Glucocorticoid receptor interaction with TrkB promotes BDNF-triggered PLC-γ signaling for glutamate release via a glutamate transporter

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An increase in glucocorticoid levels and down-regulation of BDNF (brain-derived neurotrophic factor) are supposed to be involved in the pathophysiology of depressive disorders. However, possible crosstalk between glucocorticoid- and BDNF-mediated neuronal functions in the CNS has not been elucidated. Here, we examined whether chronic glucocorticoid exposure influences BDNF-triggered intracellular signaling for glutamate release via a glutamate transporter. We found that chronic exposure to dexamethasone (DEX, a synthetic glucocorticoid) suppressed BDNF-induced glutamate release via weakening the activation of the PLC-γ (phospholipase C-γ)/Ca^{2+} system in cultured cortical neurons. We demonstrated that the GR (glucocorticoid receptor) interacts with receptor tyrosine kinase for BDNF (TrkB). Following DEX treatment, TrkB-GR interaction was reduced due to the decline in GR expression. Corticosterone, a natural glucocorticoid, also reduced TrkB-GR interaction, BDNF-stimulated PLC-γ, and BDNF-triggered glutamate release. Interestingly, BDNF-dependent binding of PLC-γ to TrkB was diminished by DEX. SiRNA transfection to induce a decrease in endogenous GR mimicked the inhibitory action of DEX. Conversely, DEX-inhibited BDNF-activated PLC-γ signaling for glutamate release was recovered by GR overexpression. We propose that TrkB-GR interaction plays a critical role in the BDNF-stimulated PLC-γ pathway, which is required for glutamate release, and the decrease in TrkB-GR interaction caused by chronic exposure to glucocorticoids results in the suppression of BDNF-mediated neurotransmitter release via a glutamate transporter.

BDNF | GR | PLC | depression | cortical neurons

Most patients with depression exhibit prolonged elevation of a glucocorticoid stress hormone, cortisol (1, 2). The blood level of glucocorticoids (cortisol in humans and corticosterone in rodents) is regulated by the hypothalamic-pituitary-adrenal (HPA) axis (2). When excessive stress is prolonged, abnormally increased amounts of glucocorticoids may damage the CNS and cause depressive symptoms, which can be decreased with antidepressants (3–5).

Glucocorticoids function via the glucocorticoid receptor (GR), which regulates gene transcription. Glucocorticoids contribute to glucose homeostasis, cell differentiation, and inflammation (6). Additionally, glucocorticoids and the GR influence neuronal functions such as hippocampal long-term potentiation/depression (7–9) and cognitive function governed by the prefrontal cortex (10). The GR potentiates the response to NMDA in dopamine-sensitive neurons in the ventral tegmental area (11) and modulates the NMDA receptor function in the spinal cord following peripheral nerve injury (12), suggesting that the GR is involved in synaptic plasticity.

Beyond the promotion of cell differentiation, nerve growth, and neuronal survival, brain-derived neurotrophic factor (BDNF) plays a crucial role in synaptic function (13–15). For instance, BDNF increases neurotransmitter release (16, 17). We reported that BDNF rapidly induces glutamate transporter-mediated glutamate release via phospholipase C-γ (PLC-γ)/Ca^{2+} signaling and that antidepressants enhance PLC-γ/Ca^{2+} signaling (18, 19). Growing evidence has suggested a close relationship between BDNF and the pathophysiology of depression (20, 21). The BDNF level was low in the brains of suicide victims, most of whom had depressive disorders (22). BDNF plays a critical role in cognition, learning, and memory, and patients with depression exhibit deficits in these brain functions (23, 24). Both BDNF and glucocorticoids/GR are involved in synaptic function and the pathophysiology of depression. However, the possible influence of glucocorticoids on the acute action of BDNF is poorly understood.

Here we report that chronic treatment with glucocorticoids suppressed BDNF-triggered PLC-γ signaling for glutamate release via a glutamate transporter. We found that the GR interacted with receptor tyrosine kinase for BDNF (TrkB), playing an important role in BDNF action.

Results

Chronic Dexamethasone (DEX) Treatment Suppressed BDNF-Induced Glutamate Release by Inhibiting PLC-γ/Ca^{2+} Signaling. We examined BDNF-induced glutamate release in cultured cortical neurons after exposure to DEX (a synthetic GR-selective agonist). DEX pretreatment (48 h) suppressed BDNF-induced glutamate release in a dose-dependent manner (Fig. 1*Ai*). After various durations of DEX (1 μM) exposure, we found that DEX exposure for 24 or 48 h inhibited BDNF-induced glutamate release, whereas shorter exposure times (10 min to 12 h) did not (Fig. 1*Aii*). When the dose-dependency of BDNF on glutamate release was examined, DEX inhibited BDNF-induced release at any dose of BDNF (Fig. 1*Aiii*). In this study, the following experiments were performed with 100 ng/ml of BDNF. Cell viability [supporting information (SI) Fig. S1 *A* and *B*] and the number of glutamatergic and GABAergic synapses (Fig. S1 *C* and *D*) were unchanged by DEX exposure for 48 h. The endogenous GR expression in vitro and in vivo during neuronal maturation is shown in Fig. S2 *A* and *B*. In this study, we applied DEX at days in vitro (DIV) 4–5 because the expressions of the GR and synaptic proteins markedly increase (Fig. S2*A*), and BDNF-induced glutamate release begins at approximately DIV 5 (18). We confirmed the inhibitory effects of TTX (a Na^+ channel blocker) and TBOA (a glutamate transporter inhibitor) on BDNF-induced glutamate release (Fig. S3*A*). Tetanus toxin, an exocytosis inhibitor, had no effect on BDNF-induced glutamate release (Fig. S3*B*). These results suggest that BDNF-

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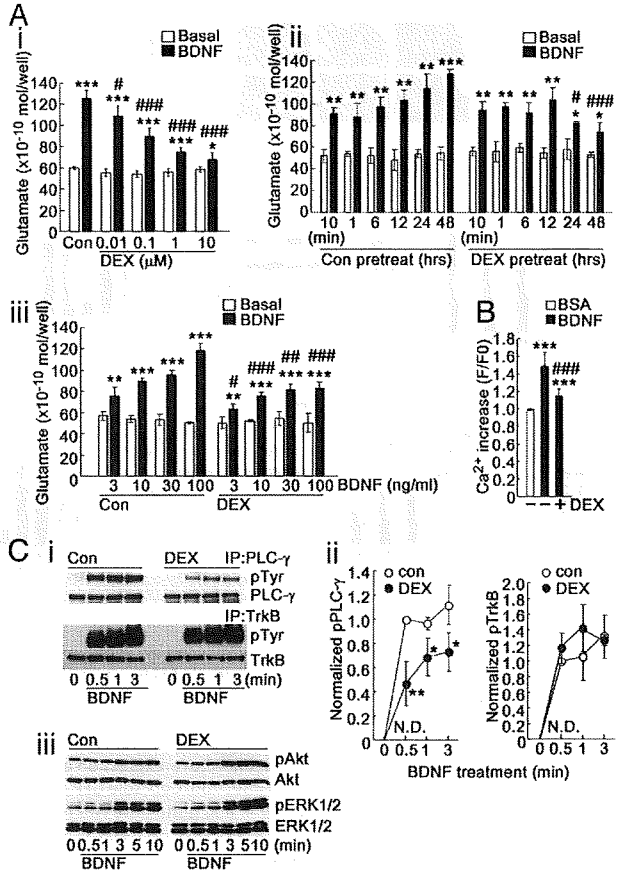

Fig. 1. Chronic DEX treatment suppressed BDNF-activated PLC-γ/Ca^{2+} signaling for glutamate release in cultured cortical neurons. (*Ai*) Dose-dependent inhibitory effect of DEX on BDNF-induced glutamate release. DEX (0.01–10 μM) was applied at DIV 4. After 48 h, BDNF (100 ng/ml, 1 min) was added. Con means no DEX treatment. Data represent mean ± SD. ($n = 4$). (*ii*) Time-course analysis of the DEX effect on BDNF-induced glutamate release. DEX (1 μM) was added at DIV 5 for the indicated durations. Data represent mean ± SD. ($n = 3$). (*iii*) Dose-dependency of BDNF on glutamate release after DEX treatment. DEX (1 μM, at DIV 4) was applied for 48 h. Then, BDNF (3–100 ng/ml) was added. Data represent mean ± SD. ($n = 5$). $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ versus basal, $^{###}P < 0.001$, $^{##}P < 0.01$, $^{#}P < 0.05$ versus BDNF-induced release in Con. (*t* test). (*B*) DEX prevented BDNF-increased intracellular Ca^{2+}. Data from 61 randomly selected cells for each experimental condition. The fluorescence ratio (F/F_0; BDNF-induced/basal) was calculated. DEX (1 μM) was applied at DIV 4. After 24 h later, Ca^{2+} imaging was performed. $^{***}P < 0.001$ versus vehicle (BSA) in the control. $^{###}P < 0.001$ versus BDNF-induced increase in the control. (*t* test). (*Ci*) Immunoprecipitation with anti-PLC-γ (*Upper*) or anti-TrkB (*Lower*) antibodies was carried out. Blotting was performed with anti-pTyr, anti-PLC-γ, or anti-TrkB antibodies. DEX (1 μM) was applied at DIV 4. After 48 h, BDNF was applied for the indicated duration. (*ii*) Quantification of pPLC-γ or pTrkB. Data represent mean ± SD. ($n = 4$). Data were normalized to the level of BDNF (0.5 min) in Con. N.D.: not detected. $^{**}P < 0.01$, $^{*}P < 0.05$ versus BDNF-induced in Con. (*t* test). (*iii*) DEX did not affect pAkt or pERK1/2. BDNF was applied for the indicated duration.

induced glutamate release occurs via a glutamate transporter in a Na^+-dependent manner as we previously reported (18).

BDNF-induced glutamate release depends on an intracellular Ca^{2+} increase via IP_3-sensitive Ca^{2+} channels (IP_3 receptor) (18). As expected, chronic DEX treatment weakened BDNF-increased Ca^{2+} (Fig. 1*B* and Fig. S4 *A* and *B*). We confirmed that U73122 (a PLC-γ inhibitor) and xestospongin C (Xest C, an IP_3 receptor inhibitor) blocked BDNF-increased Ca^{2+}, although BDNF still increased Ca^{2+} in the presence of APV (an NMDA receptor inhibitor), CNQX (an AMPA receptor inhibitor), or bicuculline (a $GABA_A$ receptor inhibitor) (Fig. S4 *C* and *D*), indicating the importance of the PLC-γ/IP_3 pathway. We confirmed that both U73122 and Xest C blocked BDNF-induced glutamate release in the control and DEX-treated cultures (Fig. S4*E*). These results suggest that BDNF-induced glutamate release depends on the PLC-γ pathway.

Next, we focused on PLC-γ activation (phosphorylation). A significant decline in BDNF-activated PLC-γ following chronic DEX exposure was observed, although TrkB (upstream of PLC-γ) was equally activated by BDNF with or without DEX (Fig. 1 *Ci* and *ii*). In other pathways activated by TrkB, DEX did not change activation of Akt (pAkt, phosphorylated Akt) or ERK1/2 (pERK1/2) stimulated by BDNF (Fig. 1*Ciii*).

Recently, activation of TrkB signaling within several hours of glucocorticoid exposure was reported (25). Indeed, TrkB, PLC-γ, Akt, and ERK1/2 were activated by short-term application of glucocorticoids (DEX or corticosterone) (Fig. S5 *A* and *B*). These activations reached their maximum at 2–4 h after the application and returned to the basal level at 6 h. As expected, BDNF induced much higher activation of TrkB signaling (including PLC-γ, Akt, and ERK1/2) compared with that induced by sole acute DEX (2 h) or corticosterone (2 h) exposure (Fig. S6 *A–C*). In contrast to chronic exposure, such a short-term treatment with DEX (2 h) or corticosterone (2 h) did not affect the exogenous BDNF-stimulated TrkB signaling, including PLC-γ. Subsequently, we focused on the suppression of BDNF-dependent PLC-γ signaling for glutamate release after long-lasting glucocorticoid exposure.

DEX-Dependent GR Down-Regulation Was Involved in the Suppressed Responses to BDNF. To investigate the mechanisms underlying the DEX-suppressed responses to BDNF, the possible involvement of the GR was examined. When DEX was coapplied with RU486 (a GR antagonist), BDNF-stimulated PLC-γ activation and glutamate release were not inhibited (Fig. 2 *Ai* and *ii* and Fig. 2*B*), suggesting that DEX acts via the GR. Thus, endogenous GR expression after DEX addition was examined. Marked down-regulation of the GR was observed following DEX application for 24 to 48 h (Fig. 2*C*). DEX induced GR down-regulation in a dose-dependent manner (Fig. 2 *Di* and *ii*), while the mineral corticoid receptor (MR, the other receptor for glucocorticoid) and TUJ1 (class III β-tubulin, a neuronal marker) expression was intact (Fig. 2*Di*). Immunocytochemistry with anti-microtubule-associated protein 2 (MAP2) and anti-GR antibodies was performed, and the quantification of the staining indicated a DEX-dependent GR reduction in neurons (Fig. 2 *Ei–vii*). As expected, RU486 recovered GR down-regulation by DEX (Fig. 2 *Fi* and *ii*). Corticosterone also down-regulated the GR and BDNF-induced glutamate release (Fig. S7 *A–C*). Moreover, the suppression of BDNF-activated PLC-γ by corticosterone was also observed (Fig. S7 *D* and *E*). We confirmed that the GR was markedly reduced in the homogenates of the cerebral cortex prepared from rats after DEX administration, although the MR level was intact (Fig. S7 *F* and *G*). These results suggested that the inhibitory action of glucocorticoid results from the down-regulation of the GR.

