
**Figure 3.** Regression lines of correlations between (*A*) praiseworthiness (*B*) blameworthiness and degree of brain activation. (*A*) There were correlations ($r = 0.82$, degrees of freedom [df] = 13, $P < 0.001$) between self-rating of praiseworthiness and degree of activation in OFC. (*B*) There were positive linear correlations ($r = -0.83$, df = 13, $P < 0.001$) between self-rating of blameworthiness and degree of activation in pSTS.

Moral depravity produced activation in the pSTS and MPFC, and the degree of pSTS activation was correlated with blameworthiness. Originally, STS was known to be activated by biological motions such as movement of eyes, mouth, hands, and body (Allison et al. 2000), and it has been suggested to have a more general function in social cognition such as detecting behavioral information that signals the intention of others (Gallagher and Frith 2003) and behavior of agents (Frith U and Frith CD 2003). MPFC appears to be responsible for inferring the cause of others' behavior, attribution. Previous studies have shown activation in the MPFC during judgments made on the basis of attributional information (Amodio and Frith 2006). It is suggested that, for the evolution and persistence of cooperation, humans have evolved neurocognitive systems that specialize in the detection of cheating and that motivate people to blame and punish those who violate social norms (Cosmides and Tooby 1992). Supporting this view, recent fMRI studies reported activation in brain regions such as the pSTS and MPFC during detection of the violation of social contracts (Canessa et al. 2005; Fiddick et al. 2005). Considering the functions of pSTS and MPFC, these regions might process intention of wrongdoings and, consequently, blameworthiness might be associated with the activation in pSTS.

The lack of activation in the pSTS and MPFC in response to moral beauty supports psychological studies in which people do not put a premium on the deliberate intention of commendable acts. Instead, correlation between the subjective ratings of praiseworthiness and the degrees of activation in the left OFC suggests that they regard positive outcome itself rather than intention of the act to be a main factor for praiseworthiness because the OFC is known to be involved in processing reward (Rolls 2006) and positive stimuli such as pictures (Northoff et al. 2000), taste (Small et al. 2003), and music (Blood and Zatorre 2001). It is also reported that the OFC was associated with maternal love (Bartels and Zeki 2004; Nitschke et al. 2004). The association between OFC activation and self-rating of praiseworthiness could be regarded as corresponding to Smith's phrase "The love of praiseworthiness" (Smith 1976).

Previous functional imaging studies have investigated the neural correlates processing facial beauty (Aharon et al. 2001; O'Doherty et al. 2003) or aesthetic beauty such as shapes or arts (Kawabata and Zeki 2004; Vartanian and Goel 2004; Jacobsen et al. 2006), and activation of reward-related subcortical and limbic areas including the OFC was reported. The connection between aesthetic judgment and moral feeling has long been emphasized in aesthetic theory (Kant 1952). Our finding could be interpreted in the context of aesthetic theory, that is, the neurocognitive system processing moral beauty might be related to that of aesthetic beauty.

We observed activation in other prefrontal areas in the left hemisphere, such as DLPFC and SMA, although activation in these unpredicted areas needs to be interpreted with caution. It is still unclear whether there is a hemispheric specialization in the processing of moral cognition, but it is suggested that frontal regions in the left hemisphere are associated with approach behavior, whereas frontal areas in the right hemisphere are associated with avoidance (Davidson 1992). Previous studies reported activation in the motor area in response to positive stimuli such as paintings, music, money, humor, and concepts (Blood and Zatorre 2001; Elliott et al. 2003; Mobbs et al. 2003; Kawabata and Zeki 2004; Cunningham et al. 2005). Although the exact role of the motor area in such tasks is not well known, it is suggested that the positive stimuli might mobilize the motor system to take some action toward them.

Although domain-specific emotional response is suggested to play a central role in moral judgments, domain-neutral reasoning could play certain roles as well (Haidt 2001; Greene and Haidt 2002). In a predictable situation, context-independent knowledge of event is processed automatically and routinely. This domain-specific process is suggested to be mediated in the medial and ventral prefrontal cortex. On the other hand, in a less predictable situation, context-dependent knowledge of event is processed with the operation of domain-neutral reasoning, which is suggested to be mediated in the DLPFC (Greene and Haidt 2002; Moll et al. 2005). It is also widely argued that emotions evolved to promote quick and automatic reaction in life-threatening situations (Fredrickson 1998). Although these models have been well fitted for negative emotions, quick and decisive actions are not typically required in a situation that gives rise to positive emotions. Instead, a wider range of thoughts or actions is required in situations where positive emotions occur (Fredrickson 1998). The DLPFC was reported to be recruited during evaluation of natural or artistic aesthetic stimuli (Cela-Conde et al. 2004). Although the exact role of the DLPFC in aesthetic evaluation remains unclear, our results suggested that context-dependent knowledge contributes to the evaluation of moral beauty.

In conclusion, evaluation of moral excellence and moral violation might be processed differently in the human brain. However, any generalization of our findings needs to be approached with caution as the social background of the participants, such as culture, generation, religion, and education, could affect the results. Still, our results suggest that humans might have developed different neurocognitive systems for evaluating blameworthiness (cheaters) and praiseworthiness (cooperators). Our finding might contribute to a better understanding of the neural basis of human morality.

## Supplementary Matrial

Supplementary table S1 can be found at: http://www.cercor.oxfordjournals.org/.

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## Notes

*Conflict of Interest*: None declared.

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# Brain Activations during Judgments of Positive Self-conscious Emotion and Positive Basic Emotion: Pride and Joy

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**We aimed to investigate the neural correlates associated with judgments of a positive self-conscious emotion, pride, and elucidate the difference between pride and a basic positive emotion, joy, at the neural basis level using functional magnetic resonance imaging. Study of the neural basis associated with pride might contribute to a better understanding of the pride-related behaviors observed in neuropsychiatric disorders. Sixteen healthy volunteers were studied. The participants read sentences expressing joy or pride contents during the scans. Pride conditions activated the right posterior superior temporal sulcus and left temporal pole, the regions implicated in the neural substrate of social cognition or theory of mind. However, against our prediction, we did not find brain activation in the medial prefrontal cortex, a region responsible for inferring others' intention or self-reflection. Joy condition produced activations in the ventral striatum and insula/operculum, the key nodes of processing of hedonic or appetitive stimuli. Our results support the idea that pride is a self-conscious emotion, requiring the ability to detect the intention of others. At the same time, judgment of pride might require less self-reflection compared with those of negative self-conscious emotions such as guilt or embarrassment.**

**Keywords:** medial prefrontal cortex, positive emotions, pride, superior temporal sulcus, theory of mind, ventral striatum

## Introduction

Although there have been numerous neuroimaging studies on basic emotions (fear, disgust, happiness, and sadness) that have led to a better understanding of the neuroanatomical correlates of emotions (Lane et al. 1997; Phan et al. 2002), only a few studies on complex social emotions such as guilt, embarrassment, and jealousy have been reported (Shin et al. 2000; Berthoz et al. 2002; Takahashi et al. 2004, 2006).

We previously examined brain activation associated with negative self-conscious emotions, guilt, and embarrassment (Takahashi et al. 2004). Self-conscious emotions are founded in social relationship and arise from concerns about others' evaluations of self (Eisenberg 2000; Tangney and Dearing 2002; Haidt 2003; Kalat and Shiota 2006). In other words, one needs the ability to represent the mental states of others, that is, theory of mind (ToM), to recognize self-conscious emotions. Negative evaluation of self or the behavior of self is fundamental to guilt and embarrassment, whereas positive evaluation of self leads to the emotion of pride. Negative self-conscious emotions promote moral behavior and interpersonal etiquette (Eisenberg 2000; Haidt 2003). Impairment of processing these emotions could lead to amoral, socially inappropriate behaviors observed in neuropsychiatric disorders (Beer et al. 2003; Miller et al. 2003; Sturm et al. 2006).

Supporting the notion that self-conscious emotions involve inferences about others' evaluation of self (Leary 2007), judgment of guilt and embarrassment produced activations in the medial prefrontal cortex (MPFC), posterior superior temporal sulcus (pSTS), and temporal poles (Takahashi et al. 2004; Kalat and Shiota 2006), the regions implicated in ToM, social cognition (Adolphs 2001; Calarge et al. 2003; Frith U and Frith CD 2003; Gallagher and Frith 2003), and moral judgment (Greene and Haidt 2002; Moll et al. 2005).

In contrast, a positive self-conscious emotion, pride has been largely unstudied by researchers. Pride refers to self-esteem, joy, or pleasure derived from achievements. It arises when people believe that they are responsible for desired outcomes (Leary 2007). As a self-conscious emotion, pride also drives people to behave in moral, socially appropriate ways (Tracy and Robins 2004a). Specifically, the "achievement-oriented" form of pride promotes prosocial behaviors, such as caregiving and achievement (Tracy and Robins 2004b). However, the hubristic form of pride could be maladaptive, and impairment of processing pride could be related to some psychiatric disorders. Narcissistic personality disorder is characterized by a grandiose sense of self-importance and lack of empathy (American Psychiatric Association 1994). It was reported that empathy and ToM rely on common networks, the MPFC, pSTS, and temporal poles (Vollm et al. 2006). Therefore, the hubristic form of pride could be regarded as a dysfunction of ToM. Affective disorder could also be linked to impairment of the processing of pride. Manic state is a condition with inflated self-esteem, whereas depressive episode could be a condition with low self-esteem (American Psychiatric Association 1994). Studying the neural substrates associated with pride should add to the understanding of the neural basis of these neuropsychiatric disorders.

