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Research Report

Volume of left amygdala subregion predicted temperamental trait of harm avoidance in female young subjects. A voxel-based morphometry study

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ABSTRACT

We investigated the relationship between temperamental predisposition and brain structure by using a standard questionnaire and high-resolution T1-weighted magnetic resonance image (MRI) in normal young volunteers. Fifty-six subjects completed the Japanese version of the Temperament and Character Inventory (TCI, 125 items) and underwent an MRI acquisition of the brain. The gray matter (GM) was extracted from the whole brain image of the subjects and normalized to the standard brain template using statistical parametric mapping and the optimized voxel-based morphometry (VBM) method. When the score on the harm avoidance (HA) subscale was used as a dependent variable, the multiple regression analysis revealed that the HA score positively correlated with the volume of the part of left amygdala. The region-of-interest analysis showed that the correlation was significant in the female subjects but not in the male subjects. The correlation was significant even after the effects of age, depression score, and total GM volume were taken into account. The differential correlation between the sexes may be caused by differences in hormonal condition and the vulnerability of women to socio-psychological stress. In addition, the novelty seeking (NS) score positively correlated with the GM of the left middle frontal gyrus. The volume of the tail of the right caudate nucleus positively correlated with the reward dependence (RD) score. With regard to the NS and RD scores, no significant sex difference was observed in the correlation. These results indicate that the temperamental traits measured using the questionnaire may have a morphological basis in the human brain.

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1. Introduction

Previous studies have confirmed that the four dimensions of temperament – novelty seeking (NS), harm avoidance (HA), reward dependence (RD), and persistence (P) – that are independently heritable manifest early in life (Cloninger, 1987; Cloninger et al., 1993). HA is the tendency to respond intensely to signals of aversive stimuli, thereby learning to inhibit behavior to avoid punishment, novelty, and frustration. Individuals having high HA scores are characterized as "worrisome" or "pessimistic" and tend to feel anxious or depressed. NS implies the tendency toward intense excitement in response to novel stimuli or a potential reward, which leads to frequent exploratory activity in the pursuit of reward or avoidance of punishment. Individuals having high NS scores are characterized as "excitable" or "impulsive." RD implies the tendency toward signals of reward such as social approval and sentiment and the maintenance of behavior that has previously been associated with rewards or relief from punishment (e.g., warm, sentimental). P is the tendency toward maintaining a constant behavior (e.g., industrious, perfectionist).

Although the genetic basis of personality traits has been extensively investigated and some candidate genes have been reported (Savitz and Ramesar, 2004; Ebstein, 2006), the results are inconsistent across the studies. For example, in Japanese subjects, two studies reported that a polymorphism in the serotonin transporter gene regulatory region (5-HTTLPR) was associated with anxiety traits (Murakami et al., 1999) and the HA score (Katsuragi et al., 1999); however, three studies did not find a significant association between the polymorphism and personality (Nakamura et al., 1997; Kumakiri et al., 1999; Umekage et al., 2003). The limitations of using a self-rating questionnaire for investigating gene–personality relationships have been indicated (Ebstein, 2006), and the use of new experimental paradigms and measures is thought to facilitate better understanding of such relationships. For such a purpose, morphological information of the brain obtained using high-resolution magnetic resonance imaging (MRI) shows promise if the volume of a particular region or structure is associated with a personality trait.

Structural MRI studies that investigated the relationship between personality traits and brain structure using normal volunteers revealed a link between the cingulate gyrus and the HA (Pujol et al., 2002) or alexithymia (Gundel et al., 2004). The thickness of the medial orbitofrontal cortex correlated positively with the NEO-Five Factor Inventory extraversion scale (Rauch et al., 2005). A recent MRI study has revealed the relationship between personality and the gray matter (GM) concentration in the amygdala of healthy subjects (Omura et al., 2005). The authors (Omura et al., 2005) showed a positive correlation between extraversion and the GM concentration in the left amygdala and a negative correlation between neuroticism and the GM concentration in the right amygdala. In contrast, other authors (Wright et al., in press) showed that the amygdala volume did not correlate with either the extraversion or neuroticism scores. These results indicate that the variation in personality traits may be related to the morphology of the cortical and subcortical structures of the human brain.