Then, the effects of GR overexpression were examined. After viral infection, about 85% of MAP2-positive cells in either the control (GFP) or GR-overexpressing (GR and GFP) cultures were GFP-positive, indicating that the majority of neurons were infected (Fig. 3*Ai*). Blotting with anti-GR and anti-GFP antibodies showed GR overexpression (Fig. 3*Aii*), which enhanced BDNF-induced glutamate release (Fig. 3*B*). DEX failed to reduce BDNF-activated PLC-γ in GR-infected cultures (Fig. 3 *Ci* and *ii*). Next, siRNA was used to examine the function of endogenous GR. Approximately 60% of endogenous GR was depleted by GR-siRNA (Fig. 3*D*), and BDNF-induced glutamate

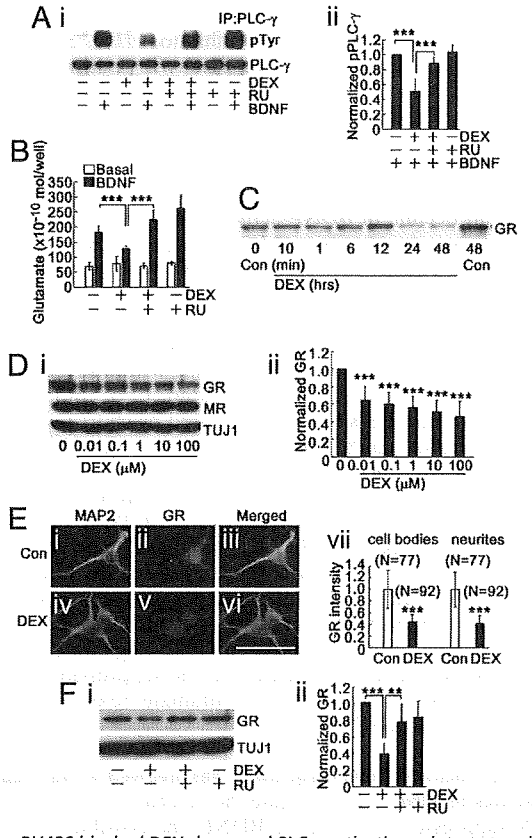

Fig. 2. RU486 blocked DEX-decreased PLC-γ activation, glutamate release, and GR expression. (*Ai*) RU486 (RU, a GR antagonist) blocked DEX-decreased PLC-γ activation. DEX (1 μM) and RU486 (1 μM) were coapplied at DIV 4. Subsequently, 48 h later, BDNF was added for 1 min. (*ii*) Quantification of pPLC-γ. Data represent mean ± SD. ($n = 4$). Normalization to the level in BDNF-stimulated PLC-γ with no pretreatment. ***$P < 0.001$ (*t* test). (*B*) RU486 blocked the inhibitory effect of DEX on BDNF-induced glutamate release. Data represent the mean ± SD. ($n = 4$). ***$P < 0.001$ (*t* test). (*C*) Time-course of DEX-decreased GR expression. DEX (1 μM, at DIV 4) was applied for 10 min or 1–48 h. (*Di*) Dose-dependency of DEX on GR down-regulation. After DEX (0.01–100 μM, at DIV 4) exposure for 48 h, GR, MR, and TUJ1 were detected. (*ii*) Quantification of the GR. Data represent mean ± SD. ($n = 5$). Normalization to the level in 0 μM. ***$P < 0.001$ (*t* test). (*Ei* and *iv*) Immunostaining with anti-MAP2 and (*ii* and *v*) anti-GR antibodies. (*iii* and *vi*) Merged images. *Upper*: control. *Lower*: DEX-treated. DEX (1 μM, at DIV 4) was applied for 48 h. (Scale bar, 50 μm.) (*vii*) GR immunoreactivities of randomly selected regions from cell bodies or neurites. Normalization to the level in Con. N indicates the number of selected regions. At least 20 neurons from 6 coverslips were examined. (*Fi*) RU486 inhibited GR down-regulation by DEX. TUJ1 is the control. (*ii*) Quantification of the GR. Data represent mean ± SD. ($n = 5$). Normalization to the level in no treatment. ***$P < 0.001$, **$P < 0.01$ (*t* test).

release was decreased in GR-siRNA-transfected cultures (Fig. 3*E*). GR-siRNA reduced BDNF-activated PLC-γ (Fig. 3 *Fi* and *ii*) but not pAkt or pERK1/2 (Fig. S8 *A–C*). These results suggest that the amount of GR expression is critical for BDNF-stimulated PLC-γ signaling and glutamate release.

DEX Decreased TrkB-GR Interaction and Binding of PLC-γ to TrkB. How does chronic DEX interrupt PLC-γ signaling? Initially, endogenous PLC-γ, TrkB, or BDNF expression after DEX exposure was examined; however, the levels of these proteins were intact (Fig. S9 *A* and *B*). The level of TrkB on the cell surface was also unchanged (Fig. S9 *C* and *D*). Subsequently, the possible interaction between GR and TrkB was investigated. Following immunoprecipitation with anti-GR antibody, coprecipitated TrkB

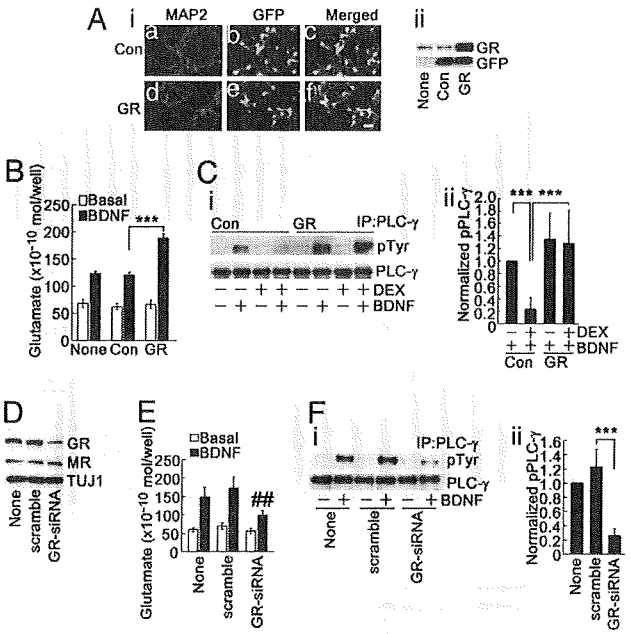

Fig. 3. BDNF-induced glutamate release was enhanced by GR overexpression and reduced by GR down-regulation. (*Aia* and *d*) MAP2- and (*b* and *e*) GFP-positive images. (*c*) Overlay of *a* and *b*. (*f*) Overlay of *d* and *e*. (*a–c*) GFP-infected control. (*d–f*) Overexpression of both GFP and GR. (Scale bar, 50 μm.) Cells were infected at DIV 4, and fixed at DIV 6. (*ii*) GR overexpression was checked by anti-GR and anti-GFP antibodies. (*B*) GR overexpression enhanced BDNF-induced glutamate release. None: no infection. Con: sole GFP. GR: both GFP and GR. Infection was performed at DIV 4, and glutamate was measured at DIV 6. Data represent mean ± SD. ($n = 4$). ***$P < 0.001$ (*t* test). (*Ci*) DEX-decreased BDNF-activated PLC-γ was recovered by GR overexpression. DEX (1 μM) was applied at DIV 5 (24 h after infection). After 24 h, BDNF (1 min) was applied. (*ii*) Quantification of pPLC-γ. Data represent mean ± SD. ($n = 7$). Normalization to the level in BDNF-activated without DEX in GFP-infected. ***$P < 0.001$ (*t* test). (*D*) Endogenous GR was decreased after GR-siRNA transfection. Scramble siRNA had no effect. MR and TUJ1 are the controls. SiRNA was transfected 48 h before lysates were collected. (*E*) BDNF-induced glutamate release in GR-siRNA-transfected cultures was reduced. Data represent mean ± SD. ($n = 4$). (*t* test). ##$P < 0.01$ versus BDNF-induced in the scramble. (*Fi*) BDNF-activated PLC-γ was decreased by the GR-siRNA. (*ii*) pPLC-γ was quantified. Data represent mean ± SD. ($n = 4$). Normalization to the level in BDNF-activated PLC-γ in None. ***$P < 0.001$ (*t* test).

was found (Fig. 4*Ai*). The degree of coprecipitated TrkB was not changed by BDNF and/or DEX (Fig. 4 *Aii* and *iii*). Significant coprecipitated GR after immunoprecipitation of TrkB was also observed (Fig. 4*Bi*). Remarkably, DEX reduced the coprecipitated GR with or without BDNF application (Fig. 4 *Bii* and *iii*). In the control, a BDNF-dependent slight increase in the coprecipitated GR was observed. To inspect the specificity of the interaction, we used a competitive peptide to block TrkB immunoprecipitation. The peptide, containing a sequence for the epitope of the antibody, blocked the immunoprecipitation of TrkB and the coprecipitation of the GR (Fig. 4*C*). Moreover, GR overexpression increased TrkB-GR interaction, and GR-siRNA transfection decreased TrkB-GR interaction (Fig. S10 *A–C*). Corticosterone also reduced TrkB-GR interaction (Fig. S10 *D* and *E*). Immunocytochemical analysis showed that the merged level of the GR- and TrkB-positive signal was diminished because chronic DEX exposure decreased the GR levels (Fig. S11 *A* and *B*). In contrast to chronic exposure, acute DEX or corticosterone had no effect on TrkB-GR interaction with or without BDNF (Fig. S12). Interestingly, BDNF-induced binding of PLC-γ to TrkB decreased due to chronic DEX (Fig. 4 *Di* and

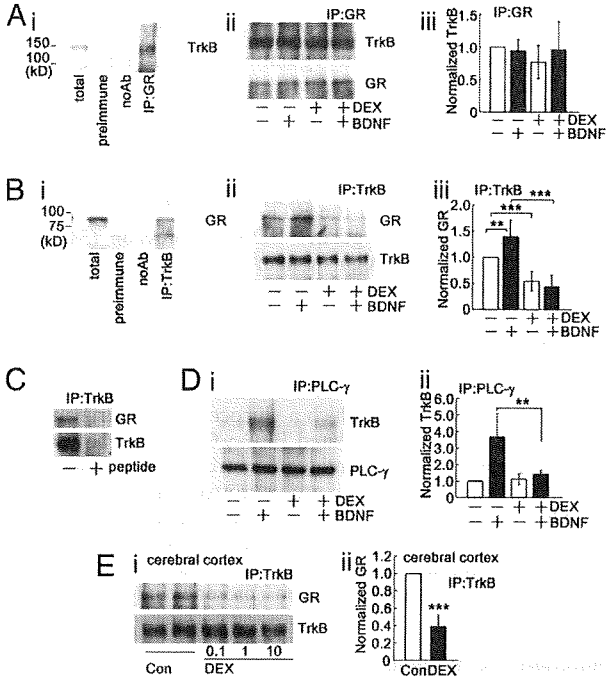

Fig. 4. TrkB-GR interaction and BDNF-dependent binding of PLC-γ to TrkB were decreased after DEX exposure. (*Ai*) After immunoprecipitation with the anti-GR antibody, blotting with the anti-TrkB antibody was performed. Lysates from DIV 6 cultures were used. Preimmune: preimmune serum control. noAb: no anti-GR antibody. Total: 10% input (total lysates). (*ii*) Immunoprecipitation of the GR was performed after BDNF stimulation with or without DEX pretreatment. DEX (1 μM, at DIV 4) was applied for 48 h. Subsequently, BDNF was added (1 min). Anti-TrkB and anti-GR antibodies were used for blotting. (*iii*) Quantification of the coprecipitated TrkB. Normalization to the level without DEX and BDNF. Data represent mean ± SD. ($n = 7$). (*Bi*) Immunoprecipitation with anti-TrkB antibody. Blotting with anti-GR antibody was performed. noAb: no anti-TrkB antibody. (*ii*) Immunoprecipitation of TrkB after BDNF stimulation with or without DEX. Blottings were performed with anti-GR and anti-TrkB antibodies. (*iii*) The coprecipitated GR was quantified. Data represent mean ± SD. ($n = 9$). Normalization to the level without DEX and BDNF. $***P < 0.001$, $**P < 0.01$ (*t* test). (*C*) Inhibition of the coprecipitation of the GR by a competitive peptide to block the immunoprecipitation of TrkB. Lysates from GR-overexpressed cortical cultures were used. (*Di*) TrkB-PLC-γ interaction after DEX treatment. After immunoprecipitation of PLC-γ, coprecipitated TrkB was detected. DEX (1 μM, at DIV 4) was applied for 48 h before BDNF addition (1 min). (*ii*) Coprecipitated TrkB was quantified. Data represent mean ± SD. ($n = 5$). $**P < 0.01$ (*t* test). Data were normalized to no treatment. (*Ei*) DEX exposure reduced TrkB-GR interaction in vivo. P7 rats received i.p. injections (0.1–10 mg/kg i.p.) of DEX or vehicle. Samples were obtained 48 h after the injections. (*ii*) The coprecipitated GR was quantified. Data were obtained from the control and 0.1 mg/kg DEX. Data represent mean ± SD. ($n = 4$). Normalization to the level in con. $***P < 0.001$ (*t* test).