We aimed to measure brain activations associated with the judgment of pride by showing scenarios, comparing them with brain activations associated with the primary positive emotion, joy, using functional magnetic resonance imaging (fMRI). We hypothesized that joy and pride conditions would show different brain activation patterns, and specifically, that joy condition would activate brain regions involved in hedonic processing, for example, the ventral striatum (Mobbs et al. 2003, 2005; Britton et al. 2006), whereas pride condition would activate the brain regions involved in social cognition (Adolphs 2001) or ToM (Calarge et al. 2003; Frith U and Frith CD 2003; Gallagher and Frith 2003), for example, MPFC, pSTS, and temporal poles.

## Materials and Methods

### *Participants*

Sixteen healthy right-handed Japanese university students (8 men, mean age 21.5 years, standard deviation [SD] = 2.2; 8 women, mean age 21.3 years, SD = 1.3) were studied. Their mean educational achievement level was 14.4 years (SD = 1.3). They did not meet any criteria for psychiatric disorders. None of the controls were taking alcohol or medication at the time nor did they have a history of psychiatric disorder, significant physical illness, head injury, neurological disorder, or alcohol or drug dependence. All subjects underwent an MRI to rule out cerebral anatomic abnormalities. After complete explanation of the study, written informed consent was obtained from all subjects, and the study was approved by the Ethics Committee.

### *Materials*

Three types of short sentences were provided (neutral, joy, and pride). Each sentence was written in Japanese and in the first person, past tense. Each sentence was expected to express joy, pride, or no prominent emotional content. We used joyful scenarios depicting hedonic, appetitive, and survival events like eating, reproduction, and economic behaviors because these stimuli are thought to be directly related to "basic" positive emotional processing. For most of the pride sentences, we used scenarios in which the protagonist was a winner of a prize or competition as a result of achievement. In order to validate our expected results, we conducted an initial survey. Other university students (20 men and 20 women, mean age 22.5 years, SD = 3.3) than the subjects participating in this fMRI study were screened. We prepared 28-32 sentences for each of 3 conditions (neutral, joy, and pride). The described situations were rated according to how joyful or proud they were using a 7-point analog scale (0 = none, 6 = extremely intense). Based on the initial survey, we selected 18 sentences for each of the 3 conditions. The selected joy sentences were judged to express joy. The mean rating of joy was 4.3 (SD = 0.5). The selected pride sentences were judged to express pride. The mean rating of pride was 4.5 (SD = 0.3). The neutral sentences were judged to express virtually no joy or pride. The mean ratings of joy and pride for neutral sentences were 0.7 (SD = 0.3) and 0.4 (SD = 0.2), respectively. Examples of the sentences are shown in Table 1. The sentences were projected via a computer and a telephoto lens onto a screen mounted on a head coil. The subjects were instructed to read the sentences silently and were told to imagine that the scenario protagonist was himself/herself. They were also told that they should rate the sentences according to how joyful or pride instilling the situations were. After reading each sentence, the subjects were instructed to press a selection button with the right index finger, indicating that they had read and understood it. The experimental design consisted of 6 blocks for each of the 3 conditions (neutral, joy, and pride) interleaved with 20-s rest periods. The order of presentation for the 3 conditions was randomized. During the rest condition, participants viewed a crosshair pattern projected to the center of the screen. In each 24-s block, 3 different sentences of the same emotional class were presented for 8 s each. After the scan, the subjects read the sentences presented during the scan, and they were asked to rate the sentences according to how they would feel if the scenario protagonist were himself/herself. The participants rated the intensity of joy, pride, and other emotions (anger, sadness, fear, disgust, and shame) for each sentence using a 7-point analog scale.

**Table 1**
Examples of sentences

| | |
|---|---|
| Neutral | I took a class at the college. |
| | I had breakfast. |
| | I watched the Olympics on TV. |
| | I recorded a baseball game on video tape. |
| | I prepared for an examination. |
| | I went to school yesterday. |
| | I watched sports news on TV. |
| | I bought a medicine for cold. |
| Joy | I won a lottery. |
| | I won at gambling at a casino. |
| | I ate my favorite cake. |
| | I had a date with my girl/boy friend. |
| | I had a delicious dinner. |
| | I received a Christmas present. |
| | I went to Hawaii with my friends. |
| | I was gifted with a bouquet on my birthday. |
| Pride | I was awarded a prize for my novel. |
| | I won the championship in a golf tournament. |
| | I got a perfect score in mathematics. |
| | I graduated at the head of my class. |
| | I won the first prize in a piano contest. |
| | I graduated from the most prestigious university. |
| | I obtained a scholarship. |
| | I won a prize at a scientific meeting. |

### *Images Acquisition*

Images were acquired with a 1.5-Tesla Signa system (General Electric, Milwaukee, WI). Functional images of 203 volumes were acquired with $T_2^*$-weighted gradient echo planar imaging sequences sensitive to blood oxygenation level-dependent contrast. Each volume consisted of 40 transaxial contiguous slices with a slice thickness of 3 mm to cover almost the whole brain (flip angle, 90°; time echo [TE], 50 ms; time repetition [TR], 4 s; matrix, 64 × 64; field of view, 24 × 24 cm). High-resolution, $T_1$-weighted anatomic images were acquired for anatomic comparison (124 contiguous axial slices, 3-dimensional [3D] spoiled Grass sequence, slice thickness 1.5 mm, TE, 9 ms; TR, 22 ms; flip angle, 30°; matrix, 256 × 192; field of view, 25 × 25 cm).

### *Analysis of Functional Imaging Data*

Data analysis was performed with statistical parametric mapping software package (SPM02) (Wellcome Department of Cognitive Neurology, London, UK) running with MATLAB (Mathworks, Natick, MA). All volumes were realigned to the first volume of each session to correct for subject motion and were spatially normalized to the standard space defined by the Montreal Neurological Institute template. After normalization, all scans had a resolution of 2 × 2 × 2 mm$^3$. Functional images were spatially smoothed with a 3D isotropic Gaussian kernel (full width at half maximum of 8 mm). Low-frequency noise was removed by applying a high-pass filter (cutoff period = 192 s) to the fMRI time series at each voxel. A temporal smoothing function was applied to the fMRI time series to enhance the temporal signal-to-noise ratio. Significant hemodynamic changes for each condition were examined using the general linear model with boxcar functions convoluted with a hemodynamic response function. Statistical parametric maps for each contrast of the $t$-statistic were calculated on a voxel-by-voxel basis.

To assess the specific condition effect, we used the contrasts of joy minus neutral (J-N), pride minus neutral (P-N), and pride minus joy (P-J). A random effects model, which estimates the error variance for each condition across the subjects, was implemented for group analysis. This procedure provides a better generalization for the population from which data are obtained. The contrast images were obtained from single-subject analysis and entered into the group analysis. A one-sample $t$-test was applied to determine group activation for each effect. To assess common activation in P-N and J-N conditions, we conducted a conjunction analysis of P-N and J-N contrasts at the second level. A statistical threshold of $P < 0.05$ corrected for multiple comparisons across the whole-brain was used, except for a priori hypothesized regions, which were thresholded at $P < 0.0005$ uncorrected (only clusters involving 10 or more contiguous voxels are reported). These a priori regions of interest included the ToM-related regions (MPFC, pSTS, and temporal poles), reward/food-related regions (striatum, insula, and orbitofrontal cortex), and emotion-related limbic regions (amygdalohippocampal regions and anterior cingulate cortex). We conducted regression analyses to demonstrate a more direct link between regional brain activities with the subjective judgments of joy and pride. Using the mean of the ratings of joy and pride for each subject as the covariate, regression analyses with the contrasts (J-N and P-N) and the covariate were done at the second level (height threshold at $P <$ 0.001, uncorrected, and extent threshold of 5 voxels). The masks of J-N and P-N contrasts from one-sample $t$-test ($P < 0.001$) were applied to confine the regions where significant activations were observed. Using the effect sizes, representing the percent signal changes, of the contrasts (J-N and P-N) at the peak coordinates uncovered in the regression analyses, we plotted the fMRI signal changes and ratings of joy and pride.

## Results

### *Self-rating*

The neutral sentences were judged as carrying no prominent emotions. The mean ratings of joy and pride for neutral sentences were, respectively, 0.7 (SD = 0.7) and 0.4 (SD = 0.4), for joy sentences 4.9 (SD = 0.7) and 1.1 (SD = 1.1), and for pride 4.1 (SD = 0.9) and 4.9 (SD = 0.6). Ratings of other emotions (anger, sadness, fear, disgust, and shame) were virtually zero. Although pride sentences were judged as containing joy, their mean ratings of pride were significantly greater than those of joy ($t = 2.9$, degrees of freedom [df] = 30, $P = 0.007$). The mean ratings of joy were significantly greater for joy sentences than for pride sentences ($t = 2.9$, df = 30, $P = 0.007$).

