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individuals'). The two genotype subgroups were age-, gender-, IQ- and task-performance-matched in each diagnostic group (Table 1).

3.2. Test for significance of [Hb] change during activation period relative to baseline

In healthy controls, a significant increase in [oxy-Hb] occurred during the task period relative to the pretask baseline at all the 52 channels (p<FDR 0.05) and a significant decrease in [deoxy-Hb] change occurred during the task period relative to the pretask baseline at 38 channels (ch2, ch13-14, ch16, ch18-20, ch22-52; FDR-corrected p: 0.0001–0.037), thus the hemodynamic cortical response to the cognitive task was confirmed. In the present NIRS study, the verbal fluency task recruited widespread regions of the prefrontal cortical surface area and superior temporal regions, which is in accordance with previous studies using fMRI and PET (Cabeza and Nyberg, 2000) (Fig. 1).

3.3. Potential confounding factors

With the sole exception of medication dosage, potential confounding factors such as age, gender, handedness, education, premorbid IQ, task performance (total number of correct words generated), and clinical symptoms were not significantly different between the two Sig-1R genotype subgroups in either diagnostic group (Table 1). The first 5-s slope and mean change of [oxy-Hb] and [deoxy-Hb] were not different between women and men for either diagnostic group (two-tailed t-test: p>FDR 0.05). In healthy controls, the first 5-s slope and mean change of [oxy-Hb] and [deoxy-Hb] were not significantly correlated with any potential confounding factors (i.e., age, handedness, education, premorbid IQ, and task performance) at any channel (Pearson's correlation coefficient: p>FDR 0.05). In patients with schizophrenia, the first 5-s slope and mean change of [oxy-Hb] and [deoxy-Hb] were also not significantly correlated with any potential confounding factors (i.e., age, handedness, education, premorbid IQ, task performance, PANSS scores, chlorpromazine equivalent dose, biperiden equivalent dose and diazepam equivalent dose) at any channel (Pearson's correlation coefficient: p>FDR 0.05). The types of antipsychotic drug of the two Sig-1R genotype subgroups were as follows: Gln/Gln individuals, only first-generation 7 (35.0%), only second-generation 6 (30.0%), both drugs 7 (35.0%); Pro carriers, only first-generation 11 (55.0%), only second-generation 5 (25.0%), both drugs 4 (20.0%); chi-square test, df = 2, p = 0.41, n.s.).

3.4. Group comparisons in [Hb] response for significant genotype effects

First, we sought to confirm a significant difference in [oxy-Hb] response between the two diagnostic groups as shown in previous

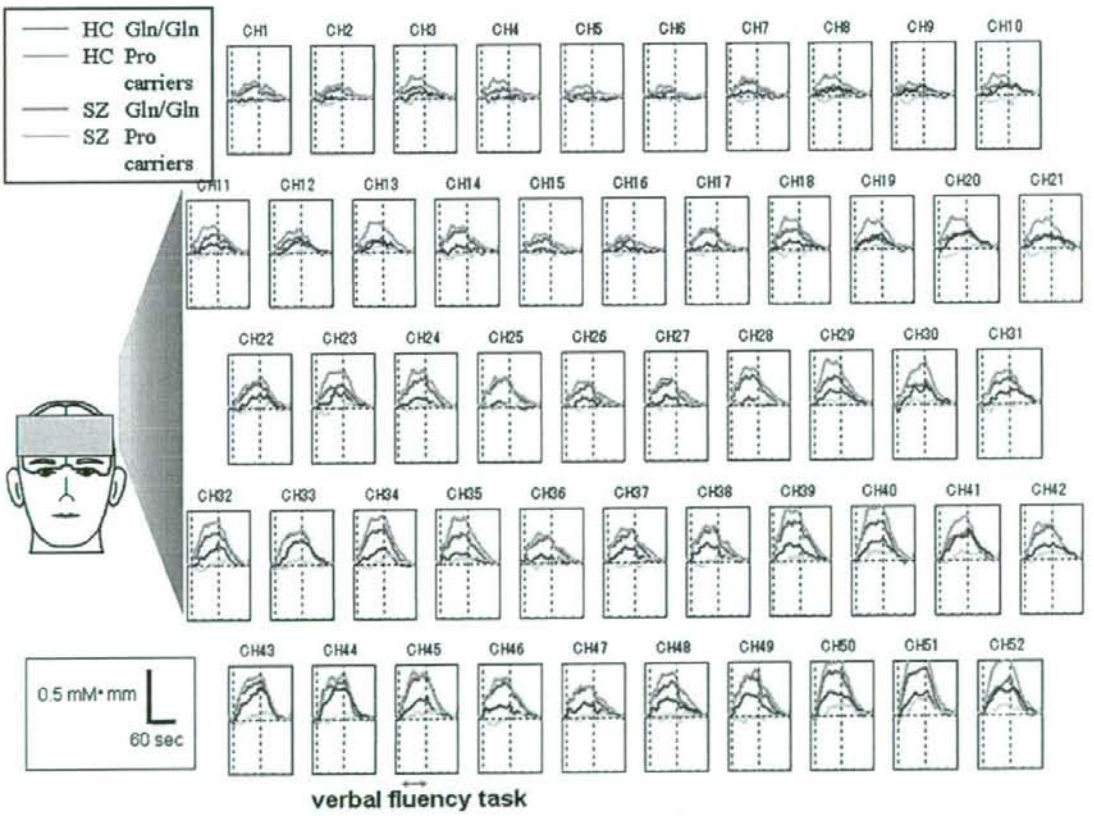

Fig. 1. Relationship between Sig-1R genotype and grand average waveforms in 52 channels in healthy controls (HC) and patients with schizophrenia (SZ). [oxy-Hb] changes during cognitive activation are presented as grand average waveforms in red (Gln/Gln individuals in HC), pink (Pro carriers in HC), blue (Gln/Gln individuals in SZ) and light-blue (Pro carriers in SZ) lines, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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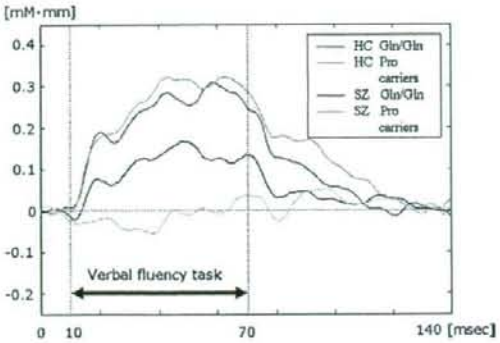

Fig. 2. Relationship between Sig-1R genotype and grand average waveforms in a representative channel (channel 38; left frontopolar region) in healthy controls (HC) and patients with schizophrenia (SZ). [oxy-Hb] changes during cognitive activation are presented as grand average waveforms in red (Gln/Gln individuals in HC), pink (Pro carriers in HC), blue (Gln/Gln individuals in SZ) and light-blue (Pro carriers in SZ) lines, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

NIRS literatures (Suto et al., 2004; Takizawa et al., 2008). As predicted, the schizophrenia patients were associated with a significantly lower [oxy-Hb] increase during the 60-s task period than the healthy controls at all 52 channels (p<FDR 0.05) (Fig. 2). The patients with schizophrenia were associated with a significantly larger [deoxy-Hb] decrease during the 60-s task period than the healthy controls at 21 channels (ch19, ch24, ch28-29, ch31, ch33-35, ch37-45, ch 47, ch49-51; FDR-corrected p: 0.0001–0.020). We then investigated the differences between the two genotype subgroups in each diagnostic group.

In the healthy controls, the first 5-s [oxy-Hb] slope and mean [oxy-Hb] changes during the 60-s task period showed no significant difference at any channel between the Gln/Gln individuals and the Pro carriers (p>FDR 0.05) (Figs. 1 and 2).

In the patients with schizophrenia, the [oxy-Hb] increases in the Gln/Gln individuals were significantly greater than those in the Pro carriers at 11 channels that corresponded approximately to the bilateral FP, the left DL and left VL regions and the left premotor cortical area ([PM]) (FDR-corrected p: 0.0001–0.0011; [FP]: ch37 (d=0.87; 95%CI 0.16, 1.54), ch38 (d=1.11; 95%CI 0.39, 1.78), ch47 (d=0.92; 95%CI 0.21, 1.58), ch48 (d=1.27; 95%CI 0.51, 1.96); [DL]: ch8 (d=1.05; 95%CI 0.24, 1.80), ch18 (d=0.94; 95%CI 0.24, 1.60); [VL]: ch39 (d=1.16; 95%CI 0.46, 1.82), ch49 (d=1.16; 95%CI 0.42, 1.83), ch50 (d=1.05; 95%CI 0.31, 1.74); [PM]: ch10 (d=0.99; 95%CI 0.16, 1.76), ch21 (d=1.20; 95%CI 0.44, 1.90)) over the prefrontal cortex (Figs. 2–3 and Table 2). The first 5-s [oxy-Hb] slopes were not significantly different between the two genotype subgroups (p>FDR 0.05).

However, the first 5-s slope and mean changes of [deoxy-Hb] during the 60-s task period showed no significant difference at any channels between the Gln/Gln individuals and the Pro carriers in either diagnostic group (p>FDR 0.05).

3.5. Genotype effects controlling for dose of medication in schizophrenia

We performed additional analyses in patients with schizophrenia because chlorpromazine equivalent dose (CPZ) was not well matched in the larger sample. First, the results of a one-way analysis of covariance (ANCOVA) with CPZ as a covariate in the significant channels ([FP]:ch37, ch38, ch47, ch48; [DL]: ch8, ch18; [VL]:ch39, ch49, ch50; [PM]: ch10, ch21) also showed a significant main effect of Sig-1R genotype (ch37 (F=9.350, df=1, p=0.004), ch38 (F=8.673, df=1, p=0.006), ch47 (F=7.762, df=1, p=0.009), ch48 (F=16.524, df=1, p<0.000); [DL]: ch8 (F=15.325, df=1, p<0.000), ch18 (F=11.554, df=1, p=0.002); [VL]: ch39 (F=11.965, df=1, p=0.001), ch49 (F=9.845, df=1, p=0.004), ch50 (F=6.210, df=1, p=0.018); [PM]: ch10 (F=7.036, df=1, p=0.013), ch21 (F=14.798, df=1, p=0.001)).