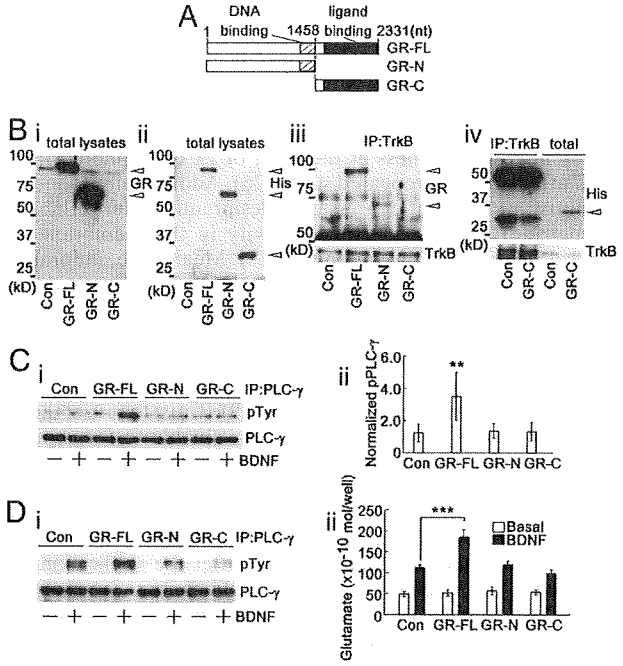

Fig. 5. The N-terminal region of the GR was required for interaction with TrkB. (*A*) Three types of GR plasmids, GR-FL (nt1- nt2331), GR-N (nt1- nt1458), and GR-C (nt1459- nt2331), were constructed. (*B*) GR plasmids and GFP plasmid (con) were transfected into SH-SY5Y cells. The exogenous GR in total lysates was detected by (*i*) anti-GR and (*ii*) anti-His antibodies. As the anti-GR antibody recognized GR-FL and GR-N but not GR-C, blotting with the anti-His antibody was also performed. (*iii*) After TrkB immunoprecipitation, blotting was performed with anti-GR and anti-TrkB antibodies. GR-FL and GR-N were coprecipitated. (*iv*) GR-C failed to interact with TrkB. Blotting with the anti-His antibody was conducted. (*Ci*) GR-FL overexpression potentiated BDNF-activated PLC-γ in SH-SY5Y cells. BDNF was applied for 15 min. (*ii*) pPLC-γ was quantified. Data represent mean ± SD. ($n = 7$). The ratio (BDNF-stimulated/basal) of pPLC-γ was calculated. $**P < 0.01$ versus BDNF-stimulated in con. (*t* test). (*D*) Three types of GR plasmids and a GFP plasmid (con) were transfected into cortical neurons. BDNF-dependent (*i*) pPLC-γ and (*ii*) glutamate release were enhanced by GR-FL transfection. Data represent mean ± SD. ($n = 4$). $***P < 0.001$ (*t* test).

ii). Marked reduction in TrkB-GR interaction in the homogenates of the cerebral cortex prepared from rats treated with DEX injection was confirmed (Fig. 4 *Ei* and *ii*).

To further assess TrkB-GR interaction, 3 types of GR plasmids containing His tags were constructed (Fig. 5*A*). GR-FL (full length), GR-N (including DNA binding site), GR-C (including ligand binding site), and GFP (con) were transfected into SH-SY5Y cells. As the anti-GR antibody recognized GR-FL and GR-N but not GR-C (Fig. 5*Bi*, the epitope for the antibody exists in the N-terminal region of GR), blotting with anti-His antibody was also performed with total lysates (Fig. 5*Bii*). After immunoprecipitation of TrkB, blotting with anti-GR (Fig. 5*Biii*), anti-TrkB (Fig. 5 *Biii* and *iv*), and anti-His antibodies (Fig. 5*Biv*) was conducted. GR-FL and GR-N were coprecipitated; however, GR-C failed to interact with TrkB, indicating the importance of the N-terminal region of the GR. BDNF-activated PLC-γ in GR-FL-transfected SH-SY5Y cells was enhanced compared with the control, whereas such enhancement was not detected in GR-N- or GR-C-transfected cells (Fig. 5 *Ci* and *ii*). Finally, the responses to BDNF in cortical neurons transfected with these GR plasmids were examined. PLC-γ activation and glutamate release in GR-FL-transfected neurons were reinforced; in contrast, neither PLC-γ activation nor glutamate release was enhanced by GR-N and GR-C transfection (Fig. 5 *Di* and *ii*). These results suggest that the N-terminal region of the GR interacts with TrkB; however, the C-terminal region is also required to boost BDNF-activated PLC-γ.

Discussion

We have shown that chronic pretreatment with DEX disturbs BDNF-stimulated PLC-γ signaling for glutamate release via a glutamate transporter. Chronic DEX caused marked GR down-regulation. GR overexpression recovered the reduction in BDNF-activated PLC-γ, and siRNA transfection for endogenous GR mimicked the inhibitory effect of DEX. Corticosterone also reduced the GR level and suppressed BDNF-stimulated PLC-γ and glutamate release. Interestingly, we found that the GR interacted with TrkB and that the TrkB-GR interaction and

the BDNF-dependent binding of PLC-γ to TrkB decreased following DEX exposure.

BDNF-activated PLC-γ was specifically down-regulated by DEX or corticosterone whereas the activation of TrkB, Akt (a component of the PI3K pathway), and ERK signaling was not affected. A study on an animal model of depression showed a different responsiveness at the level of PI (phosphoinositide)-PLC after single vs. repeated stress (26). Additionally, long-term administration of antidepressants (desipramine, fluoxetine, and phenelzine) decreases PI-PLC activity in the membrane and cytosol fractions of the rat cortex (27). Meanwhile, imipramine activates PLC-β1 in the rat frontal cortex membrane (28). Recently, we reported that BDNF-activated PLC-γ was increased by imipramine (19). These studies suggest that PLC activity is critical in the pathophysiology of depression and the effects of antidepressants. In isolated rat islets, DEX suppresses PLC activation and insulin secretion (29). Thus, to reveal the cell biology of stress hormones, it may be valuable to focus on the PLC/Ca^{2+} system in neuronal or nonneuronal cells.

Glucocorticoids acutely activate TrkB signaling via the genomic function of the GR (25). Activation of TrkB, PLC-γ, Akt, and ERK was increased by short-term application of DEX and corticosterone, reaching the maximum at 2–4 h after the application, and returned to the baseline in our cultures. In contrast, no change in the BDNF-stimulated activations of TrkB signaling, including PLC-γ, was observed after such a short-term pretreatment with DEX or corticosterone. In this study, decreased responses to BDNF (not response to the glucocorticoid alone) after long-term glucocorticoid exposure (24–48 h) were discovered. Collectively, these results suggest that glucocorticoids play various functions depending on exposure time and that the mechanism underlying the down-regulation of BDNF-dependent PLC-γ signaling after chronic glucocorticoid exposure differs from the activation of TrkB signaling by acute exposure.

Down-regulations of the GR and TrkB-GR interaction caused by chronic DEX or corticosterone were observed. Such down-regulation was also observed in vivo. It is possible that the GR down-regulation may simply result in the decrease of the GR/TrkB complex. GR-overexpressing neurons showed a high response to BDNF, and siRNA for the GR mimicked the action of DEX, suggesting that moderate levels of GR expression may be essential for the BDNF/TrkB/PLC-γ system. In this study, a BDNF-dependent slight increase in TrkB-GR interaction in the control cultures was observed. In contrast, fluctuation in TrkB-GR interaction by GR overexpression or down-regulation was observed without BDNF stimulation, and endogenous BDNF was not changed by DEX. Thus, both BDNF (or phosphorylation of TrkB)-dependent and -independent mechanisms might be involved in TrkB-GR interaction.

Glucocorticoids have a rapid influence on intracellular signaling (not via transcriptional activity), and this rapid action is presumed to be mediated via membrane-bound and nonmembrane-bound GR (classical GR) or putative G protein-coupled receptors on a plasma membrane (30, 31). We speculate that classical GR is involved in TrkB/PLC-γ signaling because RU486 blocked the inhibitory effect of DEX, and the overexpression and down-regulation of the GR influenced TrkB/PLC-γ signaling. Raf-1 and 14–3-3 interact with liganded- or nonliganded GR (32), implying that the GR directly influences signaling pathways in cytosol. Moreover, the GR affects the plasma membrane receptor in immune T-cells (30). The T cell receptor (TCR) makes a protein complex including the GR. After the glucocorticoid is bound to the GR, the GR dissociates from the complex, and TCR signaling is inhibited. A similar mechanism might be involved in TrkB-GR interaction. Interestingly, FMS-like tyrosine kinase 3 (Flt3), another member of the receptor tyrosine kinase (RTK) family, interacts with the GR in hematopoietic cells (33). Flt3 interacts with the N-terminal region of the GR in the presence and absence of glucocorticoid. In the present study, TrkB/PLC-γ signaling was potentiated by GR overexpression and declined by GR down-regulation without DEX, implying that the GR has a positive effect on PLC-γ signaling in a ligand-independent fashion. In our system, the N-terminal region of the GR interacts with TrkB; however, the C-terminal region is also required for the full activation of PLC-γ. These results, including those of our study, indicate an important role of GR in the signaling of the RTK family.

The influx of Ca^{2+} regulates BDNF expression (34, 35). In addition, glutamate can regulate neurotrophin expression (36, 37). BDNF is produced and released in an activity-dependent manner (38, 39). Collectively, it is possible that suppression in BDNF-stimulated PLC-γ/Ca^{2+} signaling for glutamate release may be followed by a reduction in BDNF protein. Previously, we found that antidepressants potentiated BDNF-stimulated PLC-γ/Ca^{2+} (19). Therefore, our system, in which a glucocorticoid exerts an inhibitory effect on BDNF-stimulated PLC-γ/Ca^{2+}, may be useful for evaluating novel analogs as antidepressant candidates. Interestingly, the high-affinity interaction of pro-neurotrophin with a low-affinity receptor p75 was reported (40). It may be valuable to study, not only with respect to the expression of mature BDNF but also in terms of the neurotrophin form (pro-/mature) and the affinity of the ligand/receptor/adaptor interaction (present study) during stress hormone exposure.

Materials and Methods

Cells, Survival Assay, Ca^{2+} Imaging, Immunoprecipitation, Immunoblotting, Immunocytochemistry, and Animals. Cortical neurons were prepared from P2 rats as previously reported (18). Cell viability was examined with an MTT assay. In Ca^{2+} imaging, in which fluo-3 dye was used, the ratio (F/F_0) of fluorescence was calculated based on the intensities of fluorescence before and after BDNF was added. Immunoprecipitation, immunoblotting, and immunocytochemistry were performed as previously described (18). For the in vivo approach, P7 rats received an i.p. injection of (0.1–10 mg/kg i.p.) DEX or vehicle (sesame oil). After 48 h, the brains were removed from the deeply anesthetized rats, and the cerebral tissues were homogenized. All animals were treated according to the institutional guidelines for the care and use of animals. Details of these experiments are available in *SI Materials and Methods*.

DEX Pretreatment. After the cortical cultures were maintained for 4 or 5 days, DEX (1 μM, BIOMOL International L.P.) or corticosterone (1 μM, SIGMA) was added to the neurons by bath application. Subsequently, the cultures were incubated for 24 or 48 h in the presence of DEX or corticosterone before amino acid measurement, Ca^{2+} imaging, immunoprecipitation, immunoblotting, and immunocytochemistry were performed. DEX or corticosterone was dissolved in DMSO. Sole DMSO (vehicle) had no effects compared with no treatment (data not shown). RU486 (1 μM, LKT Laboratories) was applied 20 min before adding DEX.

Detection of Amino Acid Neurotransmitters. HPLC was used to analyze amino acid neurotransmitters as described previously (18). Details can be found in *SI Materials and Methods*.

Viral GR Construct, GR Deletion Plasmids, and siRNA. Detailed procedures for constructing the sindbis virus, producing GR plasmids, and transfecting siRNA are described in *SI Materials and Methods*.

Statistical Analysis. Data are expressed as mean ± SD. Statistical significance was evaluated by student's *t* test, and probability values of less than 5% were considered significant.

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Functional near-infrared spectroscopy reveals altered hemispheric laterality in relation to schizotypy during verbal fluency task

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ABSTRACT

Previous functional neuroimaging studies have demonstrated that patients with schizophrenia and those with schizotypal personality disorder (SPD) show reduced laterality, or relative right hemispheric dominance, during the performance of cognitive activation tasks; however, neuroimaging studies looking at non-clinical schizotypy have been few. We have recently reported that schizotypal traits at a non-clinical level are associated with right prefrontal dominance during a letter version of the verbal fluency task (VFT), but it is unknown whether such relationship between schizotypy and functional laterality would be observed across various cognitive tasks. Here we examined the relationships of schizotypal traits as measured by the Schizotypal Personality Questionnaire (SPQ) in healthy adults with hemispheric lateralization of prefrontal activation during letter and category VFTs, using near-infrared spectroscopy. Thirty-two participants were divided into high- ($n=16$) and low- ($n=16$) SPQ groups by the median split of the total SPQ score. The high-SPQ group, but not low-SPQ group, showed significantly right-greater-than-left asymmetry of prefrontal activation during letter VFT, whereas such pronounced hemispheric asymmetry in relation to schizotypy was not found during category VFT. These results indicate that non-clinical schizotypy is related to right prefrontal preference during the letter version of VFT in particular, suggesting that the association between schizotypal traits and functional laterality may vary depending on cognitive activation tasks.