### *fMRI Result*

Pride condition relative to neutral condition (P-N) produced greater activations in the right pSTS, left temporal pole (Table 2 and Fig. 1*A*). We did not find significant activation in the MPFC. Joy condition relative to neutral condition (J-N) produced greater activations in the ventral striatum including the nucleus accumbens, anterior cingulate cortex, hippocampal regions, and insula/operculum (Table 2 and Fig. 1*B*). P-J condition produced greater activations in the right pSTS ($x = 42$, $y = -66$, $z = 22$; $t = 7.39$; 92 voxels). A conjunction analysis of P-N and J-N contrasts revealed no significant activations.

Regression analyses revealed positive linear correlations between the self-rating of pride and the degree of activation in the pSTS (middle temporal gyrus, $x = 44$, $y = -66$, $z = 20$; $t = 5.25$; 14 voxels) (Figs 2*A* and 3*A*). There were positive linear correlations between the self-rating of joy and the degree of activation in the ventral striatum (nucleus accumbens, $x = -12$, $y = 2$, $z = -6$; $t = 6.26$; 6 voxels) (Figs 2*B* and 3*B*).

## Discussion

This study demonstrated that the brain activations during judgments of the positive self-conscious emotion, pride, showed different patterns from those of the basic positive emotion, joy. Pride conditions relative to neutral condition produced greater activity in the right pSTS and left temporal pole, the components of neural substrates of social cognition or ToM (Allison et al. 2000; Adolphs 2001; Frith U and Frith CD 2003; Gallagher and Frith 2003; Moll et al. 2005). In contrast, joy conditions relative to neutral condition produced greater activity in the key nodes of processing hedonic and appetitive stimuli, the ventral striatum including the nucleus accumbens (Breiter and Rosen 1999; Salamone et al. 2003; Cardinal and Everitt 2004) and insula/operculum (Britton et al. 2006; Porubska et al. 2006; Rolls 2006). In addition, regression analyses showed that the subjective ratings of pride and joy correlated with the degrees of activation in the pSTS and ventral striatum, respectively.

Pride, by definition, is subsumed by basic emotion, joy (Tracy and Robins 2004a). In fact, our behavioral rating results showed that ratings of joy for pride sentences were high, although they were lower for pride sentences than for joy sentences. Therefore, it was expected that activations in the regions related to basic emotions, for example, the ventral striatum, might be observed. However, significant activation in such regions was not found, and the conjunction analysis of P-N and J-N did not find common activation in these regions, suggesting that joy derived from pride scenarios was not high enough to activate these regions. We used joyful scenarios containing hedonic and appetitive events that usually motivate biological behaviors like eating, reproduction, and economic behaviors. The mesolimbic dopamine system from the ventral tegmental area to the nucleus accumbens mediates the motivation to obtain reward. In other words, dopamine systems are more necessary for "wanting" incentives than for "liking" them (Berridge and Robinson 1998). Motivational processes are important for positive emotions such as happiness and joy (Lyubomirsky 2001). In an fMRI environment, it is difficult to induce liking, but participants might have felt "wanting" for reward such as money or food, leading to activation in the ventral striatum (Breiter and Rosen 1999; Salamone et al. 2003; Cardinal and Everitt 2004). In contrast, although pride sentences were articulated as joyful, their lack of hedonic contents might account for the lack of activation in such regions.

**Table 2**
Brain activations in pride condition and joy condition relative to neutral condition

| Brain regions | L/R | Coordinates | | | *t*-score |
|---|---|---|---|---|---|
| | | *x* | *y* | *z* | |
| Pride-neutral | | | | | |
| pSTS | R | 42 | −66 | 20 | 4.30 |
| Temporal poles | L | −50 | 20 | −24 | 4.62 |
| Joy-neutral | | | | | |
| Ventral striatum | R | 4 | 4 | −6 | 4.5 |
| Anterior cingulate cortex | L | −6 | 38 | 12 | 4.6 |
| Hippocampal regions | L/R | −32 | −16 | −18 | 4.94 |
| Insula/operculum | L/R | 40 | −28 | 18 | 5.39 |

Note: L, left; R, right. Coordinates and *t*-score refer to the peak of each brain region.

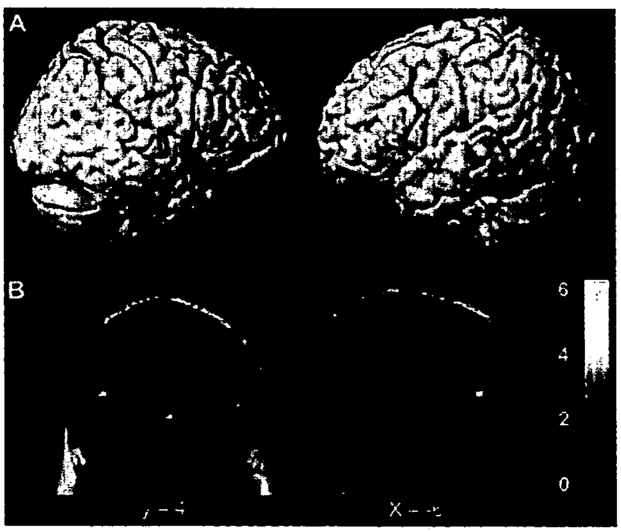


**Figure 1.** Images showing brain activation in joy and pride conditions relative to neutral condition. (*A*) Pride minus neutral. Activated regions were in the right posterior STS and left temporal pole. (*B*) Joy minus neutral. Activations in the ventral striatum, insula/operculum, and anterior cingulate were shown. Significant differences were recognized at a height threshold ($t > 4.07$; $P < 0.0005$, uncorrected) and extent threshold (10 voxels).

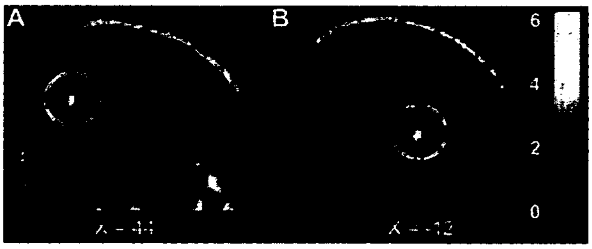


**Figure 2.** Correlation between brain activation and the self-ratings of pride and joy, with height threshold ($P < 0.001$) and extent threshold (5 voxels). (*A*) There was positive linear correlations between self-rating of pride and the degree of activation in the pSTS. (*B*) There was positive linear correlations between self-rating of joy and the degree of activation in the ventral striatum. The bar shows the range of the *t*-score. Within the image, L indicates left. Numbers in the bottom low indicate the *z*-coordinates of the Montreal Neurological Institute brain.

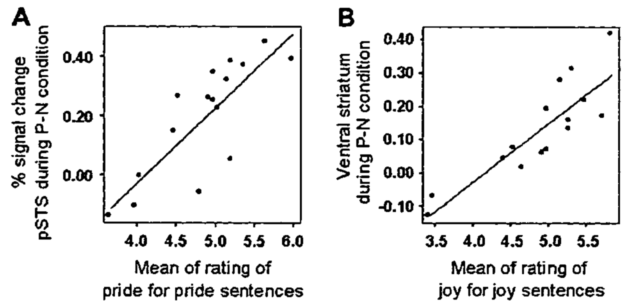


**Figure 3.** Plots and regression lines of correlations between self-ratings and the degree of activation in the brain regions. (*A*) Positive correlations ($r = 0.81$, df = 14, $P < 0.001$) between self-rating of pride and the degree of activation in the pSTS. (*B*) Positive linear correlations ($r = 0.86$, df = 14, $P < 0.001$) between self-rating of joy and the degree of activation in the ventral striatum.

Furthermore, as discussed below, unfamiliarity with some events depicted in pride scenarios might attenuate wanting for such events.

Our previous study has shown activation in the 3 key regions of ToM, the MPFC, pSTS, and temporal poles (Frith U and Frith CD 2003; Gallagher and Frith 2003) during the evaluative process of negative self-conscious emotions such as guilt and embarrassment (Takahashi et al. 2004). In addition, a recent clinical study reported that patients with frontotemporal lobar degeneration had impaired processing of negative self-conscious emotions (Sturm et al. 2006). Therefore, we expected that a positive self-conscious emotion would also recruit these regions. Although activations in the pSTS and temporal poles by pride scenarios were in agreement with our prediction, in disagreement was the lack of significant activation in the MPFC.

Although the precise roles of these 3 regions remain unclear, it was suggested that the pSTS and temporal poles are more concerned with the nature of socially relevant stimuli (Gallagher and Frith 2003; Decety and Grezes 2006). In other words, these regions are involved mainly in the early stage of social cognition, initial appraisal of socially relevant stimuli that support ToM ability, but not in ToM reasoning per se (Frith U and Frith CD 2003; Gallagher and Frith 2003).