On the other hand, several functional MRI (fMRI) studies have demonstrated that the blood oxygen level dependent (BOLD) signal, measured during tasks involving working memory (Kumari et al., 2004) and the judgment of humorous cartoons (Mobbs et al., 2005), correlated with the personality scale. In particular, the BOLD signal change associated with working memory load was greater in individuals with high extraversion scores than in those with low extraversion scores (Kumari et al., 2004). The degree of amygdala activation in response to humorous stimuli as compared to non-humorous stimuli correlated significantly with the introversion score (Mobbs et al., 2005). These findings suggest that the variation of personality traits might partially account for the variation in neural responses in a particular brain region.

The amygdala has been implicated in conditioned aversive learning (Ono et al., 1995) and emotional memory in humans (LaBar and Cabeza, 2006) and animals (McGaugh, 2004). Since HA is the tendency to respond intensely to signals of aversive stimuli, the responses in the amygdala of subjects with high HA scores would be greater than those in the amygdala of subjects with low HA scores. A recent functional neuroimaging study has found that the HA score has a significant positive correlation with signal change in the amygdala regions (Most et al., 2006). Therefore, it is possible to hypothesize that excessive neural activation in the amygdala in response to emotional experiences may be associated with functional and morphological changes within this region. In fact, several animal studies have shown that chronic immobilization stress induced dendrite elongation (Vyas et al., 2002) and spine formation (Mitra et al., 2005) in the basolateral nucleus of the amygdala.

A large community-based study in a clinical setting demonstrated that the HA score and the subjective scale of depressive symptoms correlated positively (Grucza et al., 2003). Patients with a major depressive disorder who were in remission had significantly higher HA scores than the control subjects; this indicated that this temperament and depression were closely related (Smith et al., 2005). The results of two neuroimaging studies have shown that patients with depression had enlarged amygdala volumes as compared to individuals in the control group (Frodl et al., 2003; Lange and Irle, 2004). Considering the results of these neuroscience and clinical studies, we predicted that a subject with a high HA score would have a larger amygdala volume than a subject with a low HA score.

It is hypothesized that NS is associated with the brain pathways controlling cognitive and attentional functions, reward sensitivity, and emotional expressions, and it involves corticolimbic circuitry including the prefrontal cortex and amygdala (Kelley et al., 2004). An fMRI study in normal subjects revealed that prefrontal activation during working memory tasks was greater in individuals who had high extraversion scores, a personality dimension similar to NS, than in those who had low extraversion scores (Kumari et al., 2004). Therefore, we predicted that the NS score would correlate positively with the GM volume in the prefrontal cortex.

Recent animal studies have suggested that neurons in the caudate nucleus play a role in linking reward expectation to behavioral responses (Gold, 2003). In human fMRI studies, cognitive tasks involving monetary rewards activated BOLD responses in the caudate nucleus (Delgado et al., 2003, 2004; Zink et al., 2004). In particular, neural activity in the dorsal striatum was parametrically modulated by the reward condition (Delgado et al., 2003). O'Doherty et al. found that activity in the dorsal striatum was involved in the modulation of response–reward associations (O'Doherty et al., 2004). Based on these findings, we predicted that the volume in the caudate nucleus, particularly in the dorsal part, would correlate positively with the RD score. Since an fMRI study in normal subjects indicated that the lateral orbital and medial prefrontal cortices may be linked to persistence (Gusnard et al., 2003), we hypothesized that these regions were associated with the P score.