1. Introduction

Schizotypy can be viewed as a dimensional trait ranging from non-clinical people to schizophrenic spectrum patients (Claridge, 1985) and the extent to which individuals in the general population exhibit schizotypal traits also varies on a continuum (Kendler et al., 1991). Although this dimensional model of schizotypy seems plausible, the notion of dimensionality is not entirely unequivocal such that some investigators have argued for the competing theory, namely the categorical approach to schizotypy. For example, Meehl (1962) proposed the concept called "schizotypy taxon", i.e., a bimodal distribution of two taxa, those with and without schizotypy. This Meehl's dichotomous approach to schizotypy has been supported and further explored by other workers (reviewed in Lenzenweger and Korfine, 1995). Still, however, a large number of schizophrenia/schizotypy researchers have substantiated the dimensional model of schizotypy (Claridge and Beech, 1995; Siever and Davis, 2004). This dimensional model of schizotypy assumes that non-clinical schizotypy, schizotypal personality disorder (SPD) and schizophrenia qualitatively resemble each other but quantitatively differ in the manner that the latter, particularly chronic schizophrenia, lies at the extreme end of the continuum. Among these, non-clinical schizotypy, which by definition is not confounded by potential consequences of psychiatric diagnoses such as antipsychotic treatment and institutionalization, offers a unique opportunity to disentangle and isolate the pathophysiology intrinsic to schizophrenia. In accord with this view, patients with SPD and those with schizophrenia have been demonstrated to share similar, but not completely identical, neurophysiological (Siever and Davis, 2004), neurocognitive (Mitropoulou et al., 2005; Siever and Davis, 2004) and neuroimaging (Dickey et al., 2002; Siever and Davis, 2004), impairments. Non-clinical schizotype has also been shown to display neurophysiological (Kiang and Kutas, 2005; O'Driscoll et al., 1998) and neurocognitive (Gooding et al., 2006; Lenzenweger and Korfine, 1994; Noguchi et al., in press; Park and McTigue, 1997) deficits which are similar to, albeit less severe than, those in schizophrenia

Abbreviations: ANCOVA, Analysis of covariance; CFT, Category version of verbal fluency task; deoxy-Hb, Deoxygenated hemoglobin; DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders, third edition, revised; IQ, Intelligence quotient; LFT, Letter version of verbal fluency task; LT, Left; NIRS, Near-infrared spectroscopy; oxy-Hb, Oxygenated hemoglobin; PFC, Prefrontal cortex; RT, Right; SPD, Schizotypal personality disorder; SPQ, Schizotypal personality questionnaire; VFT, Verbal fluency task.

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patients and in patients with SPD; however, neuroimaging studies looking at non-clinical schizotypes have been scarce.

One of the repeatedly reported findings in functional neuroimaging studies of schizophrenia spectrum disorders including schizophrenia is the reduced hemispheric laterality, or relative right hemispheric dominance. Using a variety of cognitive activation tasks, this reduced laterality has been well documented in schizophrenia patients (Fallgatter and Strik, 2000; Razafimandimby et al., 2007; Sommer et al., 2003) as well as in patients with SPD (Buchsbaum et al., 1997; Folley and Park, 2005). Since the left hemisphere is normally specialized for processing verbal material (Hellige, 1993), it has been proposed that schizophrenia could be seen as an anomaly of language function due to a failure of hemispheric lateralization for language (Crow, 1997).

A verbal fluency task (VFT), in which subjects are asked to generate as many words as possible within a designated time period by either a phonological cue (i.e., letter VFT) or a semantic cue (i.e., category VFT), is a widely used neuropsychological test known to activate a distribute set of brain regions including the prefrontal cortex (PFC); however, letter and category VFTs are considered to demand somewhat different cognitive processing. The former requires unfamiliar lexical search based on phonology whereas the latter necessitates semantic search based on organization of semantic memory networks. In patients with schizophrenia, findings on overall extent of frontal activation during VFT are somewhat controversial such that some researchers have found attenuated frontal activity (Ehlis et al., 2007; Takizawa et al., 2008; Yurgelun-Todd et al., 1996), whereas others have observed overall preserved activation with altered distribution of activated regions (Artiges et al., 2000; Weiss et al., 2006). On the other hand, reduced hemispheric lateralization during this task has been consistently reported (Artiges et al., 2000; Sommer et al., 2001,2003; Weiss et al., 2006). Moreover, VFT is considered to tap not only the frontally-mediated executive function, which is found to be substantially impaired in schizophrenia, but also a certain aspect of creativity or divergent thinking (Nemoto et al., 2005; Weinstein and Graves, 2001), which may in turn relate to a relatively benign facet of schizotypal traits (Folley and Park, 2005; Weinstein and Graves, 2002). In this context, VFT would be suited to investigate the possible relationship between schizotypy and functional laterality.

Near-infrared spectroscopy (NIRS), a noninvasive optical neuroimaging technique which measures changes of the hemoglobin concentration in the human brain with a high time resolution, has been extensively used to assess brain function in healthy subjects as well as in various medical conditions including psychiatric diseases (Ehlis et al., 2007; Fallgatter and Strik, 2000; Folley and Park, 2005; Kubota et al., 2005; Kuwabara et al., 2006; Suto et al., 2004; Takizawa et al., 2008). The majority of these studies employed VFT as a cognitive activation task (Ehlis et al., 2007; Kubota et al., 2005; Kuwabara et al., 2006; Suto et al., 2004; Takizawa et al., 2008), partly because NIRS enables investigators to monitor brain activity during overt word generation. The most consistently reported finding in functional NIRS studies is task-related activation of PFC with an increase in oxygenated hemoglobin (oxy-Hb) and a small decrease in deoxygenated hemoglobin (deoxy-Hb).

Two previous NIRS studies administered both letter and category VFTs to patients with schizophrenia and healthy controls and found that patients exhibited more pronounced attenuation in prefrontal activation during letter VFT than during category VFT (Ehlis et al., 2007; Kubota et al., 2005); while on a behavioral level as indexed by the number of words produced, schizophrenia patients have been found to be disproportionately deficient in category vis-à-vis letter VFT (Bokat and Goldberg, 2003). As we have recently shown that schizotypal traits in healthy women are related to the relative right prefrontal dominance during performance of letter VFT (Hori et al., 2008a), it would be of interest to further examine whether the two VFTs are distinctively associated with the functional laterality in relation to schizotypy.

Moreover, another line of research has found non-clinical schizotypal traits to be associated with abnormal typicality of responses in category VFT (Kiang and Kutas, 2006), pointing to the alteration in semantic memory organization in non-clinical schizotypes. Given the putative relations of creativity to schizotypy and to reduced hemispheric laterality (Folley and Park, 2005; Weinstein and Graves, 2001,2002), this finding of an association between non-clinical schizotypy and abnormal typicality may account for a potential route whereby schizotypy is linked to reduced laterality. A further question may therefore be raised as to what the relationships between schizotypy, functional laterality, and typicality of responses in category VFT are.

The present study aimed 1) to investigate whether letter and category VFTs would induce similar or different patterns of functional laterality in relation to schizotypal traits as assessed with the Schizotypal Personality Questionnaire (SPQ; Raine, 1991) and 2) to examine the possible relationships between schizotypy, functional laterality and typicality of responses in category VFT, by using NIRS.

2. Methods

2.1. Subjects

Thirty-two healthy volunteers (age range, 22–59 years; male/female: 10/22) participated in this study. Of the volunteers, 14 were employees of a small company who were engaged in intellectual occupations. For these subjects, the whole experimental procedure was carried out in their office. The remaining 18 participants were recruited through flyers and advertisements and by word of mouth. They were administered the experimental procedure at our laboratory. Assessment of intelligence quotient (IQ) of the former participants was left out, while IQ of the latter was estimated with the Japanese Adult Reading Test (Hori et al., 2008b; Matsuoka et al., 2002), a Japanese version of the National Adult Reading Test (Nelson and Wilson, 1991), and those whose estimated-IQ score was less than 85 were not enrolled in the study. All participants were interviewed using the Japanese version of the Mini-International Neuropsychiatric Interview (Otsubo et al., 2005; Sheehan et al., 1998) by research psychiatrists (H.H. and Y.O.), and only those who demonstrated no history of psychiatric illness were enrolled in this study. Participants were excluded if they had a prior medical history of central nervous system disease or severe head injury. Participants who had one or more first-degree relatives with schizophrenia spectrum disorders or bipolar disorder were also excluded. All subjects were right-handed according to the Edinburgh Handedness Inventory (Oldfield, 1971). After the nature of the procedures had been fully explained, written informed consent was obtained from all subjects. The study was approved by the ethics committee of the National Center of Neurology and Psychiatry, Japan.

2.2. Schizotypy measure

The SPQ (Raine, 1991) is a 74-item validated self-report questionnaire with a "yes/no" response format that incorporates DSM-III-R (American Psychiatric Association, 1987) criteria for a diagnosis of SPD. All items endorsed "yes" are scored 1 point. The questionnaire consists of 9 subscales, which have been found to load onto 3 factors: cognitive–perceptual (comprising the ideas of reference, odd beliefs/magical thinking, unusual perceptual experiences, and suspiciousness/paranoid ideation subscales), interpersonal (social anxiety, no close friends, constricted affect, and suspiciousness/paranoid ideation), and disorganized (eccentric/odd behavior and odd speech) factors (Raine et al., 1994).

The Japanese version of the SPQ translated by Fujiwara (1993) was used in the present study. The questionnaire had been administered to 258 Japanese college students in a validation study (Someya et al.,

1994), and the reliability and validity of this Japanese version of SPQ were demonstrated to be similar to those of the original version of Raine (1991), whereas this validation study showed a lower mean total SPQ score of around 10 (Someya et al., 1994). Our study sample was split by median of the total SPQ score (median = 11; range, 1–40) into high- (n = 16) and low- (n = 16) SPQ groups. The company employees/other participants ratios of the high- and low-SPQ groups were 6/10 and 8/8, respectively (χ^2 = 0.51, p = 0.48). All comparisons in this study, including comparisons of NIRS data, were made between the two groups unless otherwise specified.

2.3. Task procedure

First, participants sat on a chair near the NIRS machine with their eyes open. Then they were given practice with mimic tasks using a certain syllable (/a/) for letter VFT and a certain semantic category (fruit) for category VFT. Both VFTs consisted of a 30-s rest, a 30-s pretask, a 60-s task condition and a 60-s posttask. During the task condition, participants were required to verbally generate as many words as possible beginning with given syllables (/ki/, /nu/, /ra/, each for 20-s) for the letter VFT and belonging to given semantic categories (four-footed animals, vegetables, vehicles, each for 20-s) for the category VFT. These phonological and semantic cues were visually presented on a computer screen. During the pretask and posttask periods in both VFTs, participants were asked to repeatedly pronounce the syllables /a/,/i/, /u/, /e/, and /o/ at approximately the same speed as an example provided by an examiner during the practice time. The order of the two VFTs was counterbalanced among subjects. To prevent artifacts subjects were asked to avoid body movements, particularly head movements, during the NIRS measurements. The movements of subjects were monitored throughout the examination by an experimenter (H.H.). The phonological cues employed in letter VFT were different from those used in the majority of previous studies conducted in Japan including ours (e.g., Hori et al., 2008a; Kuwabara et al., 2006; Suto et al., 2004; Takizawa et al., 2008), while the semantic cues in category VFT were determined according to prior studies (Ehlis et al., 2007; Kiang and Kutas, 2006; Kubota et al., 2005). The verbal responses during the two VFTs were recorded using an integrated circuit recorder.

2.4. Analyses of behavioral data

For both letter and category VFTs, the number of correct words (no repetitions) generated during the task period was used as a measure of behavioral performance. In addition, the typicality of responses, a measure indicative of the extent to which the each individual's response set corresponds to the ranking in the category production norms, was calculated only for category VFT. We used the category production norms for Japanese compiled by Ogawa (1972) that lists the ranking of the frequencies of occurrence for words which belong to each of 52 categories including the four-footed animal, vegetable and vehicle (e.g., for vegetable, the ranking in the norms is the following order: *carrot*, *Chinese cabbage*, *radish*, *cabbage*, *tomato*, *cucumber*, *spinach*, and so on). Based on the study by Kiang and Kutas (2006), overall typicality of responses (typicality index t) for each of the three categories employed herein was calculated using the following equation:

$$t = \frac{\sum_{i=1}^{n} [f_i/i]}{n}$$

where n = the total number of responses for each category, i = the ordinal position of each response, and f = its position in the ranking in the norms of Ogawa (1972). For instance, as for vegetable, f ranged from 1 for *carrot* to 37 for *bamboo shoot*. If an individual's response was not found in the norms but actually belonged to the designated category, its position (i.e., f) was treated as one greater than the total number of items listed for the category in the norms-for example, as for vegetables, since the total number of items in the norms is 37, f of an individual's response unlisted in the norms (e.g., *butterbur*) was treated as 38. Thus, lower values of t represent higher typicality, with the minimum possible value of t being 1 where an individual's responses exactly match the ranking in the norms. In addition to the t of each category, the average of these three typicality indices ("average t") was calculated. When examining the possible association between typicality and hemispheric laterality, total subjects were split into two groups by the median value of "average t" and these two groups were compared for the laterality.