Originally, the STS was known to be activated by biological motions such as movement of eyes, mouth, hands, and body (Allison et al. 2000), and it has been suggested to have a more general function in social cognition such as detecting explicit behavioral information that signals the intention of others (Gallagher and Frith 2003) and behavior of agents (Frith U and Frith CD 2003). The higher order association cortices including the pSTS mature in the last stage of brain development (Gogtay et al. 2004), and this might be associated with the fact that, like all self-conscious emotions, pride emerges later in the course of development than basic emotions like fear and joy (Tracy and Robins 2007). In addition, impairments in recognizing self-conscious emotions have been reported in children with autism (Capps et al. 1992; Kasari et al. 1993), in which STS abnormalities are highly implicated (Zilbovicius et al. 2006).

Bilateral temporal poles with greater effect on the left side have also been consistently recruited during ToM task (Calarge et al. 2003; Frith U and Frith CD 2003; Gallagher and Frith 2003). Although the left temporal pole contributes to the composition of sentence meaning (Vandenberghe et al. 2002), the temporal pole activation in P-N condition cannot simply be attributed to the use of sentences because neutral stimuli also require sentence comprehension. The temporal poles are generally engaged in retrieving episodic memories such as emotional and autobiographical memory (Fink et al. 1996; Dolan et al. 2000; Sugiura et al. 2006). In ToM task, the retrieval of episodic memories enables us to understand and simulate the mental state of others (Gallagher and Frith 2003). This role of memory process in understanding others' mental state might result in activation in the temporal pole in the P-N condition. Additionally, a recent study has suggested that this region is involved in storage and recall of contextual information (Mobbs et al. 2006). Because the subjects might not have direct experience of all the pride scenarios, the activation in the temporal pole may suggest that the subjects were reminded of contextual information of themselves or others (e.g., famous person) associated with pride scenarios (Mobbs et al. 2006; Sugiura et al. 2006).

The MPFC appears to be responsible for ToM reasoning or mentalizing, the ability to represent others' perspective (Frith U and Frith CD 2003; Gallagher and Frith 2003; Amodio and Frith 2006). This ability allows us to infer the cause of others' behavior, attribution. Previous studies have shown activation in the MPFC during judgments made on the basis of attributional information (Amodio and Frith 2006), and it is suggested that the MPFC is activated when cues that have been processed in an early stage of social cognition are used in a particular way, that is, to infer the intention (Gallagher and Frith 2003; Ochsner 2004) and emotional state (Aichhorn et al. 2006) of others. The lack of activation in the MPFC might stem from pride scenarios such as used in the present study. Most pride scenarios described situations in which the protagonist was a winner of a prize or competition as a result of achievement. Winning a prize or competition, by definition, is a symbol that inevitably indicates others' positive evaluations or judgments for one's own achievement. Therefore, in order to detect how one is evaluated by others in these situations, one might have less necessity to "infer" the mental state of others by using cues that have been processed in the early stage of social cognition. Another explanation for the lack of significant activation in the MPFC during judgments of pride might be possible. The argument regarding the role of the MPFC in ToM is mainly based on classical, explicit ToM tasks that usually used false belief stories (Frith U and Frith CD 2003; Gallagher and Frith 2003), whereas our task was an implicit ToM task in which the subjects were not explicitly instructed to represent the mental state of others, and the pSTS rather than MPFC plays a more central role (Saxe and Kanwisher 2003). A body of psychological studies has demonstrated that people have self-positivity biases, tendencies to have a positive attitude toward self. People tend to accept responsibility for desired outcomes but to attribute negative events to external causes (Greenwald and Banaji 1995; Leary 2007). Self-positivity biases are known to operate implicitly and automatically without conscious reflection (Greenwald and Banaji 1995; Leary 2007). The MPFC is a key node of a neural system subserving explicit reflection of self (Johnson et al. 2002). Therefore, the subjects might have judged some scenarios as pride ones without elaborate self-reflection.

This study has some limitations. First, as mentioned above, a complex self-conscious emotion could be accompanied by basic emotion. Although we understand that it is not feasible to assess a "pure" form of emotion, the results of regression analysis tell us that brain activations during pride condition could not simply be accounted for by the accompanying emotion. Second, self-conscious emotions depend on society and culture (Haidt 2003). The social background of participants, such as generation, religion, and education, could be confounding factors. For example, there are some empirical studies to support the traditional view that Japanese culture is collectivistic, putting a premium on social harmony, whereas Northern American culture is individualistic, highlighting personal achievement (Kitayama et al. 2006). At the same time, individualism is increasing in contemporary Japanese society especially among the young generation (Cusick 2007). Therefore, examining the effect of generations on self-conscious emotions would be an interesting future theme, and any generalization of our findings needs to be approached with caution. Finally, self-conscious emotions are more difficult to elicit in an MRI environment than basic emotions (Tracy and Robins 2004a). For this reason, we used an emotion judgment task, not an emotion induction task. To complement fMRI studies, lesion studies that can assess real-life human social behavior are recommended.

In conclusion, we investigated the neural substrates of judgments of a positive self-conscious emotion and demonstrated a difference from those of a basic positive emotion at a neural basis level. Supporting the concept that pride could be regarded as a member of the self-conscious emotions family, judgments of pride produced activation in the components of neural substrates implicated in social cognition or ToM. At the same time, judgment of pride might require less self-reflection compared with those of negative self-conscious emotions such as guilt or embarrassment. We expect our findings regarding joy and pride to have broad implications for the neural basis of some neuropsychiatric disorders such as depression or schizophrenia characterized by anhedonia and narcissistic personality or affective disorder, characterized by inappropriate pride, respectively.

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## Notes

*Conflict of Interest*: None declared.

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# Low serum levels of brain-derived neurotrophic factor and epidermal growth factor in patients with chronic schizophrenia

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**Abstract**

Neurotrophic factors (NFs) play a pivotal role in the development of the central nervous system. They are thus also suspected of being involved in the etiology of schizophrenia. Previous studies reported a decreased level of serum brain-derived neurotrophic factor (BDNF) in schizophrenia, whereas the association of epidermal growth factor (EGF) with this illness remains controversial. Using a two-site enzyme immunoassay, we conducted the simultaneous measurement of serum BDNF and EGF levels in a group of patients with chronic schizophrenia ($N=74$) and a group of normal controls matched in age, body mass index, smoking habit and sex ($N=87$). We found that, compared to normal controls, patients with chronic schizophrenia exhibited lower serum levels of both BDNF and EGF across all ages examined (21–59 years). The serum levels of BDNF and EGF were negatively correlated in the controls ($r=-0.387$, $P=0.0002$) but not in the patients. Clinical parameters such as duration of illness and psychiatric rating scale also showed no robust correlations with the NF levels. Collectively, these results suggest that pervasive, abnormal signaling of NFs underlies the pathophysiology of chronic schizophrenia.


*Keywords:* Brain-derived neurotrophic factor; Epidermal growth factor; Neurotrophic factor; Schizophrenia

## 1. Introduction

Accumulating evidence from previous pharmacological, neuroimaging, genetic and postmortem studies has suggested that the etiology of schizophrenia should be viewed as a combination of genetic background and environmental factors, resulting in maldevelopment of the central nervous system and impaired neurotransmissions (Lewis and Gonzalez-Burgos, 2006; Nawa et al., 2000; Nawa and Takei, 2006; Rapoport et al., 2005; Ross et al., 2006; Stephan et al., 2006).

Neurotrophic factors (NFs) play a pivotal role in the survival, growth and differentiation of distinct populations of neurons. Among NFs, brain-derived neurotrophic factor (BDNF) is synthesized predominantly in

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neurons and is widely distributed in the brain, the highest expression having been identified in the hippocampus and cerebral cortex (Ernfors et al., 1990; Hofer et al., 1990; Wetmore et al., 1990). It has been suggested that BDNF possesses a potential role in promoting the function and survival of cholinergic, dopaminergic, serotonergic and GABAergic neurons (Connor and Dragunow, 1998). Another NF, epidermal growth factor (EGF), also serves as a neurotrophic molecule to stimulate the proliferation, migration and differentiation of neuronal cells, and influences synaptic plasticity, including hippocampal long-term potentiation (Ishiyama et al., 1991; Xian and Zhou, 1999). EGF has been suggested to be involved especially in the growth and survival of midbrain dopaminergic neurons (Alexi and Hefti, 1993; Casper et al., 1991; Casper and Blum, 1995; Ventrella, 1993). Thus, dysfunction in the BDNF and/or EGF systems may contribute to impairment in brain development, neuroplasticity and synaptic connectivity, leading eventually to the manifestation of schizophrenic syndrome. In fact, genetic manipulation of BDNF or neonatal perturbation of EGF signaling in mice has been reported to cause behavioral abnormalities often observed in psychiatric disorders (Chen et al., 2006; Futamura et al., 2003; Mizuno et al., 2004).