In this study, we investigated the correlation between the temperamental predisposition of normal subjects, as measured by the Temperament and Character Inventory (TCI) (Cloninger et al., 1993; Kijima et al., 1996), and the GM volume on a voxel-by-voxel basis using high-resolution MRI and optimized voxel-based morphometry (VBM) analysis (Ashburner and Friston, 2000; Good et al., 2001a). In brief, VBM is an unbiased whole brain technique for characterizing regional cerebral volume and tissue concentration differences in structural MRIs (Ashburner and Friston, 2000). In this technique, a voxel-wise statistical assessment of images that have been normalized into the standard space is performed. An optimized method (Good et al., 2001a) includes registration of the image to a customized template and the application of tissue-specific normalization parameters. The modulation step was conducted on the extracted GM partitions prior to smoothing. The aim of the modulation step was to render the final VBM statistics reflective of the "volume" changes rather than the "concentration" change in GM (Ashburner and Friston, 2000). As mentioned in the previous section, we hypothesized a positive correlation between the HA score and amygdala volume, the NS score and prefrontal GM volume, the RD score and caudate nucleus volume, and the P score and orbitomedial prefrontal cortex. In each of these regions, multiple comparison corrections were performed using a custom-made template created from the mean anatomical images of all subjects.

Finally, it is well known that the rate and course of a psychiatric illness differ between the sexes (Seeman, 1997) and that, in the western (Seeman, 1997) and Japanese population (Kitamura, 1998), the prevalence of major depressive episodes is higher in women than in men. The VBM analysis showed that there are several brain regions wherein the GM morphology differs significantly between the sexes (e.g., amygdala; Good et al., 2001b). Therefore, we examined whether the correlation between the temperament scale and brain volume would differ between the sexes.

2. Results

2.1. *Harm avoidance (HA)*

The mean and SD for the depression scale and each of the four temperament subscales are listed separately for male and female groups in Table 1. These values did not significantly differ between the sexes. The BDI score correlated significantly with the HA score ($r=0.38$, $p<0.01$) but not with other personality scores. The VBM analysis revealed that a volume of the left amygdala subregion correlated significantly and positively with the HA score, as shown in Fig. 1A and Table 2. The cluster was located in the dorsal part of the amygdaloid complex, presumably in the basal nucleus, according to the anatomical atlas (Mai et al., 2004). The cluster survived a small volume correction (SVC) in the left amygdala (FWE correction, $p=0.01$). The same results were obtained when the HA score normalized to the Z-value was used as a variable. The BDI score did not show a significant correlation with a volume in the amygdala region at a lenient threshold level ($p=0.05$, uncorrected). Furthermore, the mean volume extracted from the region-of-interest (ROI) positioned at the peak amygdaloid voxel had a significant positive correlation with the HA score of the female group ($r=0.54$, $p<0.01$) but not with that of the male group ($r=0.02$, n.s.), as shown in Fig. 1B. There was a significant difference between the correlation coefficients of the groups ($z=2.09$, $p<0.05$). A multiple regression analysis using the SPSS software also showed that the dorsal amygdala volume in the female subjects correlated with the HA score even after the effects of age, BDI score, and total GM volume were removed ($R^2=0.59$, $F(4,21)=7.43$, $p=0.0007$; β for HA score$=0.55$, $p=0.006$). The volume of the left orbital gyrus had a positive correlation with the HA score; however, the result did not survive an SVC within the left prefrontal GM ($p>0.1$). The GM volume of several other regions in the right temporoparietal area correlated positively with the HA score (Table 2); however, these clusters did not survive multiple comparisons within the whole brain ($p>0.05$).