2.5. NIRS measurements

NIRS was performed using a 31-channel spectrometer (FOIRE-3000; Shimadzu Corporation, Japan) at three wavelengths of near-infrared light (780, 805 and 830 nm), with the distance between pairs of emission and detector probes set at 3.0 cm. Probes on a 4×5 probe holder were placed on the forehead of each subject, with the lowest probes being symmetrically positioned along the Fp1–Fp2 line according to the International 10–20 system of electroencephalogram electrode placement. The measurement channel was defined as each area between pairs of emission-detector probes (Fig. 1). While NIRS data include three measures of hemoglobin concentration, namely oxy-Hb, deoxy-Hb and total-Hb, we employed concentration change (mM×cm) in oxy-Hb for main statistical analyses because it is considered the most reliable indicator of changes in regional cerebral blood flow (Hoshi et al., 2001, 2003; Kono et al., 2007); however, data of deoxy-Hb were also provided where appropriate. The time resolution was set at 0.1-s. An average of 10 right prefrontal channels (i.e. channels #1, 5, 6, 10, 14, 15, 19, 23, 24 and 28) and that of the 10 left prefrontal channels (i.e. channels #4, 8, 9, 13, 17, 18, 22, 26, 27 and 31) were designated as two representative measures for NIRS data, namely "RTmean" and "LTmean", respectively; those 20 channels included Brodmann's areas 10 and 46 (Hori et al., 2008a; Kuwabara et al., 2006). Thus, outcomes for NIRS data used in the statistical analyses were limited to 8 summary measures, i.e., "LFT-RTmean-oxy", "LFT-RTmean-deoxy", "LFT-LTmean-oxy", "LFT-LTmean-deoxy", "CFT-RTmean-oxy", "CFT-RTmean-deoxy", "CFT-LTmean-oxy" and "CFT-LTmean-deoxy" (i.e., the first 4 were the measures for letter VFT and the last 4 for category VFT). In addition, taking into account that channels on the upper area of a head covered by hair were subject to artifact, the main statistical analyses were repeated with the RTmean and LTmean being limited to an average of 4 right prefrontal channels (i.e. channels #19, 23, 24 and 28) and that of the 4 left prefrontal channels (i.e. channels #22, 26, 27 and 31), respectively, which were on a lower forehead. This criterion of channel reduction was according to previous studies (Hori et al., 2008a; Kuwabara et al., 2006).

By referring to prior studies (Hori et al., 2008a; Kubota et al., 2005; Suto et al., 2004), a period of 10-s for the pretask condition (excluding first 20-s) and a period of 50-s for the task condition (excluding first 10-s) were sampled for the calculation of the average concentration change in oxy- and deoxy-Hb. Then, analyses were performed on the change in oxy- and deoxy-Hb (from pretask baseline) during the task condition.

2.6. Statistical analyses

Averages are reported as means ± S.D. for the variables which satisfied the assumptions for parametrical testing and as medians with ranges in parentheses for those which did not. Demographic characteristics, SPQ scores, and NIRS data (i.e. LFT-RTmean, LFT-LTmean, CFT-RTmean and CFT-LTmean, each for oxy-Hb and deoxy-Hb) were compared between the high- and low-SPQ groups. Kolmogorov–Smirnov test was used to

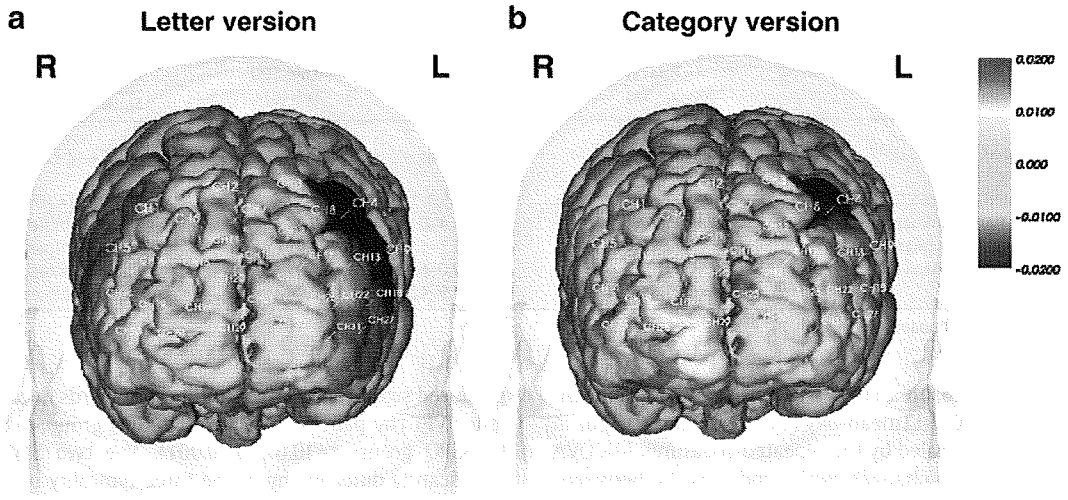

Fig. 1. Superimposed images on 3-D magnetic resonance imaging showing the differences between high- and low-SPQ groups (i.e., high-SPQ minus low-SPQ group) in oxy-Hb concentration changes during the task period (from pretask baseline) across 31 channels, by the letter (left side, a) and the category (right side, b) fluency tasks. The color bar indicates oxy-Hb concentration (mM×cm). Digits represent the numbers of the 31 channels and lines adjacent to the corresponding digits indicate the exact positions of the channels; channels # 1, 5, 6, 10, 14, 15, 19, 23, 24 and 28 were averaged into the RTmean, and # 4, 8, 9, 13, 17, 18, 22, 26, 27 and 31 into the LTmean.

examine the distribution of the data, and non-parametrical analyses, i.e. Mann–Whitney *U*-test to compare means and Spearman's ρ to examine correlations, were used where the data violated the assumptions for parametrical testing; otherwise, the *t*-test to compare means and Pearson's *r* to examine correlations were used. Specifically, age, estimated IQ, total SPQ score, numbers of words generated for both letter and category VFTs, and NIRS data for oxy-Hb, but not handedness, most of the typicality indices (*t*), most of the SPQ subscales, or NIRS data for deoxy-Hb, satisfied the assumptions for parametrical testing. Of the variables examined including demographic characteristics, estimated-IQ and VFT performance (i.e., the number of words generated), those variables that were significantly different between the two SPQ groups and significantly affected the NIRS data for oxy-Hb were considered as potential confounders. Since age has been shown to significantly influence the NIRS data (e.g., Herrmann et al., 2006; Kameyama et al., 2004), this variable was considered as a confounder regardless of the present data. Based on several previous NIRS studies that included the analysis of hemispheric laterality (Hori et al., 2008b; Kubota et al., 2005; Kuwabara et al., 2006), the NIRS data for oxy-Hb were first analyzed by the three-way repeated-measures analysis of covariance (ANCOVA), in which SPQ group was used as the between-subject factor, task condition and hemisphere as the within-subject factors, and the confounders as the covariates. When group-by-hemisphere interaction was significant in this analysis, then the hemispheric laterality was considered to be different between the two groups. Secondly, to examine the relationship between schizotypy and laterality *within* each VFT, the two-way repeated-measures ANCOVA on oxy-Hb data was performed, separately for letter and category VFTs, with SPQ group as the between-subject factor, hemisphere as the within-subject factor, and the confounders as the covariates. Similarly, to investigate the possible relationship between typicality of responses in category VFT and laterality, the two-way repeated-measures ANCOVA was performed, in which typicality group (i.e., high- and low-typicality groups) was used as the between-subject factor, hemisphere as the within-subject factor, and the confounders as the covariates. A similar ANCOVA was used to examine the potential gender difference in hemispheric laterality. To further examine the correlational relationship of schizotypy with laterality, multiple regression analyses were carried out with right-left difference measures (i.e., LFT-RTmean-oxy minus LFT-LTmean-oxy and CFT-RTmean-oxy minus CFT-LTmean-oxy) as dependent variables and the total SPQ score and confounders as independent variables. Statistical significance was set at two-tailed $p<0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo).

3. Results

3.1. Sample characteristics

Table 1 lists the demographic characteristics, estimated IQ, and SPQ scores of participants stratified by high- and low-SPQ groups. The two SPQ groups significantly differed only in SPQ scores. Compared to the low-SPQ group, the high-SPQ group had greater representation of females with statistical trend; however, gender was not considered a confounder because males and females did not significantly differ in

Table 1
Demographic characteristics and SPQ scores of the participants

Characteristic	Total subjects (n=32)	High SPQ (n=16)	Low SPQ (n=16)	Analyses (high vs. low)	
				Statistics	p
Gender, male/female	10/22	2/14	8/8	Fisher's exact	0.054
Age, years: mean±S.D.	40.6±10.8	41.1±11.8	40.2±10.1	t=0.23, df=30	0.82
Handedness, Edinburgh score: median (range)	95 (70–100)	100 (78–100)	90 (70–100)	Mann–Whitney U=102.5	0.30
Estimated IQ, mean±S.D.	110.9±10.6[a]	108.3±10.5[b]	114.2±10.4[c]	t=1.19, df=16	0.25
Total SPQ score, mean±S.D.	12.9±8.6	19.6±7.3	6.3±2.6	t=6.85, df=30	<0.001
Cognitive–perceptual factor, median (range)	3 (0–12)	5 (1–12)	2 (0–7)	Mann–Whitney U=54.0	0.005
Interpersonal factor, median (range)	4 (0–23)	7.5 (3–23)	3 (0–5)	Mann–Whitney U=18.5	<0.001
Disorganized factor, median (range)	3 (0–12)	6.5 (2–12)	1 (0–5)	Mann–Whitney U=12.0	<0.001

SPQ, schizotypal personality questionnaire; IQ, intelligence quotient.
[a] n=18.
[b] n=10.
[c] n=8.

Table 2
Behavioral data of the two verbal fluency tasks in the subjects stratified by the SPQ groups

Task performance	Total subjects (n=32)	High SPQ (n=16)	Low SPQ (n=16)	Analyses (high vs. low)	
				Statistics	p
Letter fluency task					
Number of words generated, mean±S.D.	16.3±3.2	15.9±2.8	16.7±3.5	t=0.72, df=30	0.48
Category fluency task					
Number of words generated, mean±S.D.	27.8±5.3	27.6±5.9	27.9±4.7	t=0.16, df=30	0.88
Typicality index t of four-footed animals, median (range)	2.4 (1.4–7.1)	2.4 (1.4–6.3)	2.8 (1.8–7.1)	Mann–Whitney U=84.5	0.16
Typicality index t of vegetables, median (range)	2.5 (1.7–4.3)	2.6 (1.7–4.3)	2.4 (1.8–3.8)	Mann–Whitney U=108.5	0.88
Typicality index t of vehicles, median (range)	2.8 (1.7–6.4)	2.7 (2.0–6.4)	2.8 (1.7–5.0)	Mann–Whitney U=117.0	0.91
Average t, median (range)	2.8 (1.9–4.2)	2.7 (1.9–4.1)	2.9 (2.1–4.2)	Mann–Whitney U=99.0	0.41

SPQ, schizotypal personality questionnaire.

any of the 4 NIRS oxy-Hb measures, i.e., LFT-RTmean-oxy, LFT-LTmean-oxy, CFT-RTmean-oxy and CFT-LTmean-oxy (by t-test: all $p>0.3$) or in hemispheric laterality as revealed by the repeated-measures ANCOVA on the oxy-Hb concentration changes, with gender as the between-subject factor, task and hemisphere as the within-subject factors, and age as the covariate [gender-by-hemisphere interaction: $F(1,29)=0.001$, $p=0.97$].