Previous studies have reported alterations of BDNF and EGF levels in several brain regions as well as in serum of patients with schizophrenia, although the reported changes varied among the studies (Tables 1 and 2). Postmortem studies have shown elevated BDNF levels in

Table 1
Previous studies on BDNF levels of patients with schizophrenia

| Authors (Year) | Origin of sample | Controls | | Patients | | | Remarks |
|---|---|---|---|---|---|---|---|
| | | Number | Concentration* | Number | Concentration* | Level** | |
| Takahashi et al. (2000) | Postmortem Brain | 22 | 100*** | 14 | 170*** | ↑ | In anterior cingulate |
| | | 13 | 100*** | 13 | 230*** | ↑ | In hippocampus |
| Durany et al. (2001) | Postmortem brain | 11 | 1.68±0.21 | 11 | 2.70±0.40 | ↑ | In frontal cortex |
| | | | 1.59±0.22 | | 2.93±0.53 | ↑ | In parietal cortex |
| | | | 1.39±0.18 | | 2.80±0.40 | ↑ | In temporal cortex |
| | | | 1.34±0.16 | | 2.91±0.60 | ↑ | In occipital cortex |
| | | | 4.84±0.61 | | 2.70±0.42 | ↓ | In hippocampus |
| Weickert et al. (2003) | Postmortem brain | 19 | 100*** | 12 | 60*** | ↓ | In prefrontal cortex |
| Toyooka et al. (2002) | Serum | 35 | 11.4±7.7 | 34 | 6.3±3.4 | ↓ | Number of platelets was decreased |
| Pirildar et al. (2004) | Serum | 22 | 26.8±9.3 | 22 | 14.19±8.12 (pretreatment) | ↓ | |
| | | | | | 14.53±2.93 (posttreatment) | ↓ | |
| Tan et al. (2005) | Serum | 45 | 9.9±4.3 | 81 | 7.3±2.6 | ↓ | Correlation with PANSS negative ($r$=−0.307, $P$=0.005) |
| Zhang et al. (2007) | Serum | 37 (male) | 9.7±4.5 | 91 (male) | 7.1±2.2 | ↓ | Correlation with BMI gain in females ($r$=−0.453, $P$=0.008) |
| | | 13 (female) | 9.0±4.4 | 33 (female) | 5.9±2.3 | ↓ | |
| Grillo et al. (2007) | Serum | 25 | 0.17±0.03 | 24 (typicals) | 0.10±0.05 | ↓ | Correlation with clozapine dose ($r$=0.643, $P$=0.002) |
| | | | | 20 (clozapine) | 0.13±0.04 | ↓ | No correlation with age at onset and duration of illness |
| Shimizu et al. (2003) | Serum | 40 | 28.5±9.1 | 25 (medicated) | 27.9±12.3 | n.s. | No correlation with age at onset and duration of illness |
| | | | | 15 (drug-naïve) | 23.8±8.1 | | |
| Huang and Lee (2006) | Serum | 96 | 14.17±6.86 | 126 | 14.20±6.92 | n.s. | Catatonia group ($N$=7) showed decreased BDNF levels<br>No correlation with age at onset |
| Present Study | Serum | 87 | 52.2±25.3 | 74 | 37.1±20.4 | ↓ | |

*Data indicate mean±SD of brain (ng/ml protein) and serum (ng/ml). **As compared with BDNF levels of normal controls. *** % control.
BDNF, Brain-Derived Neurotrophic Factor; PANSS, Positive and Negative Syndrome Scale; BMI, Body Mass Index; n.s., not significant.

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Table 2
Previous studies on EGF levels of patients with schizophrenia

| Authors (Year) | Origin of sample | Controls | | Patients | | | Remarks |
|---|---|---|---|---|---|---|---|
| | | Number | Concentration* | Number | Concentration* | Level** | |
| Futamura et al. (2002) | Postmortem brain | 12 | 6.3±2.0 | 14 | 4.8±2.0 | ↓ | In prefrontal cortex |
| | | 16 | 3.8±1.5 | 14 | 2.0±0.9 | ↓ | In striatum |
| | Serum | 45 | 392±344 | 45 (medicated) | 125±80.8 | ↓ | |
| | | 14 | 554±350 | 6 (drug-free) | 167±100 | ↓ | |
| Hashimoto et al. (2005) | Serum | 40 | 411±217 | 25 (medicated) | 481±241 | n.s. | Correlation with BPRS (r=0.434, P=0.005) |
| | | | | 15 (drug-naïve) | 331±226 | | |
| Present Study | Serum | 87 | 560.7±357.1 | 74 | 395.5±231.7 | ↓ | |

*Data indicate mean±SD of brain (pg/ml protein) and serum (pg/ml). **As compared with EGF levels of normal controls.
EGF, Epidermal Growth Factor; BPRS, Brief Psychiatric Rating Scale; n.s., not significant.

the anterior cingulate, hippocampus (Takahashi et al., 2000) and cerebral cortex (Durany et al., 2001), whereas decreases in BDNF levels in the hippocampus (Durany et al., 2001) and prefrontal cortex (Weickert et al., 2003) have also been reported. In the serum of treated patients, BDNF levels have been found to be decreased (Grillo et al., 2007; Pirildar et al., 2004; Tan et al., 2005; Toyooka et al., 2002; Zhang et al., 2007). Yet, other studies have shown that the serum BDNF level in patients was not significantly different from that in normal controls (Huang and Lee, 2006; Shimizu et al., 2003). As for EGF, its protein levels were found to be decreased in the prefrontal cortex and striatum of postmortem schizophrenic brains (Futamura et al., 2002). The serum EGF level was markedly reduced in patients with schizophrenia in one report (Futamura et al., 2002), whereas in another report, there was no difference between patients and normal controls (Hashimoto et al., 2005). Taking these conflicting results together, it is clear that the issue of NF levels in patients with schizophrenia requires further study.

Compared to postmortem studies, measurement of serum NFs has the obvious clinical advantage of being available from blood samples that can be drawn from living subjects as frequently as necessary. BDNF is produced in various peripheral tissues, such as retina, muscle and platelets (Radka et al., 1996), in addition to the central nervous system as described above. EGF is excreted by the pituitary gland and peripheral tissues including salivary and Brunner's gland of the gastrointestinal system (Plata-Salamán, 1991). Thus, the origins of BDNF and EGF in serum are not yet completely understood. Importantly, however, serum BDNF levels reportedly correlate with BDNF concentrations in the central nervous system (Karege et al., 2002). It has also been reported that the expression of EGF is impaired in both central and peripheral organs of patients (Futamura et al., 2002). Therefore, the serum levels of both NFs might reflect the pathophysiology and possibly the clinical outcome of schizophrenia.

In the present study, we measured the serum levels of both BDNF and EGF simultaneously in individual subjects by using a two-site enzyme immunoassay, and we examined their association with the clinical parameters of patients with schizophrenia.

## 2. Methods and materials

### 2.1. Subjects

Two groups of subjects, 74 patients with schizophrenia and 87 control subjects, participated in this study. The patients were recruited from inpatients and outpatients of Asai Hospital. Diagnoses were made by I.I., Y.O., and the attending psychiatrists on the basis of a review of their charts and a conventionally semi-structured interview. All patients also met the DSM-IV criteria for schizophrenia. Their symptoms were evaluated by Global Assessment of Functioning (GAF) and Brief Psychiatric Rating Scale (BPRS). All patients had been receiving antipsychotic drugs. Mean antipsychotic dose was 936.6±588.8 mg/day in chlorpromazine equivalents. Antipsychotic drugs administered to patients were risperidone ($N$=31), olanzapine ($N$=23), quetiapine ($N$=16), levomepromazine ($N$=15), chlorpromazine ($N$=14), haloperidol ($N$=13), zotepine ($N$=10), perospirone ($N$=7), sulpiride ($N$=6), sultopride ($N$=4), bromperidol, propericyazine ($N$=3 each), fluphenazine ($N$=2), nemonapride, perphenazine, timiperone ($N$=1 each). Of the patients, 23 were receiving monotherapy.

Healthy normal control subjects with no history of psychiatric disorders were recruited from the local community. There was no significant difference in age ($P$=0.160), body mass index (BMI) ($P$=0.920), sex ratio ($P$=0.867) and smoking habit ($P$=0.955) between

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the two groups. Their detailed demographic data are summarized in Table 3. The present study was approved by the ethics committees of all participating institutes. After complete explanation of the study, written informed consent was obtained from all subjects.