2.2. *Novelty seeking (NS), reward dependence (RD), and persistence (P)*

The GM volume in the left dorsolateral prefrontal cortex had a significant positive correlation with the NS score that

Table 1 – Mean for age, education years, depression score, and four temperamental subscales in each of the male and female group

	n	Age	Education	BDI	HA	NS	RD	P
Male	30	22.2 (3.2)	15.8 (2.1)	4.2 (4.3)	11.9 (4.6)	10.8 (3.1)	9.8 (2.1)	2.5 (1.3)
Female	26	22.4 (3.1)	16.1 (1.9)	5.5 (5.0)	11.4 (5.1)	10.0 (3.8)	10.4 (2.5)	2.6 (1.3)
p-value		0.79	0.56	0.31	0.71	0.38	0.32	0.73

SD in parenthesis; education, years; BDI, Beck depression inventory; HA, harm avoidance; NS, novelty seeking; RD, reward dependence; P, persistence; *p*-values of two-tailed *t*-test between the sexes are noted in bottom row.

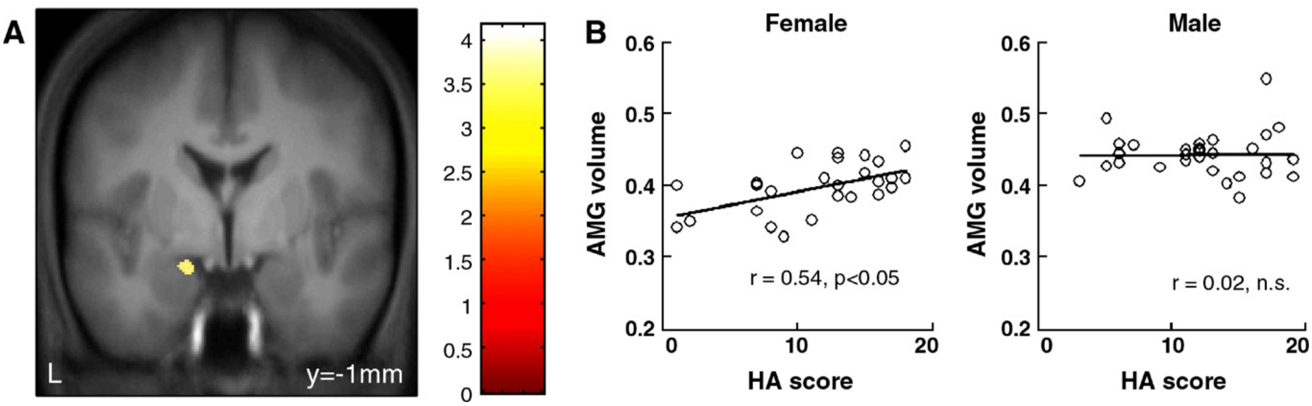

Fig. 1 – (A) A left amygdala cluster (x, y, z=−17, −1, −18, 136 voxels) that showed a significant positive correlation with the HA score is superimposed on the mean T1-weighted image of 56 subjects. (B) The correlation between the HA score (x-axis) and the mean amygdala volume (y-axis) is plotted separately for the female (left) and male (right) subject groups. The mean amygdala volume is extracted from the spherical ROI (r=12 mm) at the peak voxel. The regression line, Pearson's correlation coefficient, and the p-value are shown in the graph.

survived the multiple comparisons within the left prefrontal GM (Fig. 2). When the correlation coefficient between the GM volume extracted from the ROI and the NS score was computed separately for each sex group, the coefficient was significant for both male (r=0.40, p<0.05) and female (r=0.41, p<0.05) groups with no significant group difference (z=0.08, p=0.93). The areas that showed a significant positive correlation with the RD score were located in the tail of the right caudate nucleus, as shown in Fig. 3. The volume extracted from the ROI in the tail of the right caudate nucleus correlated positively with the RD scores of both the male (r=0.29, p>0.05) and female (r=0.47, p<0.05) groups with no significant group difference (z=0.74, p=0.45). The GM volume of several other regions in the right temporal lobe correlated positively with the RD score (Table 2); however, these clusters did not survive the multiple comparisons within the whole brain (p>0.05). There was no cluster that showed a significant positive correlation with the P score.