3.2. Behavioral data

The behavioral data on letter and category VFTs by SPQ groups are presented in Table 2. The behavioral performance as indicated by the numbers of words generated was not significantly different between the two SPQ groups, in either letter or category VFT. Both SPQ groups produced significantly larger numbers of words in category VFT than in letter VFT (by paired t-test: for high-SPQ group, $t=8.77$, $p<0.001$; for low-SPQ group, $t=10.67$, $p<0.001$). The two SPQ groups did not significantly differ in any of the three typicality indices (t) or in their average index. In addition, these behavioral measures including the numbers of words generated and typicality indices were not significantly correlated with any of the 4 oxy-Hb measures or their right-left difference measures (all $p>0.1$). Furthermore, when total subjects were split into two groups by the median of "average t", these two groups did not significantly differ in hemispheric laterality during category VFT, which was revealed by the repeated-measures ANCOVA on the oxy-Hb concentration changes, with the typicality group as the between-subject factor, hemisphere as the within-subject factor, and age as the covariate [i.e., group-by-hemisphere interaction: $F(1,29)=1.53$, $p=0.23$]. These

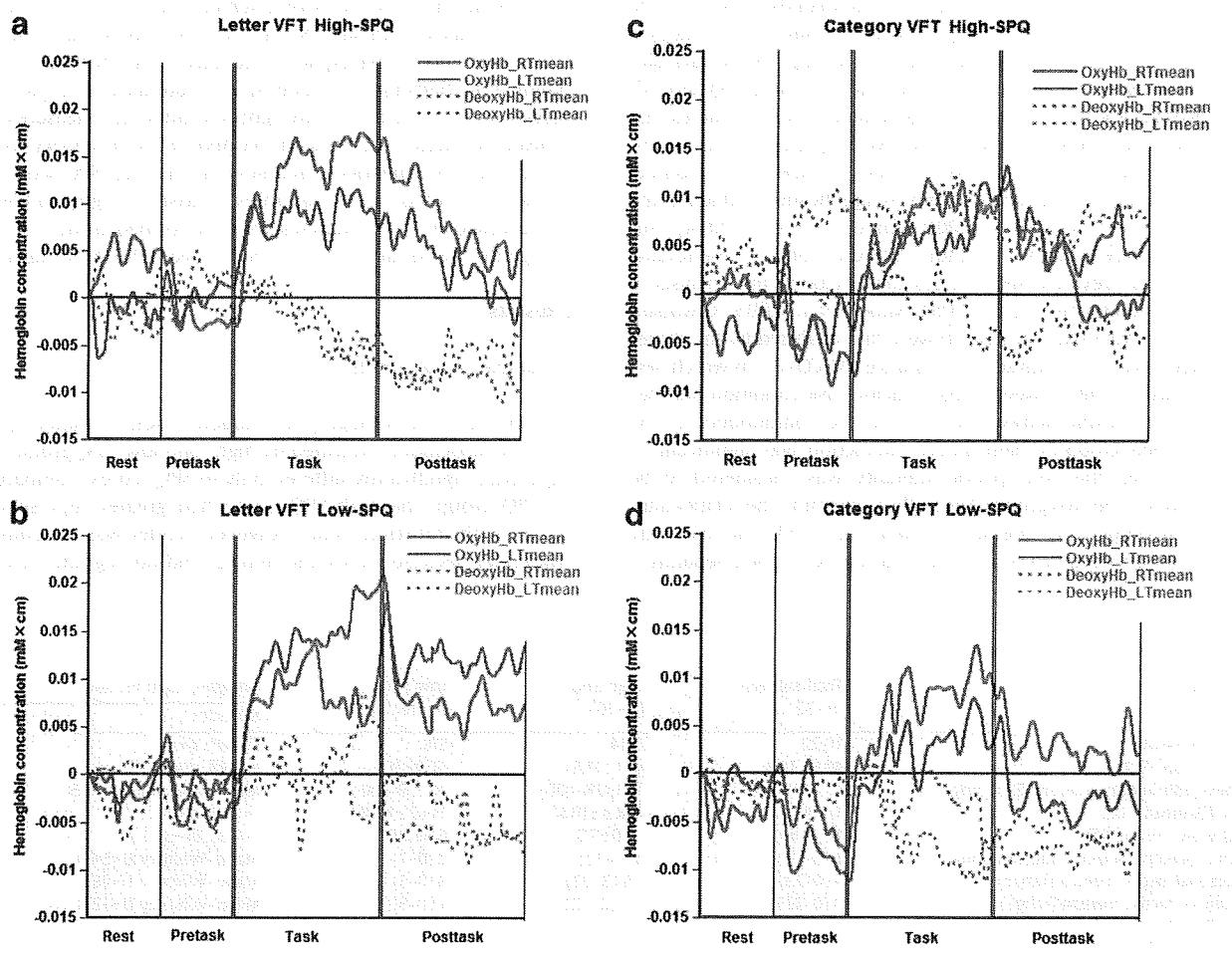

Fig. 2. Overall mean waveforms (oxy-Hb changes in solid lines and deoxy-Hb changes in dotted lines) of RTmean (red) and LTmean (blue) stratified by the SPQ group [high-SPQ group, upper half (a, c); low-SPQ group, lower half (b, d)] and by the fluency task [letter VFT, left side (a, b); category VFT, right side (c, d)].

indicated that typicality of responses did not impact upon schizotypal traits or NIRS data including hemispheric laterality.

3.3. NIRS data

3.3.1. oxy-Hb

Fig. 1 illustrates the differences between high- and low-SPQ groups (defined as high-SPQ minus low-SPQ group) in oxy-Hb concentration changes during the task period (from pretask baseline), by the letter (a) and the category (b) fluency tasks. Compared to the low-SPQ group, the high-SPQ group showed larger right and smaller left hemispheric activation in both VFTs; however, this differential hemispheric dominance pattern between the SPQ groups was more pervasive in letter VFT than in category VFT (Fig. 1). Fig. 2 shows overall mean waveforms of RTmean and LTmean, both for oxy- and deoxy-Hb, during the whole VFT period, by SPQ group and by fluency task. Clear oxy-Hb increases were consistently observed during the task period across SPQ groups, types of fluency tasks, and hemispheres. During the posttask period, both SPQ groups showed an oxy-Hb decrease from the beginning to the end of this period, in both tasks and for both hemispheres.

The initial three-way repeated-measures ANCOVA on the oxy-Hb concentration changes, with SPQ group as the between-subject factor, task condition and hemisphere as the within-subject factors, and age as the covariate, showed no significant main effect of group [$F(1,29)=0.27$, $p=0.61$], task condition [$F(1,29)=0.01$, $p=0.92$], hemisphere [$F(1,29)=1.00$, $p=0.33$], or age [$F(1,29)=0.25$, $p=0.62$]. However, the group-by-hemisphere interaction was significant [$F(1,29)=4.36$, $p=0.046$] and group-by-task-by-hemisphere interaction was at a trend-level significance [$F(1,29)=3.05$, $p=0.091$], while none of the other interactions between group, task, hemisphere and age were significant (all $p>0.3$). Similar results, including the significant group-by-hemisphere interaction [$F(1,29)=6.71$, $p=0.015$], were obtained when summary measures of NIRS data (i.e., RTmean and LTmean) were defined as an average of the lower 4 prefrontal channels. These indicated that the hemispheric laterality was significantly different between the two SPQ groups whereas neither SPQ group nor task condition nor age affected the overall component of NIRS data.

Subsequently, two-way repeated-measures ANCOVA on the oxy-Hb concentration changes was performed separately for letter and category VFTs, with SPQ group as the between-subject factor, hemisphere as the within-subject factor, and age as the covariate. It revealed that group-by-hemisphere interaction was significant for letter VFT [$F(1,29)=21.09$, $p<0.001$], but not so for category VFT [$F(1,29)=0.21$, $p=0.65$]. This analysis for letter VFT further revealed that estimated mean differences (i.e., LFT-RTmean-oxy minus LFT-LTmean-oxy), controlling for age, of the high- and low-SPQ groups were 0.0093 ($p=0.003$, 95%CI=0.0034 to 0.015; Fig. 2a) and −0.0095 ($p=0.003$, 95%CI=−0.015 to −0.0036; Fig. 2b), respectively; on the other hand, this analysis for category VFT showed that estimated mean differences (i.e., CFT-RTmean-oxy minus CFT-LTmean-oxy), controlling for age, of the high- and low-SPQ groups were 0.0066 ($p=0.30$, 95%CI=−0.0062 to 0.019; Fig. 2c) and 0.0025 ($p=0.69$, 95%CI=−0.010 to 0.015; Fig. 2d), respectively. This letter VFT-specific hemispheric asymmetry in relation to schizotypy was also found when summary measures of NIRS data were defined as an average of the lower 4 prefrontal channels.

Multiple regression analysis, with the right-left difference measure for letter VFT (i.e., LFT-RTmean-oxy minus LFT-LTmean-oxy) as a dependent variable and total SPQ score and age as the independent variables, revealed that SPQ score had a significant effect (β coefficient=0.47, $t=3.05$, $p=0.005$) while age did not (β coefficient=−0.26, $t=-1.69$, $p=0.101$). A similar analysis for category VFT again revealed that SPQ score had a significant effect (β coefficient=0.40, $t=2.35$, $p=0.026$) while age did not (β coefficient=0.004, $t=0.024$, $p=0.98$). These results indicated that the correlational relationship between schizotypy and right hemispheric bias was significant during both letter and category VFTs, which was slightly different from the results yielded by the categorical ANCOVA approach.

3.3.2. deoxy-Hb

As depicted in Fig. 2, deoxy-Hb concentration changes generally showed the opposite pattern to oxy-Hb concentration changes; however, the pattern of deoxy-Hb changes appeared to be more complicated and less consistent, across SPQ groups and tasks, than that of oxy-Hb changes (Fig. 2). Using Spearman's correlation, significant correlations of the total SPQ score were found with LFT-RTmean-deoxy ($\rho=-0.35$, $p=0.048$) and with LFT-RTmean-deoxy minus LFT-LTmean-deoxy ($\rho=-0.52$, $p=0.002$), whereas no significant correlation was found between the total SPQ score and LFT-LTmean-deoxy ($\rho=0.29$, $p=0.10$), CFT-RTmean-deoxy ($\rho=-0.046$, $p=0.80$), CFT-LTmean-deoxy ($\rho=0.16$, $p=0.39$), or CFT-RTmean-deoxy minus CFT-LTmean-deoxy ($\rho=-0.19$, $p=0.30$). These results generally corresponded to the results for oxy-Hb although the directions of the association were opposite.

4. Discussion

In the present study we examined the association between schizotypal traits in a non-clinical population and functional laterality during letter and category versions of VFT, using NIRS. We found that non-clinical schizotypy was significantly related to the right-greater-than-left asymmetry of prefrontal activation pattern during letter VFT. This relationship between schizotypy and right hemispheric bias was less clear during category VFT. There was no significant association of typicality of responses in category VFT with schizotypy or with hemispheric laterality.

The positive association of schizotypal traits with right prefrontal dominance in letter VFT as revealed by the repeated-measures ANCOVA was a replication of the finding from our previous study (Hori et al., 2008a), which had included only female volunteers from community, employed other phonological cues (/a/, /ka/ and /sa/), and used another NIRS machine (Hitachi ETG-100). This association was confirmed with the regression analysis, which may be more suited than the categorical analysis using ANCOVA for testing the study hypothesis of dimensional model of schizotypy. Moreover, in the present study the two SPQ groups were almost identical to each other in terms of age and behavioral performance on letter VFT (indexed by the number of words generated), both of which could be potential confounders in interpreting NIRS data. Therefore, the present finding, coupled with our previous one, has corroborated the relationship between schizotypy and right prefrontal dominance during letter VFT, further suggesting that schizotypy would be associated with the same *qualitative* alteration (i.e., right-greater-than-left asymmetry) as schizophrenia is. On the other hand, the relation of schizotypal traits to overall prefrontal activation during the performance of the letter VFT, as revealed by the main effect of SPQ group on the NIRS data in the repeated-measures ANCOVA, was not significant, indicating that schizotypy at a non-clinical level does not affect the overall extent of PFC activation. Taking into account that a number of NIRS studies have demonstrated that patients with schizophrenia display apparently reduced prefrontal activation during this task (Ehlis et al., 2007; Kubota et al., 2005; Takizawa et al., 2008; Watanabe and Kato, 2004), the present finding of preserved overall PFC activation in the high-SPQ group could be considered as supplementary evidence supporting the notion that schizotypal traits at a non-clinical level would be *quantitatively* different from schizophrenia.

With respect to the prefrontal activation during category VFT, like letter VFT, schizotypy was not significantly associated with overall extent of PFC activation. On the other hand, concerning the association between schizotypy and laterality during this task, the ANCOVA and regression analysis yielded somewhat different results; that is, only regression analysis revealed the significant relation of schizotypy to right hemispheric preference. It may be that schizotypy is associated

with right hemispheric dominance during category VFT, albeit to a lesser degree than during letter VFT. As for patients with schizophrenia, previous NIRS studies have yielded mixed results for the overall extent of prefrontal activation during category VFT (Ehlis et al., 2007; Kubota et al., 2005). In the study of Kubota et al. (2005) schizophrenia patients exhibited preserved or even increased PFC activation during this task compared to healthy controls while Ehlis et al. (2007) reported that patients showed reduced activation in restricted areas of PFC relative to controls. Nevertheless, these two precedent studies were compatible with each other regarding more marked prefrontal hypoactivation during letter vis-à-vis category VFT in patients. Therefore, the present results, combined with these previous findings of NIRS studies in schizophrenia, may suggest that letter VFT is more sensitive than category VFT in detecting functional alterations in PFC associated with schizotypy and/or schizophrenia spectrum, including hemispheric lateralization and overall prefrontal activation. Viewed from another angle, given the putative association between creativity and schizotypy, these differential associations of schizotypy with functional laterality between letter and category VFTs observed here might be attributed to the distinct degrees to which these two VFTs require divergent thinking (i.e., letter VFT requires it more than category VFT does), thereby the right prefrontal dominance in relation to schizotypy would be more clearly seen during letter VFT.