### *2.2. Two-site enzyme immunoassay for BDNF and EGF*

The concentrations of BDNF and EGF proteins were measured by two-site enzyme immunoassay (Futamura et al., 2002; Nagano and Suzuki, 2003). Blood samples were obtained between 10:00 and 16:00 at Asai Hospital. Samples were collected into tubes without anticoagulant and allowed to clot at room temperature. Serum was separated by centrifugation at 3000 rpm for 7 min and then stored at −80 °C until use. EIA titer plates (FluoroNunc Module, Nunc A/S, Roskilde, Denmark) were coated with primary polyclonal antibodies against BDNF (Promega, Madison, WI) or EGF (Oncogene, San Diego, CA) overnight and then blocked with EIA buffer (50 mM Tris [pH 7.5], 0.5 M NaCl, 0.3% Triton X-100, 0.4% gelatin and 0.4% bovine albumin) at 4 °C for more than 3 h. One hundred microliters of diluted serum (in duplicate) or each NF standard (1–1000 pg; in triplicate) for BDNF (Chemicon, Temecula, CA) or EGF (PeproTech, London, UK) in EIA buffer was placed into each well, and the plates were then incubated at room temperature for 7 h. After three washes with Wash-buffer (EIA buffer without bovine serum albumin), 100 µl of biotinylated antibody against human BDNF (Genzyme-Techne, Minneapolis, MN) or human EGF (R&D, Minneapolis, MN) in EIA buffer was added to the wells, and the plates were incubated for 12–18 h at room temperature. The biotinylated secondary antibody bound to BDNF or EGF was detected by incubation with streptavidin-β-galactosidase (Roche Diagnostics, Mannheim, Germany) at room temperature for 3 h. Unbound enzyme was removed by extensive washes with Wash-buffer followed by phosphate-buffered saline free of calcium and magnesium. Then, β-galactosidase activity in each well was measured by incubation with a substrate, 200 µM 4-methylumbelliferyl β-D-galactoside (Sigma, St. Louis, MO) in 50 mM sodium phosphate (pH 7.3) and 10 mM $MgCl_2$. The reaction proceeded in a dark at room temperature for 3 h, and the amount of fluorescent products was monitored by Spectraflour Plus microplate reader (Tecan, Männedorf, Switzerland) with excitation and emission wavelengths of 360 nm and 465 nm, respectively. A standard curve was obtained for each assay in a range of 1–1000 pg of recombinant BDNF or EGF. Serum NFs were measured simultaneously, as far as possible, with several standard samples to minimize inter-assay difference. The intra-assay coefficient of variation was less than 3%. There was no significant cross-reactivity among other neurotrophic factors for BDNF (Nagano and Suzuki, 2003) and the EGF family members of EGF (data not shown). The assays were all performed in a blinded fashion.

Table 3
Demographic data of patients with schizophrenia and normal controls

| | | Schizophrenia ($N$=74) | Control ($N$=87) |
|---|---|---|---|
| Gender (M/F) | | 39/35 | 47/40 |
| Age | | 41.9±11.1 | 39.8±10.7 |
| BMI (kg/m$^2$)* | | 23.6±4.7 | 23.1±2.1 |
| Atopic dermatitis (presence/absence) | | 1/22 | 3/31 |
| Smoking habit (presence/absence) | | 11/12 | 16/18 |
| Age at onset | | 22.2±6.9 | |
| Duration of illness (years) | | 19.6±11.2 | |
| Number of hospitalizations | | 4.4±3.6 | |
| Total duration of hospitalization (years) | | 8.8±9.5 | |
| Chlorpromazine equivalents (mg/day) | | 936.6±588.8 | |
| GAF** | | 39.7±10.9 | |
| BPRS** | Total | 43.8±15.5 | |
| | Positive | 11.0±4.6 | |
| | Negative | 9.8±4.6 | |

BMI, Body Mass Index; GAF, Global Assessment of Functioning; BPRS, Brief Psychiatric Rating Scale. All data were reported as mean±SD. *, $N$=44 for schizophrenia and $N$=34 for control. **, $N$=33.

### *2.3. Statistical analysis*

NF levels and demographic data of the subjects were reported as mean±SD. The Mann–Whitney $U$ test was employed for group comparisons. Linear relationship between two variables was examined by Spearman rank correlation coefficients. Pearson chi-square test was used for comparing sex ratio and smoking habit between the controls and patients, and between low and high-BDNF groups in the controls. $P<0.05$ was considered statistically significant.

## 3. Results

### *3.1. Serum BDNF and EGF levels*

Both serum BDNF and EGF levels in schizophrenia patients and normal controls were measured by two-site enzyme immunoassay. The mean serum BDNF level of patients was significantly lower than that of controls (37.1±20.4 and 52.2±25.3 ng/ml in patients and controls, respectively; $P$=0.00003; Fig. 1A). The mean serum EGF level was also significantly lower in patients than in controls (395.5±231.7 vs. 560.7±357.1 pg/ml; $P$=0.002; Fig. 1B).

The relation between serum NF levels and age was examined. The age of both patient and control groups ranged from 21 to 59 years. As shown in Fig. 1C (BDNF), Fig. 1D (EGF) and Table 4, there were no significant correlations between serum NF levels and age in either group.

Because both BDNF and EGF were measured simultaneously within the same individuals, the correlation between serum BDNF and EGF was examined in each group. In the controls, a negative correlation between BDNF and EGF levels was found ($r$=−0.387, $P$=0.0002; Fig. 2A). In contrast, there was no significant correlation between the serum BDNF and EGF levels in the patients ($P$=0.161, Fig. 2B).

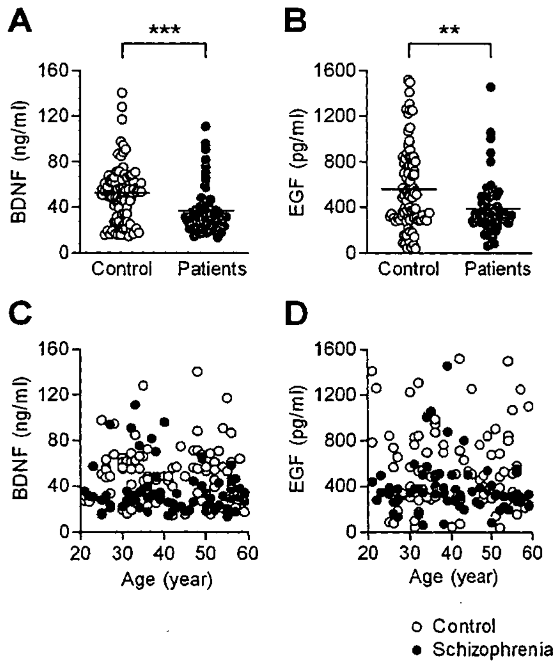


Fig. 1. Serum levels of (A) BDNF and (B) EGF measured by two-site enzyme immunoassay in normal controls ($N$=87) and patients with chronic schizophrenia ($N$=74). Compared with controls, patients exhibited lower serum levels of both neurotrophic factors (BDNF, ***$P$<0.001; EGF, **$P$<0.01). Horizontal lines indicate the mean levels. Distributions of serum (C) BDNF and (D) EGF levels in controls (open circles) and patients (filled circles) with age. No significant correlation was observed between NF levels and age (21–59 years) in the two groups. BDNF, brain-derived neurotrophic factor; EGF, epidermal growth factor.

Table 4
Correlations between levels of neurotrophic factors and clinical parameters in patients with schizophrenia

| Clinical parameters | | $N$ | BDNF | | EGF | |
|---|---|---|---|---|---|---|
| | | | $r$ | $P$ | $r$ | $P$ |
| Age | | 74 | −0.031 | 0.795 | −0.227 | 0.053 |
| Age at onset | | 74 | 0.303 | 0.009 | 0.052 | 0.644 |
| Duration of illness | | 74 | −0.196 | 0.098 | −0.281 | 0.016 |
| CPZ-EQ (mg/day) | | 74 | 0.051 | 0.520 | 0.079 | 0.327 |
| BMI (kg/m$^2$) | | 44 | 0.171 | 0.267 | −0.088 | 0.569 |
| GAF | | 33 | 0.024 | 0.843 | −0.076 | 0.727 |
| BPRS | Total | 33 | −0.099 | 0.588 | 0.349 | 0.046 |
| | Positive | 33 | −0.189 | 0.303 | 0.347 | 0.047 |
| | Negative | 33 | 0.102 | 0.558 | 0.127 | 0.468 |

CPZ-EQ, Chlorpromazine Equivalents; BMI, Body Mass Index; GAF, Global Assessment of Functioning; BPRS, Brief Psychiatric Rating Scale.

Since the distribution of BDNF in the control group appeared bimodal as shown in Fig. 2A, we examined whether the low-BDNF group (40 ng/ml of BDNF as a tentative threshold for the dichotomy; $N$=26) and high-BDNF group ($N$=61) differed in their biological parameters. Statistical analyses revealed that there were no significant differences in their BMI ($P$=0.627), age ($P$=0.959), sex ratio ($P$=0.654), and smoking habit ($P$=0.464).

### 3.2. Correlation of serum BDNF and EGF levels with clinical parameters

Overall, clinical parameters did not exhibit robust correlations with the BDNF and EGF levels ($P$>0.05/10 [=0.005], corrected for multiple comparisons in Table 4 and Fig. 2B), although age at onset was marginally correlated with the BDNF level ($r$=0.303, $P$=0.009). We also analyzed the effects of BMI and smoking habit on NF levels. There were no significant correlations between serum NF levels and BMI in patients ($P$=0.267 for BDNF, $P$=0.569 for EGF, $N$=44) or in controls ($P$=0.687 for BDNF, $P$=0.697 for EGF, $N$=34). In addition, NF levels were not significantly different between the presence ($N$=11 for patients, $N$=16 for controls) and absence ($N$=12 for patients, $N$=18 for controls) of smoking habit in patients ($P$=0.735 for BDNF, $P$=0.132 for EGF) and in controls ($P$=0.569 for BDNF, $P$=0.593 for EGF).