Second, we matched the two genotype subgroups for medication dose, which reduced the subject numbers for further analysis (N=13 for each genotype subgroup in schizophrenia). In the patients with schizophrenia, the [oxy-Hb] changes in the Gln/Gln individuals (N=13) remained significantly greater than those in the Pro carriers (N=13) at 4 channels that corresponded approximately to the left FP, the left DL and left VL regions, and the left PM (FDR-corrected p: 0.0001–0.0005; [FP]: ch48 (d=1.73; 95%CI 0.70, 2.64); [DL]: ch8 (d=1.43; 95%CI 0.34, 2.39); [VL]: ch39 (d=1.67; 95%CI 0.71, 2.51); [PM]: ch10 (d=1.42; 95%CI 0.25, 2.43)) over the prefrontal cortex. All the same, the first 5-s [oxy-Hb] slopes were not significantly different between the two genotype subgroups (p>FDR 0.05). The genotype effects on prefrontal hemodynamic response remained significant after controlling the medication effects as the two Sig-1R genotype subgroups were matched for medication dose (chlorpromazine equivalent dose: Gln/Gln individuals; 582.6 ± 383.0 mg/day,

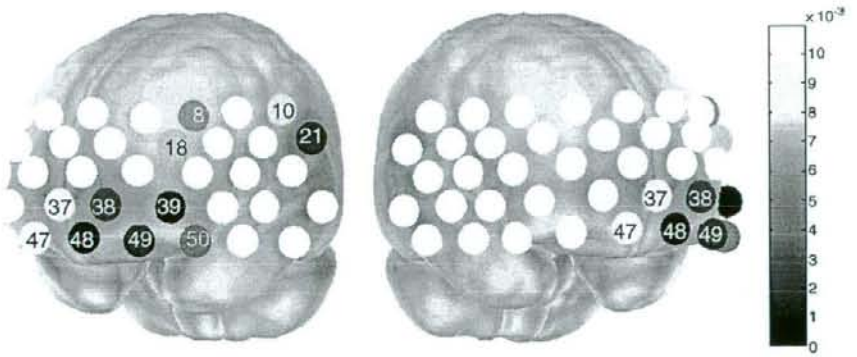

Fig. 3. Cortical distribution of significant [oxy-Hb] difference by Sig-1R genotype in patients with schizophrenia. The 52 measurement positions of the NIRS machine are superimposed on a 3D-reconstructed cerebral cortical surface from a MNI template. To illustrate the gradation in p value over the prefrontal cortical surface area, those channels with a significant difference by genotype ($p \leq$ FDR 0.05; 0.0001–0.0011) between Gln/Gln individuals and Pro carriers among patients with schizophrenia are indicated by colored areas. These areas approximately correspond to the bilateral frontopolar (BA 10), the left dorsolateral (BA 9 and 46) and left ventrolateral (BA 44, 45 and 47) PFC regions and the left premotor cortex. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Table 2
Channels with significant genotype difference in patients with schizophrenia.

Cortical region	ch	d	95%CI		FDR-corrected p-value
Frontopolar [FP]	37	0.87	0.16	1.54	.0094
	38	1.11	0.39	1.78	.0019
	47	0.92	0.21	1.58	.0096
	48	1.27	0.51	1.96	.0007
Dorsolateral [DL]	8	1.05	0.24	1.80	.0059
	18	0.94	0.24	1.60	.0068
Ventrolateral [VL]	39	1.16	0.46	1.82	.0008
	49	1.16	0.42	1.83	.0015
	50	1.05	0.31	1.74	.0044
Premotor [PM]	10	0.99	0.16	1.76	.0072
	21	1.20	0.44	1.90	.0019

Abbreviations: ch, channel; d, Cohen's effect size; CI, confidence interval; FDR, false discovery rate.

Pro carriers; 671.3 ± 325.6 mg/day; $p = .53$; biperiden equivalent dose: Gln/Gln individuals; 2.4 ± 1.7 mg/day, Pro carriers; 3.8 ± 2.2 mg/day; $p = .08$; diazepam equivalent dose: Gln/Gln individuals; 5.4 ± 4.9 mg/day, Pro carriers; 12.4 ± 19.5 mg/day; $p = .23$). The types of antipsychotic drug used for the two Sig-1R genotype subgroups were as follows; Gln/Gln individuals, only first-generation 3 (23.0%), only second-generation 5 (38.5%), both drugs 5 (38.5%); Pro carriers, only first-generation 6 (46.2%), only second-generation 3 (23.1%), both drugs 4 (30.7%); chi-square test, $df = 2$, $p = .45$, n.s.).

4. Discussion

This study demonstrated that, using 52-channel NIRS over the PFC, prefrontal hemodynamic response during a verbal fluency task was significantly associated with the effect of Sig-1R genotype in the patients with schizophrenia, but not in the healthy controls. In the patients with schizophrenia, the increase in [oxy-Hb] in the Sig-1R Gln/Gln individuals during the verbal fluency task was significantly greater than that in the Sig-1R Pro carriers in the PFC, whereas these differences were found to be not significant in the healthy controls. These impacts of Sig-1R genotype remained significant and the effect size became much larger in the patients with schizophrenia, even after controlling medication dose. Other potential confounding factors such as age, gender, handedness, education, premorbid IQ, task performance, and clinical symptoms were not likely to account for the significant effect of Sig-1R genotype on prefrontal hemodynamic response in schizophrenia.

4.1. Significant genotype effect in patients with schizophrenia

To our knowledge, this is the first imaging genetics study that implicated the significant association between Sig-1R genotype and prefrontal cortical function in patients with schizophrenia.

Gln2 allele is part of the amino acid sequence motif MQWAVGRR at the N-terminus, which is thought to be an endoplasmic reticulum retention signal (Schutze et al., 1994) and the substitution of the Gln2 allele by the Pro2 allele may perturb the appropriate regulation of the transport of Sig-1Rs from the endoplasmic reticulum to the plasma membrane. Thus, Gln2Pro polymorphism might be part of the putative endoplasmic reticulum retention signal. Also, a gene-reporter assay showed that, relative to that of the GC-241-240/Gln2 haplotype, the transcription activity of the TT-241-240/Pro2 haplotype was reduced by 42.7% in HepG2 cells, 33.9% in HeLa cells, and 33.3% in HCN-1A cells (Miyatake et al., 2004). Thus, GC-241-244TT/Gln2Pro polymorphism might be related to changes in transcriptional regulation (Ishiguro et al., 1998) and the TT-241-240/Pro2 haplotype of the Sig-1R gene results in the deficient expression of Sig-1R gene mRNA (Uchida et al., 2005). Taken together, it might be reasonable to conclude that the prefrontal hemodynamic response of Gln/Gln individuals is greater than that of Pro carriers among patients with schizophrenia.

Genetic association studies (Ohmori et al., 2000; Satoh et al., 2004) except the first such study (Ishiguro et al., 1998) have shown no significant association between Sig-1R genotype and schizophrenia. However, the present imaging genetics study revealed a significant association between Sig-1R genotype and prefrontal cognitive function in schizophrenia. These findings might partly support the notion that brain function by neuroimaging as an intermediate phenotype could be a more sensitive tool for understanding how neurobiology bridges the gap from genes to behavior and psychiatric symptoms than for studying the gene itself, as shown by association studies (Roffman et al., 2006).

4.2. No genotype effect in healthy controls

The Sigma-1 genotype effects on *in vivo* brain function were found in only the schizophrenia group not in the control group, a finding that deserves discussion. One possible explanation is as follows. The reproducible time-course pattern of changes in intracellular calcium transients in cardiomyocytes cell culture induced by selective Sig-1R agonist, with an initial decrease followed by an increase, and a final decrease, appeared to be mediated by corresponding changes in calcium influx (Ela et al., 1994). Sig-1R agonists did not cause intracellular Ca^{2+} mobilization, neuronal firing and dopamine release by themselves, and they exerted their modulatory action when the IP3 receptor, K+ channel or NMDA channel was activated by transmitters or depolarization (Hayashi et al., 2000; Monnet et al., 1990; Nuwayhid and Werling, 2003), which suggested that Sig-1R receptors appear to play an important role as a modulator or an amplifier of signal transduction in systems such as NMDA channel activity, Ca^{2+} signaling and NGF-induced cell differentiation in the brain (Su and Hayashi, 2003). In behavioral studies of memory and depression, the action of Sig-1R ligands seems to exert beneficial effects only when brain functions or neurotransmissions are perturbed (Hayashi and Su, 2004; Maurice et al., 2001). In a coherent manner with the neuromodulatory action at the cellular level, the lack of involvement of Sig-1Rs in normal memory function is presently understood as a result of its putative modulatory role. It would not be until brain functions begin to develop abnormalities that Sig-1Rs exert a cell-protective property and elicit the beneficial effect of Sig-1R genotype on transcription regulation (gene expression) and Sig-1R transport in the cell. As these abnormalities develop to a considerable extent in patients with schizophrenia, the amount of brain Sig-1Rs might diminish significantly, and indeed, this has been shown in postmortem studies (Tam and Zhang, 1988; Weissman et al., 1988). In accordance with the above discussion, there was no association between Sig-1R genotype and the binding potential of [11C]SA4503 in any of the regions of the brain in healthy male subjects (Ishikawa et al., 2007).

Another speculation is possible: an inverted-U shape in the relationship between prefrontal dopamine level and hemodynamic response (Goldman-Rakic et al., 2000) might have been modulated by Sigma-1 receptors, which resulted in a difference in the effect of sigma-1 genotype variation on NIRS signals between the patients with schizophrenia and the healthy controls. This is why the two channels that indicated a trend-level difference (uncorrected $p < 0.01$ but not remaining significant after FDR correction) were located approximately in the bilateral dorsolateral ([DL]) regions (uncorrected $p < 0.01$; ch13 ($p = 0.0076$; $d = -0.83$; 95%IC −1.44, −0.18), ch19 ($p = 0.0038$; $d = -0.89$; 95%IC −1.53, −0.22)), which showed that the prefrontal hemodynamic response of the Pro carriers was greater than that of the Gln/Gln individuals among the healthy controls. These inverse genotype effects (with nonsignificance after FDR correction but large effect size) in the normal controls suggest that individuals with a Pro allele have dopamine levels that are not necessarily more functionally optimal than Gln/Gln individuals under normal conditions, which is different from the impact of Sig-1R genetic variation on prefrontal hemodynamic response in patients with schizophrenia.