With regard to behavioral performance in the VFTs, the current findings were in line with those of numerous studies in that significantly more words were generated in category than letter VFT, demonstrating that semantic fluency would be easier to perform than letter fluency. The equivalent numbers of words generated between the two SPQ groups in both VFTs indicate that non-clinical schizotypy would not be related to apparent alteration in the level of performance on word fluency, which was also consistent with previous studies (Hori et al., 2008a; Kiang and Kutas, 2006). Regarding the other behavioral measure, namely the typicality of responses in category VFT, schizotypy was not associated with any of the typicality indices examined, which was not in agreement with the prior study (Kiang and Kutas, 2006). This discrepancy between the present and prior studies may result from the differences in semantic cues employed or from differential sample characteristics between studies. In the prior study four semantic cues (fruits, four-footed animals, articles of clothing, vehicles) were used and schizotypy was significantly related to abnormal typicality only for the fruit category; while in the present study we did not include the fruit category in the semantic cues. Concerning sample characteristics, the study of Kiang and Kutas (2006) included 60 English speakers, most of whom were undergraduates, whereas the present sample consisted of 32 adult Japanese speakers. Further investigations are therefore required to elucidate the association between schizotypy and typicality of responses.

Several limitations to the current study should be noted. First, NIRS measurement has some methodological disadvantages, as detailed elsewhere (Suto et al., 2004; Takizawa et al., 2008). Second, since each version of VFT was conducted only once, with only one epoch of the activation condition being included, quality of the NIRS data might have been lowered due to certain artifacts. The third limitation relates to the relatively low mean total SPQ score of 12.9 (S.D. 8.6) of the present sample, given that the mean total SPQ scores of non-clinical samples have been reported to be around 20 in the majority of prior studies, most of which were conducted in Western countries. This discrepancy is likely to have derived from ethnic differences (Western vs. Japanese/Asian). Actually, mean total SPQ scores in healthy Japanese populations are consistently shown to be approximately 10 (Hori et al., 2008a; Noguchi et al., in press; Someya et al., 1994; Wang et al., 2004), which means that the mean total SPQ score of the present sample was within the range of the mean total SPQ scores in healthy populations described in prior reports from Japan. Another plausible explanation for the differential SPQ scores between these samples might be the difference in mean age of participants between studies, i.e., college students in most of the precedent studies vs. adults in the present study. In support of this, a prior study, in which hundreds of Taiwanese adolescents and adults were enrolled, showed that mean SPQ scores of adolescents and adults were 20.6 and 12.9, respectively (Chen et al., 1997). Taken together, it is conceivable that the SPQ score is influenced substantially by ethnicity and to some extent by aging (i.e., lower scores in Asian and older populations) and that the present sample would be highly representative of non-clinical Asian adults; still, the present results would require confirmation by future studies employing non-clinical subjects with even higher schizotypal traits. On the other hand, from the viewpoint of the dimensional notion of schizotypy, the fact that even such degree of difference in schizotypal traits yielded the statistically significant result regarding hemispheric laterality may be regarded as the evidence in support of the continuum model of schizotypy. Fourth, we adopted the category production norms compiled more than 30 years ago (Ogawa, 1972) as there don't exist any newer versions to our knowledge, which may have erroneously caused high typicality indices. However, the problems of high typicality indices due to this issue may be subtle, if any, since typicality indices reported herein were generally lower than those described in the prior study (Kiang and Kutas, 2006). Fifth, as the sample size was not very large, possibilities of type II errors cannot be ruled out for those results which did not reach statistical significance. Finally, since the present study included only non-clinical subjects, further neuroimaging studies that investigate the association of schizotypal traits with hemispheric laterality among clinical populations (e.g., SPD patients with and without family history of schizophrenia) are needed to fully clarify the neurocognitive dimensionality of schizotypy.

5. Conclusion

Our results indicate that schizotypal traits at a non-clinical level are associated with right prefrontal dominance during the letter VFT rather than the category VFT, suggesting that the association between non-clinical schizotypy and functional laterality varies depending on cognitive activation tasks in the manner that this association might be better observed when the cognitive domain tapped by an activation task is more closely linked to the neurocognitive pathology involved in schizophrenia spectrum.

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Personality in schizophrenia assessed with the Temperament and Character Inventory (TCI)

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Abstract

The Temperament and Character Inventory (TCI) is a well-established self-report questionnaire measuring four temperament and three character dimensions. However, surprisingly few studies have used it to examine the personality of patients with schizophrenia, and none in Japan. Moreover, possible gender differences in personality among patients with schizophrenia have not been well documented. We administered the TCI to 86 Japanese patients with schizophrenia and 115 age- and gender-matched healthy controls to characterize personality traits in patients with schizophrenia and to examine their relationships with clinical variables, particularly gender and symptoms. Compared with controls, patients demonstrated significantly lower novelty seeking, reward dependence, self-directedness and cooperativeness, and higher harm avoidance and self-transcendence. Male patients showed even more pronounced personality alteration than female patients when both of them were compared with healthy people. Personality dimensions were moderately correlated with symptom dimensions assessed by the Positive and Negative Syndrome Scale (PANSS). These results, together with prior findings in several other countries, suggest that schizophrenia patients have a unique personality profile which appears to be present across cultures and that the greater alteration of personality in schizophrenia males might be related to their poorer social and community functioning.

Keywords: Schizophrenia; Personality; Temperament; Character; Gender difference

1. Introduction

Personality in schizophrenia has been of interest ever since the pioneering work of Bleuler (1950) and Kraepelin (1919). Personality is considered to be an important aspect of schizophrenia primarily because it may influence symptom expression (Lysaker et al., 1999; Guillem et al., 2002) and social functioning (Lysaker et al., 1998; Eklund et al., 2004).

The Temperament and Character Inventory (TCI, Cloninger et al., 1993) is a well-established self-report

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questionnaire measuring four temperament and three character dimensions, developed on the basis of a psychobiological model of personality. The TCI has recently been widely used in personality studies in various psychiatric disorders including mood disorders (Hansenne et al., 1999; Cloninger et al., 2006) and personality disorders (Svrakic et al., 1993, 2002). However, to our knowledge, only three studies (Guillem et al., 2002; Boeker et al., 2006; Calvo de Padilla et al., 2006) have examined the personality of schizophrenia patients in comparison with healthy controls, using the TCI. The findings from these studies on unique personality profiles of schizophrenia are, to some extent, consistent with each other; but the limited sample sizes of the studies have made it difficult to draw definitive conclusions. It is also possible that cultural differences in personality exist between these studies, in view of the fact that personality traits among the general population as measured by the TCI vary across cultures (Pélissolo and Lépine, 2000; Brändström et al., 2001). Such cross-cultural comparison of personality in schizophrenia is an under-studied topic.

The knowledge to date on the personality characteristics of schizophrenia patients has been based mostly on instruments other than the TCI (Malmberg et al., 1998; Lysaker et al., 1999; Gurrera et al., 2000; Van Os and Jones, 2001; Pillmann et al., 2003). A number of studies have been done to investigate personality in patients with schizophrenia by using the well-known NEO Five-Factor Inventory (NEO-FFI, Costa and McCrae, 1992), and the results are fairly consistent in showing higher neuroticism and lower extraversion and conscientiousness in schizophrenia patients than in healthy controls (Gurrera et al., 2000; Pillmann et al., 2003; Camisa et al., 2005). Given the close relationship of the TCI dimensions to the "Big Five" personality dimensions of the NEO-FFI (De Fruyt et al., 2000; MacDonald and Holland, 2002; Ramanaiah et al., 2002), it would be intriguing to examine whether the personality of schizophrenia patients as assessed by the TCI shows a compatible pattern with that assessed by the NEO-FFI.

Concerning the association between personality and symptom dimensions in schizophrenia, previous studies that employed the TCI (Guillem et al., 2002) as well as the NEO-FFI (Lysaker et al., 1999) found certain relationships between these two dimensions; for example, Guillem et al. (2002) reported that psychotic symptoms in schizophrenia patients were associated with specific personality dimensions of the TCI. Boeker et al. (2006), by contrast, did not find any relationships between personality and symptoms, although the sample size of this study was relatively small. Due to the paucity of material, the association between personality and symptoms in schizophrenia remains to be further clarified.

Gender difference in essential facets of a particular disorder can yield important clues to its pathogenesis. In schizophrenia, gender differences have been shown in premorbid functioning, age at onset, symptomatology, and neuropsychological functioning. In general, male patients are reported to show indications of severer illness than female counterparts (Castle et al., 1993; Leung and Chue, 2000). However, possible gender differences in personality among patients with schizophrenia have not been well documented.

In this context, the present study aimed (1) to characterize personality traits in Japanese patients with schizophrenia using the TCI and compare the results with findings from the prior TCI as well as the NEO-FFI studies, and (2) to examine whether personality is related to clinical variables, particularly gender and symptoms, in schizophrenia. The study hypotheses were as follows: (i) Japanese patients with schizophrenia would show a unique personality profile, which is similar to that found in previous TCI studies of other countries as well as NEO studies; (ii) when compared with the personality profile of healthy people, the alteration of personality in male patients would be even greater than that in female patients, as is usually the case with gender differences in schizophrenia; and (iii) the more severe the symptoms, the more prominent the personality alteration would be.

2. Methods

2.1. Subjects

Subjects were 86 patients with chronic schizophrenia who were under treatment at the National Center of Neurology and Psychiatry, Musashi Hospital, Tokyo, Japan. All met the DSM-IV criteria (American Psychiatric Association, 1994) for schizophrenia. Consensus diagnoses were made based on clinical interviews, observations and case notes by clinicians who were all senior psychiatrists. One hundred and fifteen age- and gender-matched healthy volunteers were recruited from hospital staff and their associates through flyers and by word of mouth, and also from the community through local newspaper advertisements, our website announcement, and notices posted on bulletin boards at a college. Healthy participants were interviewed for enrollment using the Japanese version of the Mini-International Neuropsychiatric Interview (MINI, Sheehan et al., 1998; Otsubo et al., 2005) by a research psychiatrist

Table 1
Demographic and clinical characteristics of patients with schizophrenia and healthy controls stratified by gender

Characteristic	Schizophrenia patients (n=86)		Analyses (male vs. female patients)		Healthy controls (n=115)		Analyses (male vs. female controls)	
	Male (n=53)	Female (n=33)	Statistics	P	Male (n=71)	Female (n=44)	Statistics	P
Age, years: mean (S.D.)	41.5 (11.8)	41.9 (10.6)	$F(1,84)=0.025$	0.87	41.2 (14.0)	41.6 (4.1)	$F(1,113)=0.046$	0.83
Education, years: mean (S.D.)	13.6 (2.6)	13.1 (1.7)	$F(1,84)=1.19$	0.28	17.3 (3.0)	14.4 (1.9)	$F(1,113)=31.9$	<0.001
Family history of psychiatric disease: yes/no	20/33	9/24	$\chi^2(1)=0.996$	0.32				
Age at illness onset, years: mean (S.D.)	23.6 (6.6)	25.1 (8.8)	$F(1,84)=0.83$	0.36				
Duration of illness, years: mean (S.D.)	17.9 (11.4)	16.8 (10.1)	$F(1,84)=0.22$	0.64				
CPZeq of total antipsychotics, mg/day: mean (S.D.)	974.5 (927.5)	837.6 (690.8)	$F(1,84)=0.53$	0.47				
Number of hospitalizations, n: mean (S.D.)	2.3 (2.1)	2.2 (2.7)	$F(1,84)=0.061$	0.81				
Outpatients/Inpatients, n	35/18	22/11	$\chi^2(1)=0.0036$	0.95				
PANSS scores (n=53): mean (S.D.)	(n=31)	(n=22)						
Positive subscale	13.3 (5.6)	15.8 (7.7)	$F(1,51)=1.79$	0.19				
Negative subscale	20.5 (6.9)	18.9 (7.0)	$F(1,51)=0.63$	0.43				
General subscale	29.0 (8.8)	30.1 (8.1)	$F(1,51)=0.20$	0.66				
Total score	62.8 (16.8)	64.8 (18.9)	$F(1,51)=0.16$	0.69				

CPZeq: Chlorpromazine equivalents.
PANSS: Positive and Negative Syndrome Scale (Kay et al., 1987).

(H.H.), and only those who demonstrated no history of psychiatric illness or contact with psychiatric services were enrolled as healthy controls. Participants were excluded from both the patient and control groups if they had a prior medical history of central nervous system disease or severe head injury, or if they met DSM-IV criteria for mental retardation, substance dependence, or substance abuse within the past 6 months. All subjects were biologically unrelated Japanese who resided in the Western part of Metropolitan Tokyo. Written informed consent was obtained from all subjects prior to their inclusion in the study, and the study was approved by the ethics committee of the National Center of Neurology and Psychiatry (NCNP), Japan.