### 3.3. Type of antipsychotic drugs and neurotrophic factor levels

Thirteen patients had been taking one or more typical antipsychotic drugs, while thirty-one other patients had been taking only atypical antipsychotic drugs. We found

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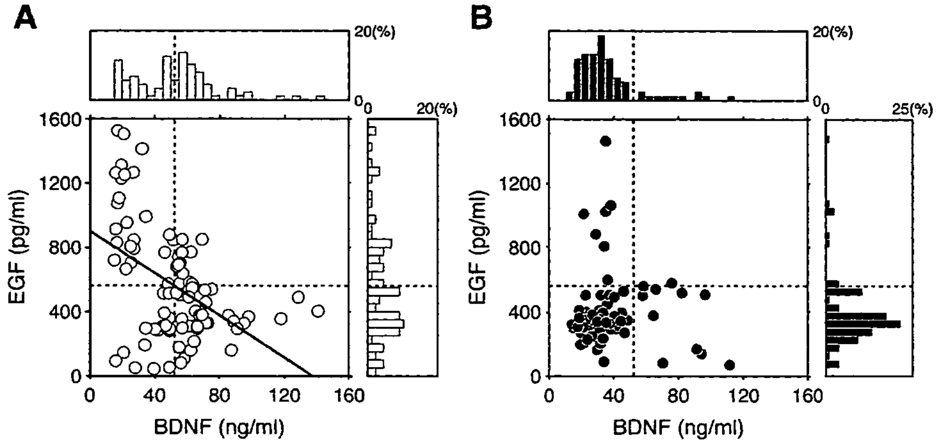


Fig. 2. Relation between the serum levels of BDNF and EGF measured simultaneously in (A) normal controls and (B) chronic schizophrenia patients. For controls, serum levels of the two neurotrophic factors were negatively correlated as shown by the line ($r=-0.387$, $P=0.0002$). The histograms above and on the right of the main plots show the fractions of subjects that fall into particular intervals of serum BDNF (in steps of 5 ng/ml) and EGF (in steps of 50 pg/ml) levels, respectively. In both histograms, dotted lines represent the mean levels of BDNF (52.2 ng/ml) and EGF (560.7 pg/ml) of normal controls, respectively. BDNF, brain-derived neurotrophic factor; EGF, epidermal growth factor.

that the levels of both BDNF and EGF did not differ between the patients taking typical and atypical antipsychotic drugs ($P>0.05$, Fig. 3A and B). In addition, there was no significant correlation between

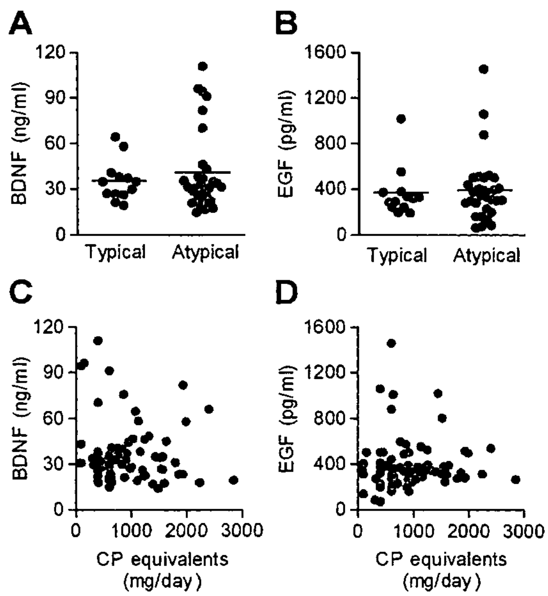


Fig. 3. Effects of antipsychotic drugs on serum (A) BDNF and (B) EGF levels. For both neurotrophic factors, no significant differences were seen between patients taking typical ($N=13$) and atypical ($N=31$) antipsychotic drugs. Horizontal lines indicate the mean levels. Antipsychotic dosages in chlorpromazine equivalents were correlated neither (C) with serum BDNF nor (D) with EGF levels ($N=74$). BDNF, brain-derived neurotrophic factor; EGF, epidermal growth factor; CP, chlorpromazine.

the chlorpromazine equivalents of medication and serum NF levels (Fig. 3C and D; Table 4).

We also analyzed the effects of anticholinergic drugs on the NF levels. Thirty-five patients had been taking anticholinergic drugs including biperiden and trihexyphenidyl in combination with antipsychotic drugs. NF levels were not significantly different between the patients with (BDNF, 37.9±20.1 ng/ml; EGF, 395.8±225.0 pg/ml; $N=35$) and without (BDNF, 36.3±20.9 ng/ml; EGF, 395.3±240.5 pg/ml; $N=39$) anticholinergic drugs ($P=0.626$ for BDNF, $P=0.475$ for EGF).

## 4. Discussion

### *4.1. Lower serum BDNF and EGF levels in schizophrenia*

As summarized in Tables 1 and 2, previous studies have mostly reported low serum BDNF levels (Grillo et al., 2007; Pirildar et al., 2004; Tan et al., 2005; Toyooka et al., 2002; Zhang et al., 2007), while changes in the serum EGF level have remained a matter of controversy (Futamura et al., 2002; Hashimoto et al., 2005). In the present study, at least, it was clearly shown that most of the chronic schizophrenia patients had lower serum levels of EGF as well as BDNF. Mean serum BDNF values were 37.1 and 52.2 ng/ml in patients and controls, respectively, in the present study. These values were higher than those in several other reports, but, as can be seen in Table 1, BDNF levels varied considerably among the studies reported. Such differences may be due to the antibodies used against neurotrophic factors, the methods of measurement, and the sampling conditions. Actually, the values in the present study fell into a range similar of values to those in the reports adopting similar methods (Toyooka et al., 2002). In addition, this decrease in NFs was observed in patients regardless of age, ranging from the early 20s to the late 50s. This observation was consistent with previous reports showing no correlation between age and serum BDNF levels (Grillo et al., 2007; Huang and Lee, 2006; Toyooka et al., 2002), lending credence to the hypothesis that schizophrenia is the behavioral outcome of aberration in the neurodevelopmental processes.

In the present work, the simultaneous measurement of NFs revealed a significant negative correlation between serum BDNF and EGF levels in controls (Fig. 2A), whereas there was no correlation between the two NF levels in patients (Fig. 2B), possibly reflecting their low levels of both BDNF and EGF. The fact that no control subjects showed high serum levels of both BDNF and EGF is of particular interest. Neurite outgrowth from EGF-responsive stem cell-derived neurons can be enhanced by treatment with BDNF (Shetty and Turner, 1999), while BDNF reportedly induced the down-regulation of EGF receptors (Huang et al., 1988). In addition, the co-application of transforming growth factor-alpha, a member of the EGF family, with BDNF blocked the BDNF-triggered up-regulation of AMPA receptor expression and currents (Namba et al., 2006). Thus, complementary roles of both factors may underlie the normal development of the nervous system. In other words, chronic schizophrenia may represent a state deficient in NF-regulated neural functions, leading eventually to various mental malfunctions.

The origins of serum BDNF and EGF are not yet completely understood. EGF reportedly enters the brain through the blood-brain barrier (BBB) in mouse (Pan and Kastin, 1999). BDNF is reported to be transported across the BBB in normal mouse (Pan et al., 1998) and rats with cerebral ischemia (Schäbitz et al., 2000), while another report has argued that the transport of BDNF is negligible (Sakane and Pardridge, 1997). EGF and BDNF are produced in various peripheral tissues (Plata-Salamán, 1991; Radka et al., 1996), in addition to the central nervous system as described above. Nevertheless, the serum levels of NFs can be used as clinical markers, since they show different distributions between patients and controls, as shown in previous studies as well as in the present study.

### 4.2. Clinical parameters and neurotrophic factors

We failed to find any clinical parameters that demonstrated robust correlation with the two NF levels. As shown in Tables 1 and 2, previous reports also examined the correlation between clinical parameters and NF levels: the BDNF level was correlated with the negative symptom subscore of the Positive and Negative Syndrome Scale (Tan et al., 2005); the serum EGF level was significantly correlated with the BPRS score (Hashimoto et al., 2005). Although the reasons for the discrepancy between the previous and present results are unclear, differences in demographic characteristics of the patients (such as age at onset, illness duration, sample size, distribution of BPRS score, and dosage of antipsychotic drugs) might provide at least a partial explanation.

Other factors than psychiatric parameters have been reported to affect serum BDNF levels. BMI (Suwa et al., 2006) and age (Ziegenhorn et al., 2007) showed positive and negative correlation with BDNF levels, respectively. Patients with atopic dermatitis have higher levels of serum BDNF in association with the severity of symptoms (Raap et al., 2005; Namura et al., 2007), while smokers have lower values as compared with non-smokers (Kim et al., 2007). We could not completely rule out the possibility that these factors affected the values in the present study, since data could not be obtained from all participants. However, the limited data suggested that neither BMI nor smoking habit affected neurotrophic levels in patients or controls.