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4.3. Clinical application and translational approach

Although there is concern about the possibility of false positive findings in imaging genetics studies when genetic variants (SNPs) of uncertain functional relevance are related to neuroimaging data, Meyer-Lindenberg et al. provided empirical data on false positive rates and concluded that type I error is well controlled and that positive neuroimaging findings in imaging genetics paradigms point toward positive neurofunctional effects that merit further study using translational approaches (Meyer-Lindenberg et al., 2008).

Sig-1R is a potential candidate genetic variant that may partly account for individual differences in the efficacy of pharmacological psychiatric treatment. In the present study, the antipsychotic medication dose in use was significantly different between the two Sig-1R genotype subgroups, which suggested that a subpopulation of patients with the Sig-1R Gln/Gln genotype might respond more favorably to antipsychotic treatment. In clinical setting, however, psychiatrists would want that information in the early stage of treatment. Since we detected a relationship between Sig-1R genotype and prefrontal function in the patients with schizophrenia in this study, the prefrontal hemodynamic response assessed by NIRS may be considered an intermediate phenotype and might be a biological indicator of treatment response and efficacy prediction in advance of treatment. To that end, longitudinal and well-controlled clinical trial studies using translational approaches will be needed in the near future. Fortunately, NIRS has several beneficial features such as being more noninvasive, more portable, lower cost (almost no running cost), less restraining and less demanding for participants than other imaging methods. These features make NIRS highly desirable for application not only to neuroimaging research with large samples, but also to repeated clinical evaluations of individual patients.

4.4. Limitations

However, some methodological limitations should be mentioned. First, NIRS detects brain activations only from cortical surface areas, and cannot detect signals from deeper brain structures, for example, the amygdala or hippocampus. Consequently, the present study leaves the relationship between Sig-1R genotype and the function of deeper brain structures unresolved. Second, because the patients with schizophrenia were all medicated, the possibility that medication has affected the functional neuroimaging findings cannot be conclusively ruled out. However, the relationship between Sig-1R genotype and prefrontal cortical function in the patients with schizophrenia remained even after controlling medication dose, and there was no significant correlation between medication dose and hemodynamic response at any channel. Also, the present findings leave open the possibility that patients with the Sig-1R Gln/Gln genotype respond more favorably to antipsychotic treatment and that Pro carriers require a higher medication dose. Future studies with drug-naive or drug-free patients with schizophrenia are necessary. Moreover, the relationship between Sig-1R functional polymorphism (Gln2Pro) and ability to metabolize antipsychotics (e.g. cytochrome P450) is also an attractive topic and should be investigated in the future. Third, in the commercially available continuous-wave NIRS system, the signals are not absolute values but measures relative to the pretask baseline and also include a differential pathlength factor (DPF). The data accuracy of NIRS in terms of DPF should be considered. "Hemoglobin concentration change * DPF" (ΔC^*L) is calculated as a solution to the simultaneous equations based on the modified Beer–Lambert law in the continuous-wave NIRS system. At present, the real-time measurement of precise DPF at each channel is technically difficult *in vivo* (Hoshi, 2003). Although ΔC^*L includes DPF values, it has been reported that the results of ΔC^*L closely agree with previous clinical data (Fallgatter and Strik, 2000; Suto et al., 2004; Takizawa et al., 2008). These results suggest that NIRS signals including DPF (ΔC^*L) have valid clinical implications. Fourth, although some researchers emphasize a [deoxy-Hb] (Obrig and Villringer, 2003), the genotype effects of Sig-1R on [deoxy-Hb] response were too small to detect in this study. A different prefrontal task design that elicits a larger [deoxy-Hb] response will be needed to investigate [deoxy-Hb] changes associated with the Sig-1R genotype in future NIRS studies. Fifth, we have not yet established a standard statistical paradigm for analyzing NIRS signals. All statistical analyses in this study were performed using FDR correction for multiple corrections. Considering a relatively large effect size (between 0.87 and 1.27) in the significant channels, FDR correction for multiple corrections of NIRS signals might be too conservative. However, because this neuroimaging method is unfamiliar to psychiatric audiences and the sample size in this study was limited, we might as well use more conservative and stricter statistical methods at present.

5. Conclusions

In conclusion, this study provides the first *in vivo* evidence of an association between Sig-1R Gln2Pro variation and prefrontal hemodynamic response in schizophrenia. The evidence of association between Sig-1R genotype and increasing risk for developing schizophrenia remains to be elusive; however, this study may have implications for the use of imaging genetics in the translational approach in psychiatry. If the functional significance of this polymorphism in the pharmacological treatment of schizophrenia is clarified in future studies, noninvasive and less demanding NIRS could potentially be used as a biological marker to aid in the evaluation and prediction of response to medication.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.pnpbp.2009.01.014.

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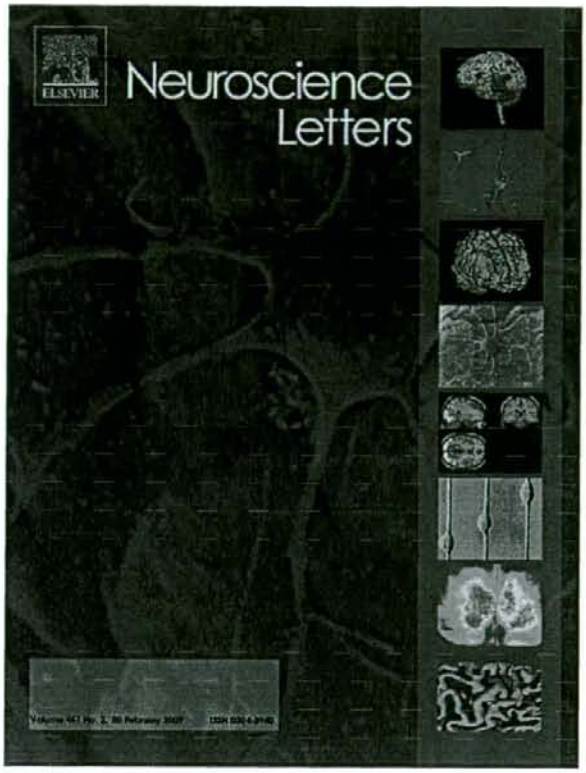

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Activation in ventro-lateral prefrontal cortex during the act of tasting: An fNIRS study

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ABSTRACT

The act of tasting is the product of inseparable integrative behavior consisting of multi-sensory processing and orolingual motor coordination. Often tasting-induced brain activity is looked at in a reductionist manner as a set of isolated components. However, brain activity as a whole during tasting may not simply be the sum of isolated brain responses; therefore, attempting to look at the cortical activation in a more holistic manner is important. Using functional near-infrared spectroscopy (fNIRS), we assessed cortical responses during tasting, contrasting observed neuronal activation of the lateral prefrontal cortex (LPFC), of 19 healthy participants before and during tasting of 8 ml of sweet-based solutions. To examine the activated brain structure, we estimated the anatomical regions of the measured location in standard brain space. We also included simple tongue tapping movement (TT) and word fluency (WF) tasks as comparative functional markers. Significant activity was found in channels (CHs) estimated to be in the bilateral oral motor areas during the TT task, and those in the LPFC, primarily in the left hemisphere, during the WF task. During the tasting task, significant activation was observed in CHs estimated to lie in the ventral part of pre- and post-central gyri as well as in the ventro-LPFC (VLPFC). The activated regions partly overlapped with those detected during TT or WF tasks, but extended more anteriorly and ventrally. Our study suggests that, in addition to tongue motor areas, the VLPFC is involved in the act of tasting.

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Tasting food involves not only taste processing, but also complex multimodal processing including oral-somatosensation, olfaction, oral motor functions, and some cognitive functions [18]. The central processing related to these functions has been studied mainly by assessing each effect separately. For example, taste-related brain functions have been assessed by comparing the cortical activation elicited by taste and tasteless solutions to exclude other sensory and motor effects [10]. Thus, cortical regions involved in each element related to tasting have been successfully elucidated [20]. However, brain responses during tasting as a whole may not be the same as their componential sum. Therefore, a more holistic approach may be needed to understand the neural basis for feeding behaviors.

In this report, we explore lateral prefrontal (LPFC) activity during tasting using functional near-infrared spectroscopy (fNIRS). To now, the major human brain mapping techniques used for taste related functions are positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and magnetoencephalography (MEG). These neuroimaging techniques often require participants to be fixed in a supine position with strict movement restrictions, and often necessitate specialized stimuli presentation methods (e.g., stimuli presentation via a small hole in a tube to a specific part of the tongue [12]). They are well designed to optimize the use of each neuroimaging technique, but are very different from the conditions under which we taste in our everyday lives. In contrast, fNIRS, an optical method that non-invasively measures cortical hemodynamic responses [11], is relatively forgiving of body movement, and is less restrictive (Fig. 1A). In fNIRS measurements, participants simply wear a set of probes on their heads, and can, therefore, taste samples in an upright position [13]. In this respect, fNIRS has better potential for examining brain responses in a holistic manner while tasting under more natural conditions than other neuroimaging techniques.

fNIRS can measure the responses of lateral cortical surfaces, but not gustatory areas such as the insular and orbitofrontal cortices, which are located deep inside the brain where the near-infrared light cannot reach. Among the regions where fNIRS can be used, the LPFC is of interest, as a recent study has suggested that taste and other food-related activities occur in this area [9]. Using fMRI, Kringelbach et al. reported activity in the dorso-LPFC (DLPFC) related to taste, and suggested that taste constantly evokes cognitive processes mediated by this region [9]. This finding evoked research interest in fields associated with feeding behaviors, such as obesity and eating disorders [26]. In our fNIRS experiments, however, we consistently observe activities in more ventral areas, in addition to the DLPFC, during tasting in contrast with resting

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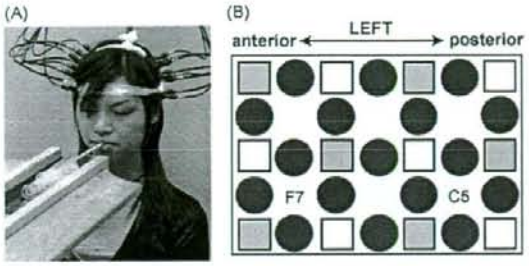

Fig. 1. Experiment setting. (A) One of the authors receiving a taste solution is monitored using multi-CH fNIRS for demonstration. She is connected to the fNIRS equipment behind her with optical fibers whose ends, consisting of illuminator-detector pairs, are attached to the surface of her head. (B) Schematic depiction of CH arrangements for the left hemisphere. A CH (gray circles) is set between each pair of detectors (gray squares) and illuminators (white squares) resulting in 17 CHs per hemisphere. Orientations of the illuminator-detector pairs and the 10–10 marker positions used as cranial landmarks are indicated.

conditions. If we widen our scope from pure taste processing to the act of tasting as a whole, the involvement of larger LPFC areas may be observed, yielding a more holistic picture of tasting.