3. Discussion

In the present study, we investigated the relationship between the scores of the four temperament dimensions and the whole brain GM volume in normal volunteers, and we found a significant positive correlation between the amygdala volume and the HA score, the prefrontal volume and the NS score, and the caudate volume and RD score. These correlations were significant after taking into the account the effects of age, depression scale, and total GM volume. The results indicated that the variation of a temperamental trait was related to the structure of the brains of the normal young subjects. In particular, the results showed that the HA score could predict the volume of the subregion of the left amygdala in the female subjects but not in the male subjects; this suggested possible sex-related differences in the development of the brain structure and socio-psychological responses.

The amygdala is involved in the processing of biologically significant stimuli, conditioned fear learning (Ono et al., 1995), and emotional memory (LaBar and Cabeza, 2006; McGaugh, 2004). In humans, it is more likely that environmental stress affects the emotional condition of individuals with high HA than individuals with low HA since the HA score correlated positively with the subjective scale of depressive symptoms in the present study and in a large community-based study (Grucza et al., 2003). As mentioned earlier, animal experiments have shown that chronic immobilization stress induces structural changes such as dendrite elongation (Vyas et al., 2002) and an increase in spine density (Mitra et al., 2005) in the neurons of the basolateral nucleus of the amygdala. Therefore, we speculate that these morphological changes form the neuroanatomical basis of the present results, showing that subjects with high HA scores had larger volumes in the amygdala subregion than those with low HA scores.

In an fMRI study that involved normal subjects watching humorous cartoons (Mobbs et al., 2005), the left amygdala activity correlated positively with the introversion scale of the personality questionnaire (NEO). Higher neural activity that appears to be coupled with enhanced blood flow and metabolism might actually result in larger amygdala volumes. Although an MRI does not differentiate among the several nuclei within the amygdala, a significant cluster appeared to be located in the dorsal part of the basal nucleus (Mai et al., 2004). A neural model based on a physiological experiment in animals indicates that functional interaction between the prefrontal cortex and amygdala basal nucleus plays a role in fear conditioning and extinction (Sotres-Bayon et al., 2004). Thus, the present findings and those of previous studies suggest that a large amygdala volume is associated with a vulnerability to socio-psychological stress in normal subjects. However, which factor, i.e., morphological change or stress vulnerability, is an originating factor remains to be elucidated. These phenomena may be caused by genetic and neurodevelopmental interactions involving HA and exposure to stress during adolescence and early adulthood.

Although the present study used normal healthy volunteers, the results may have a clinical implication since

Table 2 – Brain regions that showed a significant positive correlation with each of the four temperamental factors in 56 subjects

Temperament	Region name	R/L	Size (voxels)	Z-value	Coordinate
HA	**Amygdala**[a]	L	136	3.31	−17, −1, −18
	Orbital gyrus	L	355	3.85	−12, 61, −24
	Middle temporal gyrus	R	122	3.51	74, −35, 6
	Angular gyrus	R	142	3.39	51, −58, 60
NS	**Middle frontal gyrus**[b]	L	525	4.21	−48, 41, 35
RD	**Caudate nucleus**[c]	R	224	3.72	27, −31, 11
	Inferior temporal gyrus	R	443	3.55	71, −30, −22
	Inferior temporal gyrus	R	727	3.55	52, −37, −20
	Superior temporal gyrus	R	182	3.45	47, 24, −32
P	No significant cluster				

R/L: right/left hemisphere. Region names in **bold** indicate clusters that survived SVC in [a]the left amygdala region (p=0.01), [b]the left prefrontal gray matter (p=0.04), and [c]the right caudate nucleus (p=0.008). Other clusters are thresholds at p=0.001, uncorrected for multiple comparisons.