### 4.3. Types of antipsychotic drugs and serum neurotrophic factor levels

In the present study, the NF levels were not correlated with any types or dosages of medications. Although Grillo et al. (2007) found a significant correlation between the BDNF level and clozapine dosage, other investigators found no significant correlation between BDNF (Hori et al., 2007; Shimizu et al., 2003; Tan et al., 2005; Toyooka et al., 2002; Zhang et al., 2007) or EGF level (Futamura et al., 2002) and antipsychotic dosages. In addition, treatment with olanzapine for 8 weeks (Hori et al., 2007) or antipsychotic drugs (risperidone for most patients) for 6 weeks (Pirildar et al., 2004) did not alter BDNF levels in blood. It was recently suggested that the effects of atypical and typical antipsychotic drugs on the BDNF level were different. In animal experiments, haloperidol, a typical antipsychotic drug, decreased the BDNF expression in the hippocampus, whereas atypical antipsychotics did not affect or even up-regulated this expression (Bai et al., 2003; Chlan-Fourney et al., 2002; Parikh et al., 2004). In addition, atypical antipsychotics, but not haloperidol, stimulated neurogenesis in the subventricular zone of the rat brain (Wakade et al., 2002). Clinically, chronic treatment with haloperidol, but not olanzapine, was associated with a significant reduction in gray matter volume in schizophrenia patients with first-

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episode psychosis (Lieberman et al., 2005). However, the present study failed to show that the type of drug affects either the BDNF or the EGF serum level. This observation might indicate a limitation concerning the measurement of serum NFs for predicting their function in the brain. Nevertheless, the serum levels of NFs could be used as clinical markers from the viewpoint that they are independent of the type of medication used.

In conclusion, we showed herein that patients with chronic schizophrenia have lower serum levels of both BDNF and EGF across all ages, possibly reflecting pervasive abnormal signaling of NFs underlying the pathophysiology of schizophrenia. A future study should investigate NFs of patients with schizophrenia before pharmacological intervention or those undergoing the first-episode of the disease, thereby addressing whether this overall reduction in NFs is a common characteristic in the symptomatology of schizophrenia.

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**Contributors**

Y.I. measured the concentrations of BDNF and EGF proteins, analyzed the data and wrote the manuscript. N.Y. undertook the statistical analyses of whole data including neurotrophic factor levels and demographical data, and wrote the manuscript. M.N. developed the two-site enzyme immunoassay for BDNF and EGF and measured the concentrations of BDNF and EGF proteins. I.I, T.T and T.Y recruited the subjects for this project and collected blood samples. Y.O and H.S designed and supervised the whole study and wrote the manuscript. All authors contributed to and have approved the final manuscript.

**Conflict of interest**

All authors declare that they have no conflicts of interest.

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# GABA$_A$/Benzodiazepine receptor binding in patients with schizophrenia using [$^{11}$C]Ro15-4513, a radioligand with relatively high affinity for α5 subunit

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**Abstract**

Dysfunction of the GABA system is considered to play a role in the pathology of schizophrenia. Individual subunits of GABA$_A$/Benzodiazepine (BZ) receptor complex have been revealed to have different functional properties. α5 subunit was reported to be related to learning and memory. Changes of α5 subunit in schizophrenia were reported in postmortem studies, but the results were inconsistent. In this study, we examined GABA$_A$/BZ receptor using [$^{11}$C]Ro15-4513, which has relatively high affinity for α5 subunit, and its relation to clinical symptoms in patients with schizophrenia.

[$^{11}$C]Ro15-4513 bindings of 11 patients with schizophrenia (6 drug-naïve and 5 drug-free) were compared with those of 12 age-matched healthy control subjects using positron emission tomography. Symptoms were assessed using the Positive and Negative Syndrome Scale. [$^{11}$C]Ro15-4513 binding was quantified by binding potential (BP) obtained by the reference tissue model. [$^{11}$C]Ro15-4513 binding in the prefrontal cortex and hippocampus was negatively correlated with negative symptom scores in patients with schizophrenia, although there was no significant difference in BP between patients and controls. GABA$_A$/BZ receptor including α5 subunit in the prefrontal cortex and hippocampus might be involved in the pathophysiology of negative symptoms of schizophrenia.


*Keywords:* γ-Amino-butyric acid; Schizophrenia; Negative symptoms; Prefrontal cortex; Hippocampus; PET

## 1. Introduction

γ-Amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system.

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$GABA_A$/Benzodiazepine (BZ) receptors are heteropentamiric GABA-gated chloride channels, and mediate fast synaptic inhibition (Moss and Smart, 2001). Benzodiazepines enhance the action of the neurotransmitter GABA at $GABA_A$/BZ receptors by interaction with their modulatory benzodiazepine sites.

Dysfunction of GABA neurotransmission in the brain is thought to play a role in the pathology of schizophrenia (Simpson et al., 1989; Reynolds et al., 1990). Postmortem studies using [$^3$H]muscimol showed that binding was increased in the hippocampal formation (Benes et al., 1996a), anterior cingulate cortex (Benes et al., 1992) and prefrontal cortex (Benes et al., 1996b; Dean et al., 1999) in patients with schizophrenia. The axon terminals of chandelier GABA neurons are reported to be reduced substantially in the middle layers of the prefrontal cortex in schizophrenia (Lewis et al., 1999).

$GABA_A$/BZ receptor chloride channel complex consists of two α subunits, two β subunits and one γ subunit (Barnard et al., 1998; Lüddens et al., 1995; Mehta and Ticku, 1999). It has been reported that the diversity of α subunits is responsible for various functional properties and ligand selectivity to the $GABA_A$/BZ receptor (Barnard et al., 1998; Low et al., 2000; Mehta and Ticku, 1999; Tobler et al., 2001). α1 subunit has been suggested to be related to hypnotic and sedative amnesic actions, whereas α2, α3 and α5 subunits to anxiolytic, anticonvulsant, and antipsychotic actions, and to the function of learning and memory (Crestani et al., 2001; Mohler et al., 2001; Serwanski et al., 2006).

Alterations in individual subunits of $GABA_A$/BZ receptor in schizophrenia have been the focus of recent postmortem studies. Expression of α1 subunit was reported to increase in the prefrontal cortex of patients with schizophrenia (Ohnuma et al., 1999; Ishikawa et al., 2004), α2 subunit was reported to increase in the prefrontal cortex (Volk et al., 2002), and α5 subunit expression was reported to show no significant change (Akbarian et al., 1995) or increase (Impagnatiello et al., 1998).

Several ligands such as [$^{11}$C]flumazenil and [$^{11}$C]Ro15-4513 were developed to visualize $GABA_A$/BZ receptors by positron emission tomography (PET) (Inoue et al., 1992; Halldin et al., 1992; Pappata et al., 1988). Both [$^{11}$C]flumazenil and [$^{11}$C]Ro15-4513 have the imidazobenzodiazepine core structure. However, flumazenil is a $GABA_A$/BZ receptor antagonist while Ro15-4513 is known as a $GABA_A$/BZ receptor partial inverse agonist. A different distribution pattern has been reported for the binding of [$^{11}$C]Ro15-4513 compared to that of [$^{11}$C]flumazenil (Inoue et al., 1992; Halldin et al., 1992). Ro15-4513 was reported to have relatively higher affinity for the α5 subunit-containing $GABA_A$/BZ receptor *in vitro* (Lüddens et al., 1994; Wieland and Lüddens, 1994). [$^{11}$C]Ro15-4513 bindings in the cingulate and temporal cortical regions showed relatively higher binding to α5 subunit of $GABA_A$ receptor (Lingford-Hughes et al., 2002; Maeda et al., 2003).

A simplified method without arterial blood sampling for [$^{11}$C]Ro15-4513 in the living human brain has been evaluated recently, and it can be used in clinical studies (Asai et al., in press).

In this study, we measured [$^{11}$C]Ro15-4513 binding to examine $GABA_A$/BZ receptors with α5 subunit and their relation to clinical symptoms in patients with schizophrenia.

## 2. Methods and materials

### 2.1. Subjects

Eleven patients with schizophrenia (5 women, 6 men; 32.8±10.2 years old, mean±SD) meeting DSM-IV criteria for schizophrenia or schizophreniform disorder were enrolled in this study. Demographic and clinical data on subjects are shown in Table 1. Six of the patients (3 women, 3 men; 29.2±7.3 years old) were neuroleptic-naïve and five (2 women, 3 men; 37.2±12.2 years old) had been neuroleptic-free for at least one year before the PET measurement except one subject who took

Table 1
Demographic and clinical characteristics asstudy entry

| | N | Age (years) | Male/female | Duration of illness (months) | Schizophrenia/schizophreniform | PANSS Positive | PANSS Negative | PANSS General | PANSS Total |
|---|---|---|---|---|---|---|---|---|---|
| Patient | 11 | 32.8±10.2 | 6/5 | 1–444 | 9/3 | 24.4±5.1 | 21.4±6.0 | 44.6±10.2 | 90.4±19.6 |
| Drug-naïve | 6 | 29.2±7.3 | 3/3 | 1–36 | 3/3 | 24.8±3.9 | 20.3±8.0 | 45.3±12.0 | 90.5±23.0 |
| Drug-free | 5 | 37.2±12.2 | 3/2 | 24–444 | 6/0 | 23.8±6.8 | 22.6±2.5 | 43.8±8.8 | 90.2±17.4 |
| Normal controls | 12 | 29.0±10.2 | 12/0 | – | – | – | – | – | – |