LPFC areas, which include the motor language areas, are adjacent to oral sensori-motor areas. As fNIRS does not provide anatomical information of the measured brain, we examined the locations of tasting-related activities in relation to these well-known functional sub-regions by measuring brain activities of a simple tongue tapping movement (TT) task as well as a word fluency (WF) task. In addition, we estimated anatomical regions using a database by probabilistically registering the measured location to the standard brain space as previously described [19]. In this way, we sought to characterize the activation location related to tasting measured by fNIRS.

Nineteen participants (13 females; 6 males; average age = 32.1 years; S.D. = 6.9; range: 23–44 years) participated in this study. We excluded four participants' tasting data because of event-related noise likely due to temporal muscle movements. One participant could not attend the WF task session. Written informed consent was obtained after a complete explanation of the study. The study was approved by the National Food Research Institute's institutional ethics committee.

Participants performed three tasks in separate sessions: a tasting task, a word fluency (WF) task, and a tongue tapping (TT) task. All the tasks were performed using a block design. In all the sessions, participants sat in a quiet room with their eyes closed during the trials. An experimenter monitored the participants' behavior throughout, and the trials with event-related noise were removed from analysis. The order of the sessions was randomized across participants.

The WF and TT sessions shared the same time schedule, and consisted of 10 trials each. For each trial, task blocks were 20 s, and spaced 20–24 s apart. In WF sessions, the experimenter announced a word from one of ten categories (e.g., fruit, countries, etc.) at the onset, and participants were instructed to silently (internal speech) think of as many nouns as possible in the given category during the task period, stopping when instructed. In TT sessions, the participants touched their tongue to the back of each of their upper teeth sequentially from the right back tooth to the left back tooth and back again, at a rate of approximately 1.2 Hz during the task period. The start and the end of the task period were indicated orally using the words "start" and "stop."

Tasting sessions consisted of 6 trials, each consisting of 40 s of rest, 20 s of tasting, and 10 s of rinsing, with about 60 s inter-trial intervals. We used fewer trials and longer rest/interval blocks than the other tasks to avoid sensory fatigue, and to provide enough time for fNIRS signals to stabilize before the baseline period after the straw insertion or mouth rinsing [13]. At the beginning of the rest period, an experimenter inserted the end of a straw attached to a syringe into the participant's mouth and manually injected 8 ml of a tasting sample at the onset of the tasting period. Each participant held the sample in his/her mouth during the entire tasting block and then spit it out to avoid satiety. We used a total of 8 sugar-based taste stimuli with slightly different concentrations of sour, salty, and umami/savory tastes (Supplemental Table 1). The stimuli were made so as to avoid overall bias toward specific characteristics (e.g., not all were intense, unpleasant, etc.) to minimize and counter-balance the effect of emotional reactions. The selection of samples was counter-balanced evenly across participants, the order of presentation was randomized between trials, and the samples were served at room temperature. After the fNIRS session, we asked each participant to taste the samples again, evaluating the familiarity, pleasantness, and intensity of each. We used a scale of 1–5 (5 = very familiar/pleasant/strong, 3 = neutral, 1 = very unfamiliar/unpleasant/weak). The post-experiment ratings revealed that, on average, taste samples were perceived as neutral regarding pleasantness (mean rating score 3.0; S.D. = 0.9), intensity (mean rating score 3.4; S.D. = 1.0), and familiarity (mean rating score 3.1; S.D. = 1.0).

We used the fNIRS topography system OMM-2000 Optical Multi-channel Monitor (Shimadzu, Kyoto, Japan), which uses near-infrared light with wavelengths of 780, 805, and 830 nm. We set 6 illuminator-detector pairs (distance between illuminator and detector = 3 cm) in a 3 × 4 lattice pattern to form 17 channels (CHs), and placed one holder over the frontal region of each hemisphere, using F7 (F8) and C5 (C6) of the international 10–10 system as reference points for holder placement (Figs. 1B and 2A). We analyzed optical data based on the modified Beer–Lambert Law, and calculated signals reflecting the oxygenated hemoglobin (oxyHb) and deoxygenated hemoglobin (deoxyHb) concentration changes, in an arbitrary unit (millimolar–millimeter) as previously described [13].

Statistical analysis was performed using (task–rest) contrast for each task using a random effects summary statistics approach as previously described [13]. A boxcar function was employed at the first level to generate participants' contrasts for all CHs. At the second level, the single sample t test (one-tail) was employed to test increases of oxyHb and decreases of deoxyHb. The resulting p-values from all CHs were thresholded using the false discovery rate control (FDR) method (P < FDR 0.05) as previously described [13], for multiple testing correction.

We used our probabilistic estimation method [19] to estimate CH location to the Montreal neurological institute (MNI) standard brain space. Briefly, fNIRS optode positions, together with several scalp landmarks, were digitized using a 3D magnetic space digitizer (FASTRAK – Polhemus, Colchester, VT). Referring to our structural head MRI database, we performed a registration simulation and estimated locations of given CHs and their estimation errors for our participants (Fig. 2; Supplementary Fig. 1 and Supplementary Table 2). We anatomically labeled these locations using a Matlab function (available at http://brain.job.affrc.go.jp), which reads anatomical labeling information coded in a brain atlas constructed by Shattuck et al. [17]. Based on this estimation, we selected CHs located on the frontal lobe and the post-central gyri for the functional analysis.

We observed significant activations in different LPFC areas across different tasks (Fig. 2). In the TT task, a significant increase in oxyHb was observed in CHs located in bilateral pre- and post-central gyri (P < FDR 0.05). In the WF task, CHs covering part of the inferior frontal gyrus (IFG) and middle frontal gyrus (MFG), mainly in the left hemisphere, exhibited a significant oxyHb increase (P < FDR 0.05). In the tasting task, an increase of oxyHb was observed in CHs covering part of the precentral gyrus, MFG, and IFG in both hemispheres (P < FDR 0.05). Only the TT task induced a significant

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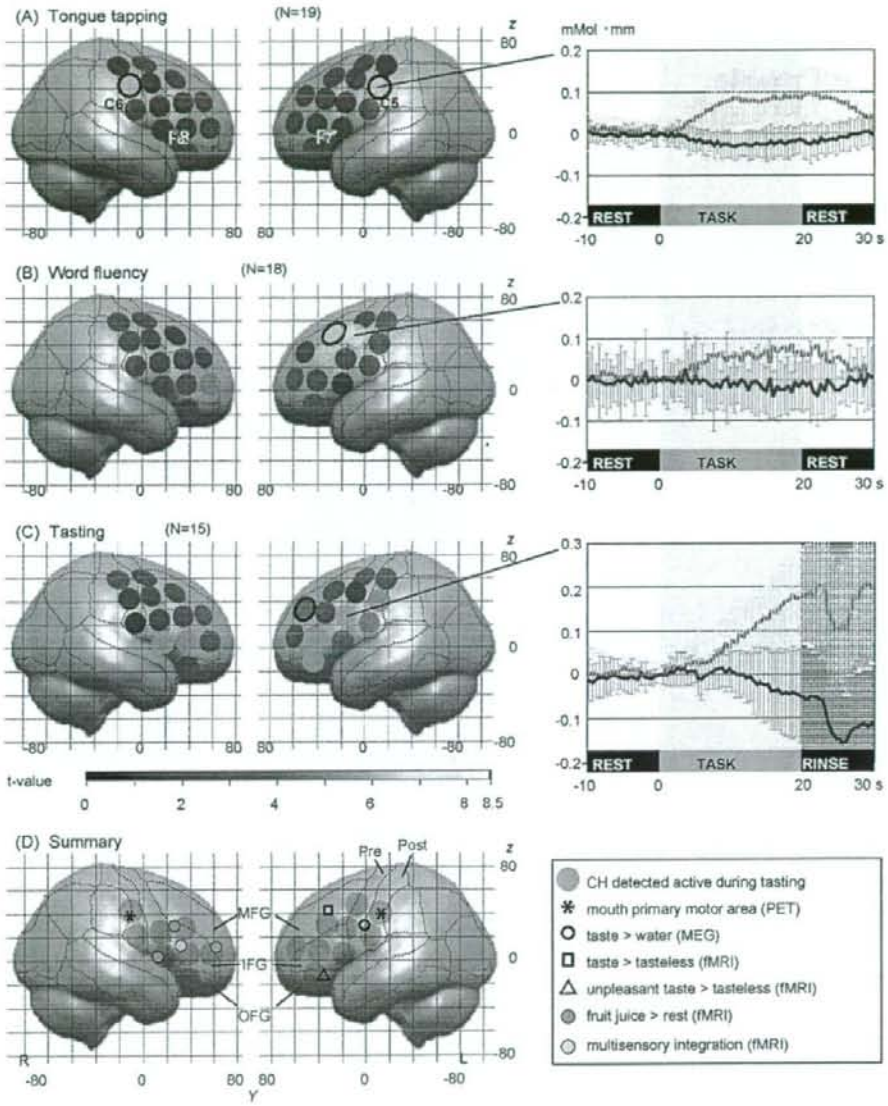

Fig. 2. Results of fNIRS analyses (A–C). CH-wise results are shown on our template brain in MNI space. The CHs (filled circles) that exhibited significant oxyHb increase (P < FDR 0.05) are colored according to the t-values, as shown in the color bar, while those below the threshold are indicated in gray. The CHs that exhibited significant deoxyHb decrease (P < FDR 0.05) are indicated with open blue circles. Pink dotted lines show borders of anatomical regions. Marker positions of the international 10–10 system we used as reference points for holder placement are indicated in (A). The time courses of oxyHb (red lines) and deoxyHb (blue lines) from the CH that exhibited the highest t-value based on oxyHb analysis in each experiment is shown on the right. Error bars indicate standard deviation across participants. Error bars for post-task rinsing period (shaded area) that includes noise due to muscle movements are truncated. (D) Locations of activation foci referred to in the discussion are shown based on MNI coordinate values reported in original papers. Activations that were neither located within 1 cm of the lateral brain surface nor reported with coordinate values are not included. The activated CHs for the tasting condition are shown as filled pink circles.

decrease in deoxyHb, centering in the same CHs where we detected a significant increase of oxyHb (P < FDR 0.05). For the WF and tasting tasks, in the CHs exhibiting significant oxyHb increase, deoxyHb tended to decrease, but inter-subject variability was large and the decrease was not significant in most of the CHs.