2.2. Personality assessment

Personality was assessed in all subjects with the Temperament and Character Inventory (TCI, Cloninger et al., 1993). TCI is a 240-item (including 14 items which are not analyzed) self-report questionnaire; each item requires a true/false answer. The term *temperament* refers to automatic emotional reactions to subjective experiences that may be genetically transmitted and therefore stable over time. Four dimensions of temperament are distinguished by the TCI: novelty seeking (NS), harm avoidance (HA), reward dependence (RD), and persistence (PS). NS, HA, and RD have been assumed to relate to dopaminergic, serotonergic, and noradrenergic neurotransmission, respectively (Cloninger, 1987). This model, therefore, may be particularly relevant in schizophrenia since such neurotransmitters are involved in symptom expression and are the main targets of antipsychotic medication (Markianos et al., 2001). The term *character* refers to concepts pertaining to the individual, focusing on personal differences in intentions, decisions and values. Three dimensions of character are distinguished: self-directedness (SD), cooperativeness (CO), and self-transcendence (ST). The reliability and validity of the original American version of the TCI in general community dwellers and in psychiatric patients have been established (Cloninger et al., 1993; Svrakic et al., 1993). Moreover, the TCI has been translated into and validated in more than seven languages including Japanese (Kijima et al., 1996, 2000), and used in many genetic (Benjamin et al., 1996; Ebstein et al., 1996) and clinical studies (Eklund et al., 2004; Cloninger et al., 2006). The Japanese version of the TCI, translated by Kijima et al. (1996), was used in the present study. The questionnaire was distributed to both patients and controls at the hospital and at our laboratory, respectively. Each subject was allowed to take as much time as needed to complete the questionnaire, then returned it to us by mail or by hand.

2.3. Clinical assessment and antipsychotic medication

Schizophrenic symptoms were assessed by an experienced research psychiatrist (H.K.) in 53 (male, 31; female, 22) of 86 patients using the Positive and

Table 2
Comparisons of TCI scores between patients with schizophrenia and control subjects

Variable		No. of items	Schizophrenia patients (n=86) Mean (S.D.)	Healthy controls (n=115) Mean (S.D.)	ANOVA[a] F	ANOVA[a] P	ANCOVA[b] F	ANCOVA[b] P
Novelty seeking	NS	40	17.4 (4.6)	20.6 (4.1)	26.69	<0.001	27.79	<0.001
Harm avoidance	HA	35	22.7 (6.0)	16.9 (5.6)	48.4	<0.001	40.87	<0.001
Reward dependence	RD	24	13.6 (3.5)	15.2 (3.7)	9.86	0.002	13.55	<0.001
Persistence	PS	8	4.1 (1.9)	4.6 (1.7)	3.81	0.052	0.36	0.55
Self-directedness	SD	44	23.6 (6.6)	30.1 (5.8)	55.07	<0.001	32.64	<0.001
Cooperativeness	CO	42	26.7 (5.4)	28.5 (5.4)	5.38	0.02	5.18	0.02
Self-transcendence	ST	33	13.8 (7.5)	10.9 (5.1)	10.79	0.001	5.24	0.02

[a] Degrees of freedom=1, 199.
[b] Education (in years) was controlled for. Degrees of freedom=1, 199.

Negative Syndrome Scale (PANSS, Kay et al., 1987); this yields a total score in addition to scores on positive, negative, and general psychopathology subscales. All patients with schizophrenia were receiving antipsychotic agents and were clinically stable at the time of the personality evaluation. Daily doses of antipsychotics, including depot antipsychotics, were converted to chlorpromazine equivalents (CPZeq) using published guidelines (American Psychiatric Association, 1997; Inagaki et al., 1999).

2.4. Statistical analyses

Demographic characteristics and TCI scores were compared between groups. Means and categorical variables were compared using analysis of variance (ANOVA) and the χ^2 test, respectively. Pearson's r was used to examine correlations. One-way ANOVA with Bonferroni correction, allowing for multiple comparisons, was performed to examine differences between three groups. Analysis of covariance (ANCOVA) was used to compare TCI scores between groups, controlling for confounding variables. Statistical significance was set at two-tailed $P<0.05$. Analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 11.0 (SPSS Japan, Tokyo).

3. Results

3.1. Sample characteristics

Table 1 shows the characteristics of patients with schizophrenia and healthy controls (both are stratified by gender), respectively. Patients with schizophrenia and healthy controls did not differ in age ($F(1,199)=0.033$, $P=0.86$) or gender ($\chi^2(1)=0.00026$, $P=0.99$), but patients demonstrated significantly fewer years of education as compared with controls ($F(1,199)=51.1$, $P<0.001$). Schizophrenic males and females did not significantly differ in any of the characteristics examined. Control males and females did not differ in age, but control males had received significantly more years of education than females. Education was significantly correlated with RD ($r=-0.22$, $P=0.02$) and PS ($r=0.35$, $P<0.001$) in healthy controls; thus, in ANCOVA it was used as a covariate where appropriate.

3.2. TCI scores of patients vs. controls

TCI scores of patients with schizophrenia and control subjects are presented in Table 2. All personality dimensions except PS, namely six dimensions, were

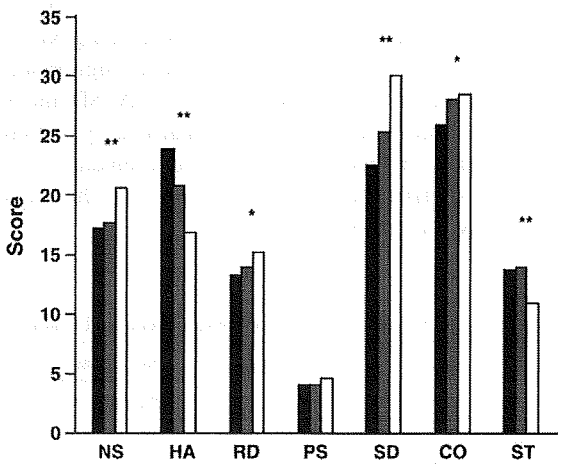

Fig. 1. Mean scores on 7 dimensions of TCI. ■, schizophrenia males; ▒, schizophrenia females; □, total controls (male and female combined). *Only schizophrenia males significantly differed from controls; **Both schizophrenia males and females significantly differed from controls.

Table 3
Correlations between TCI and PANSS scores

	NS	HA	RD	PS	SD	CO	ST
Positive subscale	0.02	−0.14	−0.10	0.23	−0.17	−0.04	0.34*
Negative subscale	0.04	0.11	−0.34*	−0.27*	−0.25	−0.28*	−0.25
General subscale	0.26	0.004	−0.37**	0.03	−0.32*	−0.29*	0.16
Total score	0.15	−0.01	−0.35**	−0.01	−0.32*	−0.27	0.11

Each figure represents Pearson's *r*.
* <0.05, **P<0.01.

significantly different between patients and controls using ANOVA; patients showed significantly higher scores on HA and ST and lower scores on NS, RD, SD and CO than controls. These differences between patients and controls in the six dimensions all remained significant after ANCOVA was performed with years of education as a covariate.

3.3. Gender differences in TCI scores

3.3.1. TCI scores of male patients vs. female patients

When TCI scores were compared between male and female patients using ANOVA, male patients showed significantly higher HA (F(1,84)=5.23, P=0.025) than female patients. In addition, male patients demonstrated lower SD (F(1,84)=3.78, P=0.055) and CO (F(1,84)=3.46, P=0.066) than female patients with statistical trend.

3.3.2. TCI scores of male patients vs. female patients vs. controls (male and female combined)

Fig. 1 shows comparisons of three groups (male patients/female patients/total controls) using one-way ANOVA with Bonferroni correction. Regarding RD and CO, male patients, but not female patients, significantly differed from controls. Concerning NS, HA, SD and ST, both male and female patients significantly differed from controls. In this analysis, male and female patients did not significantly differ in any of the seven personality dimensions.

3.4. Correlations between TCI scores and clinical variables (including symptoms) of patients

Duration of illness showed a significantly negative correlation with NS (r=−0.23, P=0.04). CPZeq medication dosage showed a significantly positive correlation with PS (r=0.23, P=0.04) and ST (r=0.22, P=0.04). Correlations between scores on the TCI and the PANSS are presented in Table 3. Family history of psychiatric disease, age at onset, and number of hospitalizations were not correlated with any of the TCI dimensions.

3.5. Comparisons of TCI scores in patients and controls between prior studies and ours

Table 4 shows a comparison of our TCI results and those of the two previous studies (Guillem et al., 2002; Boeker et al., 2006) which examined the personality of patients with schizophrenia using the TCI with a cross-sectional case–control design. All directions of differences between patients and controls in TCI dimensions, except for RD, were consistent in these three studies. Lower SD in patients with schizophrenia was the most consistent finding. Lower NS and CO and higher HA and ST in patients were quite consistent findings. Lower PS in patients was also a consistent finding, but not of great statistical significance. In addition, Calvo de Padilla et al. (2006) reported with an indigenous sample of Argentina that patients with schizophrenia showed significantly lower

Table 4
Comparisons of TCI results in schizophrenia patients and healthy controls between prior studies and ours

	Country (City)	No. of sample		Matching status	TCI results (patients vs. controls)[a]						
		Patients	Controls		NS	HA	RD	PS	SD	CO	ST
The present study	Japan (Tokyo)	86	115	Age/gender	−3.2**	5.8**	−1.6**	−0.5	−6.5**	−1.8*	2.9**
Guillem et al. (2002)	Canada (Montreal)	52	25	Age	−4.4**	8.1**	−0.9	−1.4*	−8.1**	−3.6**	2.4
Boeker et al. (2006)	Germany (Magdeburg)	22	22	Age/gender	−0.4	2.9	0.4	−0.4	−5.9**	−2.8*	4.1*

Each figure in these 7 columns was calculated as follows: ⟨mean of patients⟩−⟨mean of controls⟩
[a] Differences in sub-dimensions of TCI between patients and controls of each study.
* Patients showed a significant difference from controls (P<0.05).
** Patients showed a significant difference from controls (P<0.01).

RD, SD and CO compared with community controls (their data are not included in Table 4 because mean TCI scores were not presented in their report).

4. Discussion

In this study we report personality, as assessed with the TCI, in patients with schizophrenia compared to healthy subjects. Patients with schizophrenia demonstrated marked alteration of personality. Male patients seemingly showed greater alteration than female patients.

4.1. Personality traits in patients with schizophrenia

Our results indicate that patients with schizophrenia have pervasively altered personalities. Furthermore, the findings of the present and prior two studies (Guillem et al., 2002; Boeker et al., 2006), as shown in Table 4, are fairly consistent with each other. Guillem et al. (2002) reported that patients with schizophrenia showed significantly higher HA, and lower NS, PS, SD and CO compared to healthy controls, all of which were congruent with the present study, although the lower PS in our patients just failed to reach statistical significance. In contrast, RD and ST showed significant differences between the two diagnostic groups only in the present study. These could mainly be attributed to the larger sample size in the present study since the patterns of differences in mean scores on RD and ST between patients and controls were similar in these two studies. Moreover, Boeker et al. (2006) found that patients with schizophrenia showed significantly lower SD and CO and higher ST than healthy subjects, all of which corroborated our results. In addition, Calvo de Padilla et al. (2006) reported that patients with schizophrenia showed significantly lower RD, SD and CO compared to controls, all of which were also in line with our results. In general, our findings confirmed and extended the prior ones in that patients with schizophrenia have unique personality profile, in which lower SD is the most prominent abnormality. These findings may be of clinical importance, taking account of the studies that reported TCI scores, especially SD, were related to level of functioning and psychological health (Eklund et al., 2004) and to subjective quality of life (Hansson et al., 2001) in patients with schizophrenia.

On the other hand, cross-cultural differences in personality assessed with the TCI may exist (Pélissolo and Lépine, 2000; Brändström et al., 2001). Indeed, mean scores for both patients and controls on each dimension of TCI were substantially different between our subjects and the prior ones. Further, these differences of TCI scores between studies within the same diagnostic groups were of comparable size to the differences between patients and controls within each study.

The most plausible explanation may be that although personality itself may vary across cultures, it may be a worldwide phenomenon that patients with schizophrenia collectively have markedly different personality profiles from healthy people in their own cultural group, especially regarding NS, HA, SD, CO and ST.

Moreover, the fact that the NEO-FFI findings higher neuroticism and lower extraversion and conscientiousness are well established in schizophrenia (Gurrera et al., 2000; Pillmann et al., 2003; Camisa et al., 2005), coupled with the substantial overlap between NEO-FFI and TCI dimensions (e.g., positive correlation between neuroticism and HA, negative correlation between neuroticism and SD, and positive correlation between conscientiousness and PS) (De Fruyt et al., 2000; MacDonald and Holland, 2002; Ramanaiah et al., 2002), would theoretically predict the following TCI results in schizophrenia patients: high HA, low PS, low SD. Indeed, these predictions are largely in accord with our actual results as well as with previous TCI findings. Regarding NS, positive correlation with extraversion and negative correlation with conscientiousness have simultaneously been reported (De Fruyt et al., 2000; MacDonald and Holland, 2002; Ramanaiah et al., 2002); however, since both extraversion and conscientiousness are low in schizophrenia, it is impossible to examine the compatibility of this TCI dimension with the NEO findings. In short, the personality profile of schizophrenia patients as assessed by the TCI showed a compatible pattern with that assessed by NEO-FFI. All in all, hypothesis (i) has largely been supported.

4.2. Gender differences in personality among schizophrenia patients

Hypothesis (ii) was partly supported in that male patients showed even greater personality alteration than female patients (when both groups are compared to controls) for the two dimensions, RD and CO (Fig. 1). These results are in harmony with a precedent study that reported schizophrenic males showed greater abnormality in premorbid personality than schizophrenic females (Foerster et al., 1991). Gender differences have already been reported concerning other important variables in schizophrenia such as age at illness onset, premorbid functioning, symptomatological characteristics, and neuropsychological function (Castle et al., 1993;