment using fNIRS may reveal whether they are executed with compatible functional modes which can be observed as similar cortical activation patterns. In this sense, fNIRS as an instrument for assessing functional localization would also serve as a valuable tool for localization of universal language tasks.

Statement of significance

Reflecting structural differences in language, a verbal fluency task in a moraic language, Japanese, recruited different cortical areas than that in syllable-alphabet-based languages. Category fluency tasks activated the bilateral fronto-temporal language-related regions with left-superior lateralization, while the mora/letter fluency tasks led to more widespread activation including the bilateral supramarginal gyri.

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