These results are in line with previous studies. Regarding location, the areas activated by the TT task correspond to those identified as oral sensory-motor areas by meta-analysis of PET studies [4], and left lateralized activation in the LPFC region during the WF task is in line with earlier fMRI studies using this task (earlier studies are listed in [3]). Regarding the behavior of each Hb signal, simultaneous recordings of fNIRS and fMRI during motor tasks have produced oxyHb and deoxyHb changes consistent with fMRI signals in primary sensori-motor areas corresponding to the task [24]. As with these studies, both oxyHb and deoxyHb changes were observed in oral motor areas during our TT task. For language tasks,

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fNIRS studies have detected increases of oxyHb in the left LPFC area where fMRI signal increases have been detected, but a decrease of deoxyHb was not always observed [2]. Similarly, oxyHb signal changes were observed in the left LPFC during our WF task.

For the tasting task, the taste-rest contrast we examined includes multiple effects: taste, oral somato-sensory, and oral movement. As expected for this contrast, we detected oxyHb increase in the same oral sensory-motor area as for the TT task [4] (Fig. 2D blue asterisks) as well as in more ventral regions in both hemispheres. Somatotopically, the pre- and post-central regions ventral to the tongue region represent the region for the laryngopharyngeal organ [14]. Activations observed in the ventral parts of pre- and post-central gyri are also consistent with activations related to taste stimuli [12] (Fig. 2D, blue circle), and salivation [15]. The portion of activations observed in the DLPFC may be related to the taste-related activation reported by Kringelbach et al. [9] (Fig. 2D, blue square).

However, the areas where we found oxyHb increases extended ventrally and anteriorly in the IFG and part of the MFG: areas not often associated with oral sensori-motor, or taste processing, but which are located over the frontal operculum, a structure known to be involve in gustatory processing. It may be that activity in the frontal operculum itself was observed; however, this is unlikely as it is too deep to be measured using fNIRS [5]. As the bilateral activation observed did not overlap completely with that occurring during the WF task, internal speech evoked during tasting, if any, cannot be the only factor for this activation.

Of the few taste-related studies reporting activation in the ventro-LPFC (VLPFC) (including the IFG), one fMRI study reported activation related to aversive taste [21] (Fig. 2D, blue triangle). However, this does not seem to be the case here because subjective rating scores indicate that our taste stimuli were, on average, neutral regarding their pleasantness. Other studies have included brain response to electrically evoked taste [1], and to taste or flavor stimuli in contrast to weak-tasting solutions [23] or water [6], but activation foci reported in these studies appear to be deeper than the lateral brain surface, according to the reported coordinates.

Taste-related VLPFC activation may not be detected using fMRI, whose signal is considered to mainly reflect changes of deoxyHb. However, this does not explain why PET and MEG studies have not detected activation in this area. Also, regarding the VLPFC area, changes of oxyHb, rather than deoxyHb, was reported to agree more closely with fMRI results [27]. Therefore, the lack of significant deoxyHb changes does not sufficiently explain the difference of our fNIRS results and earlier taste-related neuroimaging results.

Alternatively, the activation we observed may correspond to that subtracted out in other taste studies using a different baseline to define contrasts: a tasteless solution as opposed to a simple resting state. Interestingly, there is an fMRI study reporting activation in the VLPFC (Fig. 2D, green circles) while participants tasted fruit juice. The baseline in their study was taken to be a resting activity rather than activity elicited by a tasteless solution. This may imply that this part of the brain is active when liquid is "tasted" in the mouth regardless of whether the liquid has taste or not. The VLPFC has been implicated in many other functions including integration of sensory information with the control of oral movements [7], and multi-sensory integration for creating flavor representation [22] (Fig. 2D, yellow circle). Although we cannot identify the specific role of these areas in tasting, such multi-sensory and motor integration functions may be involved during tasting, and may explain the activation we found.

In this study, there was only one CH where deoxyHb decreased significantly during the tasting task. This is possibly due to higher individual differences of behaviors of deoxyHb than that of oxyHb, as shown by studies using both animals [8] and humans [16], especially in the prefrontal area (summarized by Cannestra et al., in their introduction [2]). Considering our observations and these reports, deoxyHb behavior may be rather complicated, leading to the inconsistent trends we observed.As demonstrated, using fNIRS we detected activation not only in the oral-motor area, but also in the VLPFC when participants tasted 8 ml of aqueous taste solutions. Although more experiments with wider experimental conditions are needed to generalize our findings, our results indicate the possible involvement of these areas in tasting when the scope is broadened to include holistic taste processing. While it is important to examine the cortical process for each element separately, in order to understand the neural basis for tasting, examining the brain response to tasting as a whole is required. Such holistic tasting effects might also be of interest in clinical areas related to feeding behaviors such as obesity, eating disorders, or dysphasia: recently, using fNIRS, Uehara found that VLPFC activity differed between patients with eating disorders and healthy control participants during a word fluency task [25]. Whether such differences occur in tasting tasks may be worth future exploration.The compactness and flexibility of fNIRS allows for brain measurements under relatively natural tasting conditions, and for its use in clinical settings. The current findings are a step towards understanding the role of the LPFC in tasting, and fNIRS will further contribute to this issue, including possible clinical applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2008.12.016.

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Dose-dependent effect of the Val66Met polymorphism of the brain-derived neurotrophic factor gene on memory-related hippocampal activity

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Abstract

Brain-derived neurotrophic factor (BDNF) plays a critical role in activity-dependent neuroplasticity underlying learning and memory in the hippocampus. Recent human studies have indicated that a common single nucleotide polymorphism of the BDNF gene, the Val66Met polymorphism, has impact on episodic memory, hippocampal morphology and memory-related hippocampal activity measured by functional magnetic resonance imaging (fMRI). However, two issues remain to be clarified: (1) whether the genotype effect of this polymorphism on memory-related brain activity is allele dose dependent and (2) whether the effect of this polymorphism in Asian population is the same as effects observed in Caucasian sample. To clarify these issues, we studied the relationship of the Val66Met polymorphism genotype and hippocampal activity during episodic memory task using fMRI in healthy 58 biologically unrelated Japanese. Although there was no genotype effect on episodic memory function obtained by behavioral assessments, fMRI measurements revealed a significantly negative correlation between the dose of Met-BDNF allele and encoding related brain activity in the bilateral hippocampi and right parahippocampal gyrus. There was no genotype effect on retrieval related brain activity. These data indicated a genetic mechanism for normal variation in human memory and suggest effects of BDNF signaling on hippocampal function in humans.
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Keywords: Brain-derived neurotrophic factor (BDNF); Hippocampus; Human memory; Gene; Polymorphism; fMRI

1. Introduction

Brain-derived neurotrophic factor (BDNF), a member of neurotrophin family, has important roles in hippocampal plasticity and hippocampal-related learning and memory through long-term potentiation (LTP) (Poo, 2001). It is also important for long-term developmental phenomena, such as neuronal survival, differentiation, and activity-dependent refinement of synaptic architecture (Huang and Reichardt, 2001; Malcangio and Lessmann, 2003). A common single nucleotide polymorphism (SNP) of the BDNF gene producing an amino acid substitution of valine to methionine (Val66Met) affects intracellular packaging and activity-dependent secretion of BDNF (Egan et al., 2003; Chen et al., 2004). A previous study revealed that normal individuals with the Met-BDNF allele had poorer episodic memory accompanying with lesser engagement of the hippocampi during memory task observed in a functional magnetic resonance imaging (fMRI) when compared to individuals homozygous for the Val-BDNF allele (Hariri et al., 2003). They also demonstrated that Met-BDNF

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carriers had smaller hippocampus when compared to individuals homozygous for the Val-BDNF allele (Pezawas et al., 2004; Bueller et al., 2006).

Although these results strongly suggest that the Val66Met polymorphism of the BDNF gene should contribute inter-individual differences of human episodic memory function and memory-related brain structures and brain activity, at least two issues remain to be clarified. First, because of relatively small sample size, particularly homozygous for Met-BDNF allele, the previous study might demonstrate different brain activity related to memory task between homozygous for Val-BDNF allele and Met-BDNF carriers (Hariri et al., 2003). It has been unclear whether the genotype effect of the Val66Met polymorphism on memory-related brain activity is dose dependent. Indeed, their behavioral study with larger sample demonstrated dose-dependent effect of the Val66Met polymorphism on episodic memory performance in normal individuals (Egan et al., 2003). Second, some studies suggested racial differences of effects of the Val66Met polymorphism, such as discrepancy in associations between BDNF polymorphism and the prevalence of neuropsychiatric diseases (Sklar et al., 2002; Kunugi et al., 2004). In terms of brain morphology, our previous structural MR study demonstrated that effects of the Val66Met polymorphism on brain morphology in Japanese were different from those in Caucasians; i.e. the effect of the Val66Met polymorphism was noted in not hippocampal volume but volumes of the parahippocampal gyrus and caudate nucleus (Nemoto et al., 2006). Taken together, it would be possible that genotype effects of the Val66Met polymorphism on brain activity related to episodic memory task in Asian population may differ from those in Caucasians.

To clarify these two issues, we performed fMRI measurements with memory task in larger samples consisted of normal Japanese individuals.

2. Methods

2.1. Subjects

Fifty-eight healthy subjects participated in the study. Written informed consent was obtained from all the subjects in accordance with ethical guidelines in place at local ethical committee. All the subjects were recruited from local advertisements and underwent a Wechsler Adult Intelligence Scale-Revised (WAIS-R), Wechsler Memory Scale-Revised (WMS-R) and MRI scanning. They were also screened by a questionnaire on medical history and excluded if they had neurological, psychiatric or medical conditions that could potentially affect the central nervous system, such as substance abuse or dependence, atypical headache, head trauma with loss of consciousness, asymptomatic or symptomatic cerebral infarction detected by T2 weighted MRI, hypertension, chronic lung disease, kidney disease, chronic hepatic disease, cancer, or diabetes mellitus. All the subjects were biologically unrelated Japanese. Table 1 shows characteristics of subjects. The age range of the sample was 22–63 years (mean 36.4, standard deviation [S.D.] 10.6). According to genotypes, subjects were categorized into three groups; the homozygous Val-BDNF group ($n = 17$, male:female = 5:12, one was left-handed), the Val/Met-BDNF group ($n = 29$, male:female = 5:24, two were left-handed), and the remaining, homozygous Met-BDNF group ($n = 12$, male:female = 2:10, all were right-handed). The genotype distribution of this SNP was not deviated with Hardy–Weinberg equilibrium (chi-square = 0.003, $p = 0.95$). The one way analysis of variance revealed that there was no significant difference of age, education years, full scale IQ, or scores of WMS-R between groups (all $p > 0.05$). The chi-square test also revealed that there was no significant difference of distribution of gender ratio (d.f. = 2, chi-square = 1.1, $p = 0.57$), handedness (d.f. = 2, chi-square = 0.85, $p = 0.65$) or the ApoE genotype (d.f. = 2, chi-square = 2.9, $p = 0.24$).

2.2. Genotyping

Venous blood was drawn from the subjects and genomic DNA was extracted from whole blood according to the standard procedures. Genotyping the Val66Met SNP (dbSNP accession: rs6265) was performed with the TaqMan 5′-exonuclease allelic discrimination assay, described previously (Nemoto et al., 2006). Primers and probes for detection of the SNP (TaqMan SNP Genotyping assays on demand) were purchased from Applied Biosystems (ABI, Foster City, CA, USA). PCR cycling conditions were: at 95 °C for 10 min, 45 cycles of 92 °C for 15 s and 60 °C for 1 min. ApoE genotypes were determined by the polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) method. We used the primers designed by Hixson and Vernier (Hixson and Vernier, 1990). The procedures have been previously described in detail (Asada et al., 1996). Hha I—digested PCR fragments were subjected to gel electrophoresis and visualized by staining with ethidium bromide.

2.3. fMRI study

Cerebral activation was measured with fMRI using blood oxygen level-dependent contrast. After automatic shimming, a time course series of 145 volumes was obtained using single-shot gradient-refocused echo-planar imaging (TR = 4000 ms, TE = 60 ms, flip angle = 90°, in-plane resolution

Table 1
Characteristics of subjects

	Val/Val-BDNF	Val/Met-BDNF	Met/Met-BDNF	Genotype F (p)	Chi square (p)
Number of subjects	17	29	12		
Age	37.6 (2.9)	35.1 (10.1)	38.0 (10.6)	0.47 (0.63)	
Gender (M/F)	5/12	5/24	2/10		1.1 (0.57)
Handedness (R/L)	16/1	27/2	12/0		0.85 (0.65)
Education	15.4 (2.7)	16.4 (2.4)	15.9 (2.7)	0.97 (0.36)	
ApoE epsilon4 allele (non-carrier/carrier)	15/2	21/8	11/1		2.9 (0.24)
Full scale IQ (WAIS-R)	108.9 (13.7)	108.3 (12.2)	110. 8 (13.2)	0.12 (0.89)	
WMS-R					
Verbal memory	117 (12.4)	108.3 (12.8)	113.08 (11.8)	2.51 (0.09)	
Visual memory	109.5 (11.2)	108.9 (9.1)	106.3 (9.1)	0.41 (0.67)	
General memory	116.8 (11.7)	109.4 (12.0)	112.7 (10.7)	2.05 (0.14)	
Attention/concentration	106.6 (16.3)	104.1 (14.2)	105.9 (15.9)	0.14 (0.97)	
Delayed recall	116.9 (13.5)	110.4 (11.9)	108.3 (16.7)	1.81 (0.17)	

WMS-R (Wechsler memory scale-revised); mean value (standard deviation).

3.44 mm × 3.44 mm, FOV = 22 cm, contiguous 4-mm slices to cover the entire brain) with a 1.5 T MAGNETOM Vision plus MR scanner (Siemens, Erlangen, Germany) using the standard head coil. The first five volumes of each fMRI scan were discarded because of non-steady magnetization, with the remaining 140 volumes used for the analysis. Head motion was minimized by placing tight but comfortable foam padding around the subject's head. Before the collection of fMRI data for each subject, we acquired a reference EPI scan and visually inspected it for artifacts (i.e. ghosting) as well as for good signal across the entire volume of acquisition, including the medial temporal lobes.

The fMRI paradigm consisted of the encoding and subsequent retrieval of novel, complex scenes. Stimuli were presented in a blocked paradigm; seven encoding blocks were followed by seven retrieval blocks in an interleaved design with a passive viewing of scrambled picture condition (control condition). During encoding blocks, subjects viewed five images, presented serially for 4 s each, and were asked to remember them. All scenes were of neutral emotional valence and were derived from the International Affective Picture System (Lang et al., 1997). During subsequent retrieval blocks, subjects again viewed five images, presented serially for 3 s each, and determined whether each scene was "new" (presented during the encoding blocks) or "old" (not presented during the encoding blocks). In each retrieval block, half of the scenes were "old" and remaining half were "new". During the interleaved rest blocks, subjects were instructed to fixate on a centrally presented cross-hair. During scanning, all subjects responded by button presses with their dominant hand, allowing for the determination of accuracy and reaction time.

Analysis of the fMRI data were completed using statistical parametric mapping 2 (SPM2). Images for each subject were realigned to the first volume in the time series to correct for head motion, spatially normalized into a standard stereotactic space (Montreal Neurological Institute template) using a 12 parameter affine model and smoothed with a Gaussian filter, set at 8 mm full-width at half-maximum. Voxel-wise signal intensities were ratio normalized to the whole-brain global mean. Task-related activations were calculated using a t-statistic, producing a statistical image for the contrasts of encoding versus control and retrieval versus control for each subject. These individual contrast images were then used in second-level random effects models, which account for both scan-to-scan and subject-to-subject variability, to determine task-specific regional responses at the group level for the entire sample (main effects of tasks). We used $p < 0.001$, corrected for multiple comparisons with false discovery rate (FDR) < 0.05 as a statistical threshold for these whole-brain search. To test dose-dependent genotype effects of the BDNF gene, we performed correlational analysis. In this analysis, we treated Met-BDNF gene dose (i.e. the number of Met-BDNF allele in a person's BDNF genotype) as a covariate of interest and treated the genotype of the ApoE gene, age, and gender as nuisance variables. Because

Table 2
Brain activity related to encoding and retrieval

Brain regions	Brodmann area	Cluster size	Tarailach coordinates			Corrected p value	T value
			x	y	z		
Activity related to encoding (Fig. 1)							
Rt. Middle frontal gyrus	46	36	44	17	21	$<5.0 \times 10^{-4}$	4.5
Lt. Middle frontal gyrus	46	38	−44	17	25	5.0×10^{-3}	3.8
Rt. Parahippocampal gyrus, hippocampus	30/36/19	2617	36	−32	−22	$<5.0 \times 10^{-4}$	11.8
Lt. Parahippocampal gyrus, hippocampus	30/36/19	2617	−24	−39	−11	$<5.0 \times 10^{-4}$	8.9
Rt. Precuneus	19	2617	32	−72	33	$<5.0 \times 10^{-4}$	8.3
Lt. Precuneus	19	2617	−28	−76	37	$<5.0 \times 10^{-4}$	7.5
Lt. Superior parietal lobule	7	2617	−24	−72	44	$<5.0 \times 10^{-4}$	6.9
Rt. Fusiform gyrus	19/37	2617	28	−39	−11	$<5.0 \times 10^{-4}$	10.5
Rt. Middle temporal gyrus	19	2617	40	−81	19	$<5.0 \times 10^{-4}$	13.1
Rt. Angular gyrus	39	2617	36	−76	30	$<5.0 \times 10^{-4}$	9.7
Lt. Fusiform gyrus	37	2617	−48	−59	−17	$<5.0 \times 10^{-4}$	13.7
Lt. Middle temporal gyrus	19	2617	−40	−81	19	$<5.0 \times 10^{-4}$	9.7
Rt. Lingual gyrus	19	2617	32	−58	−4	$<5.0 \times 10^{-4}$	7.7
Rt. Middle occipital gyrus	37	2617	44	−70	7	$<5.0 \times 10^{-4}$	11.6
Lt. Superior occipital gyrus	39	2617	−32	−76	30	$<5.0 \times 10^{-4}$	8.2
Lt. Middle occipital gyrus	18/19	2617	−36	−86	−2	$<5.0 \times 10^{-4}$	17.5
Activity related to retrieval (Fig. 2)							
Lt. Middle frontal gyrus	9	74	−40	13	25	$<5.0 \times 10^{-4}$	6.4
Rt. Middle frontal gyrus	9/46	56	44	13	25	$<5.0 \times 10^{-4}$	5.4
Rt. Parahippocampal gyrus, hippocampus	19/30/36/37	2393	16	−50	3	$<5.0 \times 10^{-4}$	9.4
Rt. Posterior cingulate	29	2393	4	−42	6	$<5.0 \times 10^{-4}$	9.2
Lt. Parahippocampal gyrus, hippocampus	30/35/36/37	2393	−24	−47	−8	$<5.0 \times 10^{-4}$	6.2
Lt. Posterior cingulate	29	2393	−4	−42	6	$<5.0 \times 10^{-4}$	10.5
Rt. Precuneus	7	2393	4	−71	51	$<5.0 \times 10^{-4}$	7.3
Lt. Precuneus	7	2393	−4	−75	52	$<5.0 \times 10^{-4}$	6.4
Rt. Fusiform gyrus	20/37/39	2393	36	−55	−14	$<5.0 \times 10^{-4}$	14.8
Lt. Fusiform gyrus	37	2393	−44	−59	−17	$<5.0 \times 10^{-4}$	13.0
Rt. Middle occipital gyrus	18/19	2393	40	−85	4	$<5.0 \times 10^{-4}$	18.1
Lt. Lingual gyrus	18	2393	−8	−82	−13	$<5.0 \times 10^{-4}$	9.9
Lt. Middle occipital gyrus	19	2393	−28	−89	15	$<5.0 \times 10^{-4}$	15.6
Lt. Inferior occipital gyrus	18	2393	−40	−82	−6	$<5.0 \times 10^{-4}$	15.0
Genotype effects on brain activity related to encoding							
R Parahippocampal gyrus, hippocampus		44	27	−35	−5	$<5.0 \times 10^{-4}$	4.8
L Hippocampus		52	−36	−20	−9	2.0×10^{-3}	4.0
Genotype effects on brain activity related to retrieval							
Not significant							

of our *a priori* hypothesis regarding the differential response of the hippocampus and parahippocampal gyrus, a statistical threshold of $p < 0.05$, a small volume correction for multiple comparisons within the *a priori* volume of interest, was used to identify dose-dependent genotype effects on brain activity. For this hypothesis-driven analysis, we used the Wake Forest University PickAtlas (Maldjian et al., 2003). Whole-brain correlational analyses were also calculated using second-level random effect models. Because we had no *a priori* hypotheses regarding the activity of brain regions outside of the memory-related areas, we used a statistical threshold of $p < 0.05$, corrected for multiple comparisons across all supra-threshold voxels, for these whole-brain correlational analyses.

3. Results

3.1. Task performance during fMRI measurements

The percentage of correct answers (S.D.) for the retrieval task in each genotype was as follows; 98.9% (2.13) for Val/Val-BDNF group, 97.2% (6.06) for Val/Met-BDNF group, and 96.7% (3.89) for Met/Met-BDNF group, respectively. There was no significant genotype effect on the percentage of correct answers of the retrieval task ($F = 0.8$, $p = 0.45$). The mean reaction time (S.D.) of the retrieval task in each genotype was as follows; 1239 (232) ms for Val/Val-BDNF group, 1227 (175) ms for Val/Met-BDNF group, and 1312 (219) ms for Met/Met-BDNF group, respectively. There was no significant genotype effect on reaction time for the retrieval task ($F = 0.76$, $p = 0.48$).

3.2. Results of brain activity

When all subjects were treated one group, we found a significant activation in the bilateral hippocampus, parahippocampal gyri, visual association areas, parietal association areas including precunei and the dorsolateral prefrontal cortices during encoding period (Table 2 and Fig. 1). During retrieval period, a significant activation was also found in the bilateral hippocampus, parahippocampal gyri, posterior cingulate cortex (PCC), visual association areas, parietal association areas and the dorsolateral prefrontal cortices (Table 2 and Fig. 2).

Regarding brain activity related to encoding, a significantly negative correlation between the dose of Met-BDNF gene and brain activity was noted in the bilateral hippocampi and right parahippocampal gyrus (Table 2 and Figs. 3 and 4). On the other hand, a weak negative correlation between dose of Met-BDNF gene and brain activity related retrieval in the bilateral parahippocampal gyri at a lenient statistical threshold (uncorrected $p < 0.05$), however, no voxels could survive after the correction for multiple comparisons within the ROI (corrected $p > 0.05$). Since the previous study demonstrated genotype effect on retrieval related hippocampal activity in the group comparison (Val/Val BDNF subjects versus Met-BDNF carriers), we additionally performed the same analysis to estimate genotype effect on retrieval related activity. With a lenient statistical threshold (uncorrected $p < 0.05$), Val/Val-BDNF group showed stronger activation during retrieval in the

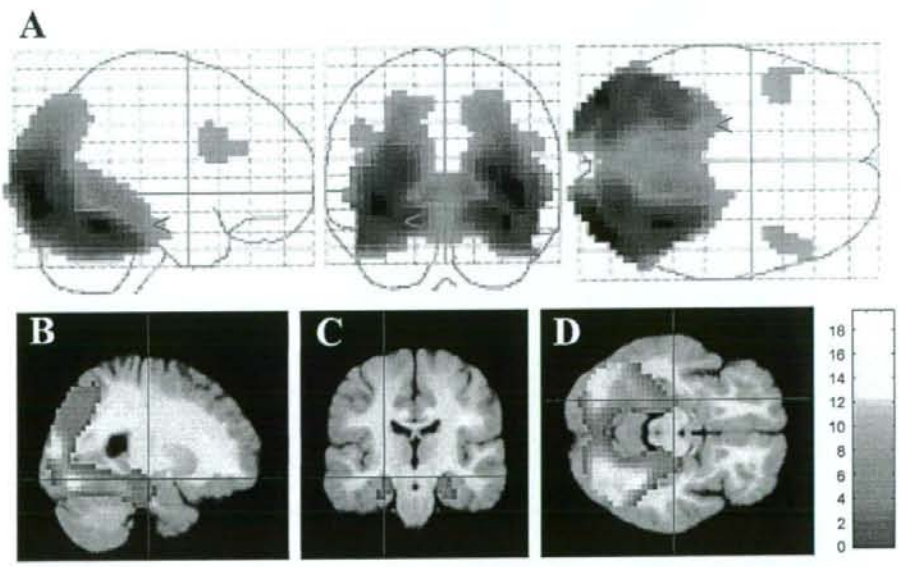

Fig. 1. Brain activity associates with encoding in the whole group. (A) The SPM {*t*} is displayed in a standard format as a maximum-intensity projection (MIP) viewed from the right, the back and the top of the brain as SPM2 "glass brain" representation. The anatomical space corresponds to the atlas of Talairach and Tournoux. Representation in stereotaxic space of brain areas with significant activation was demonstrated. Task-related activations were calculated using a *t*-statistic of SPM2. A significant activation associated with encoding is noted in the bilateral hippocampus, parahippocampal gyri, visual association areas, parietal association areas including precunei and the dorsolateral prefrontal cortices ($p < 0.05$, corrected for multiple comparisons) and anatomical localization, as projected on (B) sagittal, (C) coronal, and (D) axial sections of a normal MRI, spatially normalized into the Montreal Neurological Institute template. The scale of *T* value is indicated on the bottom right.

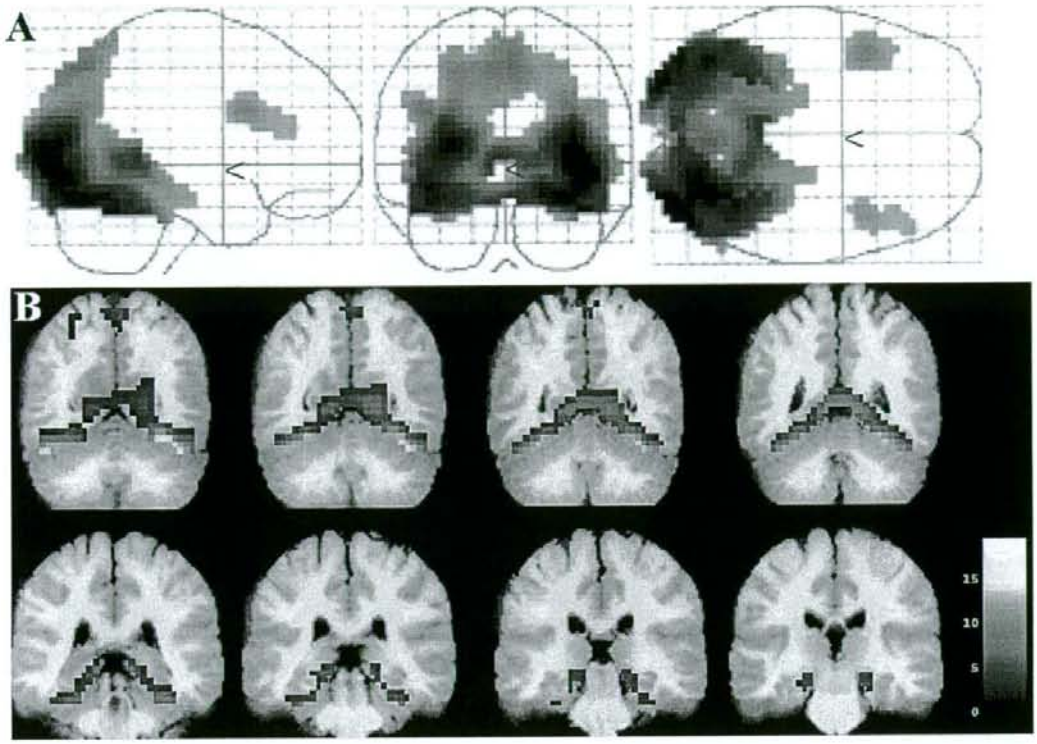

Fig. 2. Brain activity associates with retrieval in the whole group. (A) The SPM {t} is displayed in a standard format as a MIP viewed from the right, the back and the top of the brain as SPM2 "glass brain" representation. The anatomical space corresponds to the atlas of Talairach and Tournoux. Representation in stereotaxic space of brain areas with significant activation was demonstrated. Task-related activations were calculated using a t-statistic of SPM2. A significant activation was also found in the bilateral hippocampus, parahippocampal gyri, posterior cingulate cortex, visual association areas, parietal association areas and the dorsolateral prefrontal cortices ($p < 0.05$, corrected for multiple comparisons) and anatomical localization, as projected on (B) coronal sections of a normal MRI, spatially normalized into the Montreal Neurological Institute template. The scale of T value is indicated on the bottom right.

bilateral parahippocampal gyri and hippocampi. However, no voxels could survive after the correction for multiple comparisons within the ROI (corrected $p > 0.05$). Whole brain correlational analyses demonstrated that the Val66Met polymorphism had no impact on activity within the distributed cortical network, such as visual association areas, parietal association areas and the dorsolateral prefrontal cortex (corrected $p > 0.05$).

As age range of our subjects is relatively broad, age might be a possible confounding factor. Thus, we analyzed the data including age as a covariate. We further re-analyzed the data using subjects leaving out the subjects older than 55 years (Val/Val = 3, Val/Met = 1 and Met/Met = 0) and obtained essentially same results in all analysis (data not shown).

4. Discussion

In the whole subjects' analysis, we found significant bilateral activation in the hippocampus and parahippocampal gyrus during both encoding and retrieval. The data was well concordant with previous fMRI studies of episodic memory. We also replicated significant bilateral activations in the inferotemporal, parietal, and frontal cortices, a distributed network critical for visuospatial information processing (Ungerleider and Haxby, 1994) during both encoding and retrieval. In addition, we found significant activation in the bilateral PCC during retrieval. Several studies have indicated that the PCC have prominent response properties associated with episodic memory, particularly, successful retrieval (Wagner et al., 2005). A significant activation in the PCC associated with retrieval in the present study is well consistent with previous studies.

In terms of effects of the Val66Met polymorphism of the BDNF gene on memory-related brain activity, we obtained two main findings: (1) the Val66Met polymorphism of the BDNF gene affects encoding related neuronal activity in the hippocampal regions with a dose-dependent manner; i.e. a negative correlation between the number of Met allele and the degree of activation in the bilateral hippocampi and parahippocampal gyri during encoding and (2) the Val66Met polymorphism of the BDNF gene did not affects retrieval related neural activity in the hippocampal regions. Further, this

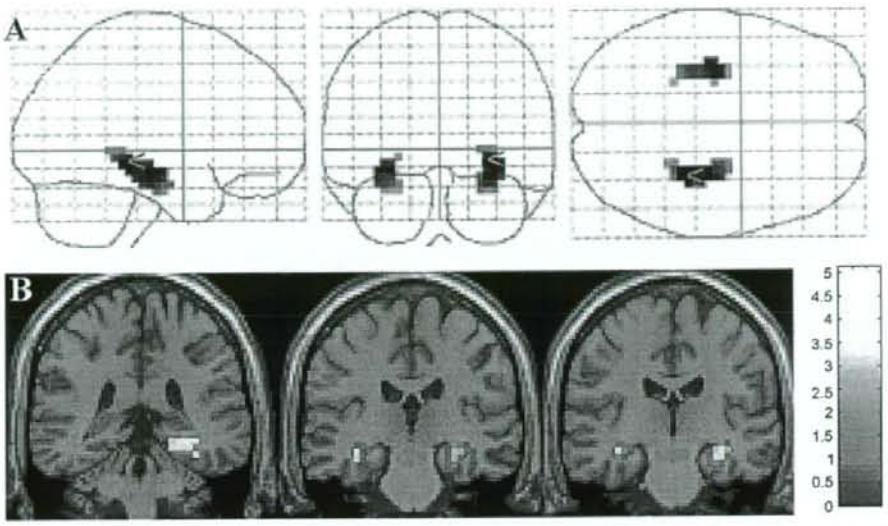

Fig. 3. Genotype effect of the Val66Met polymorphism of the BDNF gene on encoding related hippocampal activity. (A) The SPM {t} is displayed in a standard format as a MIP viewed from the right, the back and the top of the brain as SPM2 "glass brain" representation. The anatomical space corresponds to the atlas of Talairach and Tournoux. Representation in stereotaxic space of brain areas with significant activation was demonstrated. A significant negative correlation between numbers of Met allele and degree of activation is noted in the bilateral hippocampal regions calculated by correlational analysis of SPM2 ($p < 0.05$, corrected for multiple comparisons) and anatomical localization, as projected on (B) coronal sections of a normal MRI, spatially normalized into the Montreal Neurological Institute template.

polymorphism had no effects on activity within the other distributed cortical network, such as visual association areas, parietal association areas and the dorsolateral prefrontal cortex involved with general visuo-spatial information processing. Consistent with the previous fMRI study of the Val66Met polymorphism (Hariri et al., 2003), our data also suggest that this polymorphism could have impact on memory-related neuronal activity of the hippocampal region in an Asian sample. In addition, our data indicate Met-BDNF dose-dependent

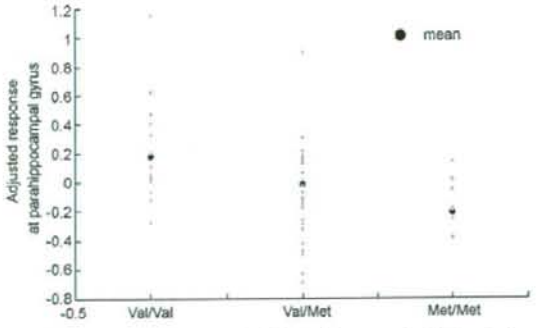

Fig. 4. Dose-dependent effect of Met allele on hippocampal activation. Activation in parahippocampal gyrus during encoding task was calculated in each genotype individual (Val/Val = 17, Val/Met = 29 and Met/Met = 12) by the SPM2. *X*-axis: adjusted response at parahippocampal gyrus; *Y*-axis: genotype group. A gray dot indicates each individual response and a black dot indicates a mean response in the genotype groups. Negative correlation between activation associated with encoding and genotype was calculated by correlational analysis of SPM2 ($p < 0.05$, corrected for multiple comparisons).

effects of the Val66Met polymorphism on hippocampal activity during encoding. The specific effect of BDNF on hippocampal activity is well concordant with the expression pattern of BDNF in the brain, which is highest in the hippocampus (Murer et al., 2001). This effect of the Val66Met polymorphism on BOLD response in the hippocampal region may be mediated through alterations in activity-dependent hippocampal processes requiring BDNF-regulated secretion, particularly the abnormal intracellular trafficking and regulated secretion of BDNF in Met carriers, which may result in impaired hippocampal LTP that could underlie encoding (Egan et al., 2003; Chen et al., 2004). Most neuroimaging studies also have demonstrated genotype effects on hippocampal region, i.e. Met allele is associated with reduced hippocampal volume and impaired hippocampal function (Hariri et al., 2003; Pezawas et al., 2004; Bueller et al., 2006). However, owing to the relatively small sample size and the low frequency of the Met allele, these studies, including the previous memory fMRI study, used combined Met/Met and Val/Met subjects (Hariri et al., 2003; Pezawas et al., 2004; Bueller et al., 2006). On the other hand, our study with a relatively larger sample, particularly individuals with Met-allele, revealed dose-dependent genotype effects of the Val66Met polymorphism on encoding-related hippocampal activity. Our data support and strengthen the results of the previous fMRI study.

The previous study demonstrated that the Val66Met polymorphism affected both on encoding and retrieval related hippocampal activity (Hariri et al., 2003), however, we could not found genotype effects on retrieval related hippocampal activity. Considering the possible underlying mechanism of

genotype effect of the Val66Met polymorphism on memory-related neuronal activity; i.e. abnormal intracellular trafficking and regulated secretion of BDNF in Met carriers may result in impaired hippocampal LTP or analogous synaptic events that may underlie encoding reflect decreased hippocampal activity, it seems to be plausible that effects of the polymorphism was found on encoding related hippocampal activity rather than retrieval related hippocampal activity. However, we cannot deny the possibility that inconsistencies between our study and the previous study may relate to sample differences, methodological differences, and also to possible genetic and allelic heterogeneity. These uncertainties cannot be addressed with the current data.

We did not detect the significant genotype effects on the memory performances during fMRI measurements, although previous studies reported the genotype effects. This could be explained by the difference of the difficulty between the two tasks and/or a ceiling effect of our task performance, as the mean hit rate of our retrieval task is above 95% and that of previous study was 88% (Val group, 91.6 ± 1.5; Met group, 84.5 ± 2.6). Thus, more difficult tasks would yield different findings. Our WMS-R results, although not reaching significance, do not always tend to be dose-dependent. Previous studies demonstrated genotype effect of the Val66Met polymorphism on human episodic memory on the behavioral level (Egan et al., 2003; Hariri et al., 2003), however, a recent study also demonstrated no association between episodic memory (estimated by logical memory) and BDNF genotype (Harris et al., 2006). Further studies should be conducted to clarify whether the Val66Met polymorphism have apparent effect on human episodic memory function.

Someone may argue that genotype effects of the Val66Met polymorphism on encoding related hippocampal activity observed in this study seem inconsistent with the result of behavioral measures; i.e. no significant BDNF genotype effects on memory-related behavioral measures. In general, it is considered that the difference of a missense polymorphism, i.e. the BDNF Val66Met polymorphism and the Val158Met polymorphism of the catechol-*O*-methyl transferase (COMT) gene, firstly causes the difference of function in the protein level, and then that the distinct protein function influences neuronal functions in the cellular level. Distinct cellular functions might affect brain activities in the network level and these differences of brain activities could result in the distinct performance in the behavioral level. Thus, genotype effects could be stronger in order of the protein level, the cellular level, the network level and the behavioral level. The difficulty to replicate the genotype effects in the behavioral level is well known particularly in the phenotype of psychiatric disorders (Preston and Weinberger, 2005). Thus, the brain activity might reflect genotype effects more directly than the behavior does in our study. A similar phenomenon – no significant effect of the COMT Val158Met polymorphism on working memory performance but significant effects on brain activities during a working memory task – was reported (Ho et al., 2005). Since there was no behavioral effect but effect observed in fMRI in hippocampus/parahippocampal gyrus (HPHG) region, this raises another possibility that the fMRI effect of Val/Met is not relevant to memory but epiphenomenal, secondary to effects either in other, undetected brain regions projecting to HPHG or to HPHG activity unrelated to memory. The present study suggests that measurement of brain activity related to particular tasks should be useful to investigate relationships between several functional SNPs and human cognitive functions.

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Disrupted integrity of the fornix is associated with impaired memory organization in schizophrenia

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Abstract

Background: The fornix is a major projection of the hippocampus to and from other brain regions. A previous diffusion tensor imaging (DTI) study has reported disrupted integrity of the fornix in patients with schizophrenia. However, functional significance of the DTI abnormalities of the fornix in schizophrenia has not been fully studied yet. We investigated an association between DTI abnormalities of the fornix and impairment of memory organization in schizophrenia.
Methods: Thirty-one patients with schizophrenia and 65 age- and gender-matched healthy controls underwent DTI, and fractional anisotropy (FA) and mean diffusivity (MD) were measured in cross-sections of fornix tractography. In addition, all of the patients and 32 controls performed a verbal learning task specialized for evaluating memory organization, the verbal memory subscale of the Wechsler Memory Scale-Revised, the category- and letter fluency tests, and the Japanese version of National Adult Reading Test.
Results: Statistically significant reduction of FA and increase of MD were found in the fornix of patients with schizophrenia compared with controls with no significant lateralization. A significant patients-specific correlation was found between increased MD in the left fornix and lower scores on utilization of semantic organization in the verbal learning task. In addition, increased MD in the right fornix showed a patients-specific association with poorer performance on the category fluency test, which indexes organization of long-term semantic memory. These patients-specific correlations, however, were not statistically lateralized to either hemisphere.
Conclusions: These results indicate that disrupted integrity of the fornix contributes to impaired memory organization in schizophrenia.
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Keywords: Schizophrenia; Fornix; Memory organization; Diffusion tensor imaging; Verbal learning task; Category fluency test

1. Introduction

The fornix is a major projection of the hippocampus to and from other brain regions such as the mamillary

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