several neuroimaging studies have investigated the relationship between depression and amygdala volume. These studies showed inconsistent results (Drevets, 2003); however, two studies have reported that patients with depression had enlarged amygdala volumes as compared to individuals in the control group (Frodl et al., 2003; Lange and Irle, 2004). The authors studied patients with recent-onset depression (Frodl et al., 2003) and first-episode depression (Lange and Irle, 2004). They suggested that the amygdala is enlarged in the early stage of the disease and that its size may decrease due to prolonged disease duration and/or treatment. One hypothesis is that the amygdala volume increased prior to the onset of disease in individuals who had stress vulnerability and were predisposed to depression. On the other hand, there is an argument that small amygdala volume is the primary cause of high HA rather than the result of socio-psychological stress because the amygdala volume is affected by a genetic polymorphism involving the serotonin system (Pezawas et al., 2005). The authors have found that second allele carriers of the 5-HTTLPR polymorphism showed reduced amygdala volume compared to l/l genotype subjects (Pezawas et al., 2005). The 5-HTTLPR polymorphism has been associated with HA (Katsuragi et al., 1999) and anxiety traits (Murakami et al., 1999) in the Japanese population. Therefore, under genetic influences, individuals with a small amygdala volume may tend to have a depressed or anxious personality.

The difference in the correlation between the sexes is intriguing since the mean HA and BDI scores did not significantly differ between the groups. It has been known that women are more prone than men to anxiety disorders, unipolar depression, and dysthymia (Seeman, 1997). With regard to major depressive episodes, in the Japanese population, women had a higher rate of the illness than men (Kitamura, 1998). In a study by Frodl et al. (2003) that showed larger amygdala volumes in patients with depression as compared with the control subjects, only female subjects were included. These results indicate that females may have biological mechanisms that lead to a vulnerability to stressful life events and an enlargement of amygdala volume. A functional MRI study has shown that, in women, there was a significantly stronger activation of the left amygdala during a memory task for emotional pictures than in men (Cahill et al., 2004). Normal female subjects had enhanced activation in the left amygdala in response to the administration of psychoactive drugs such as procaine

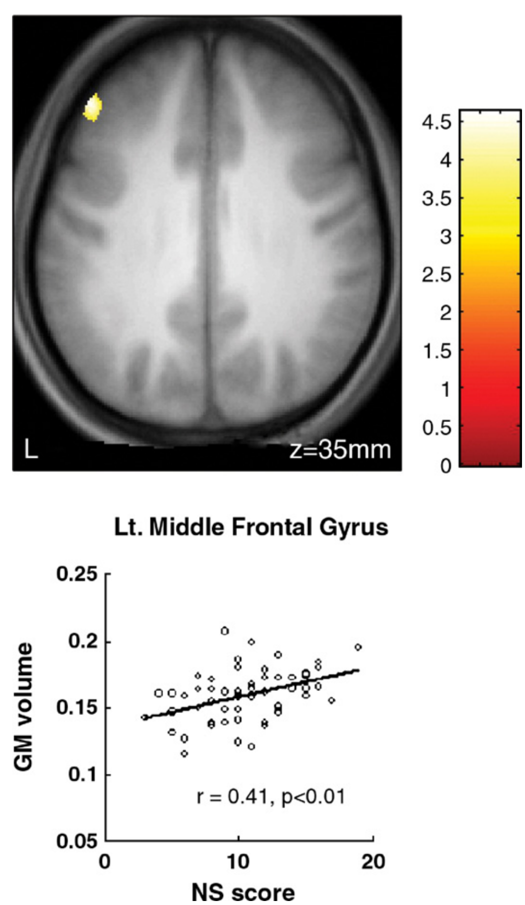

Fig. 2 – The GM volume in the left middle frontal gyrus (x, y, z=−48, 41, 35, 525 voxels) and the NS score had a positive correlation that did not differ between the sexes. The prefrontal cluster is superimposed on the mean T1-weighted image of 56 subjects. The correlation between the NS score (x-axis) and the mean prefrontal volume (y-axis) for all subjects is plotted. The regression line, Pearson's correlation coefficient, and the p-value are shown in the graph.

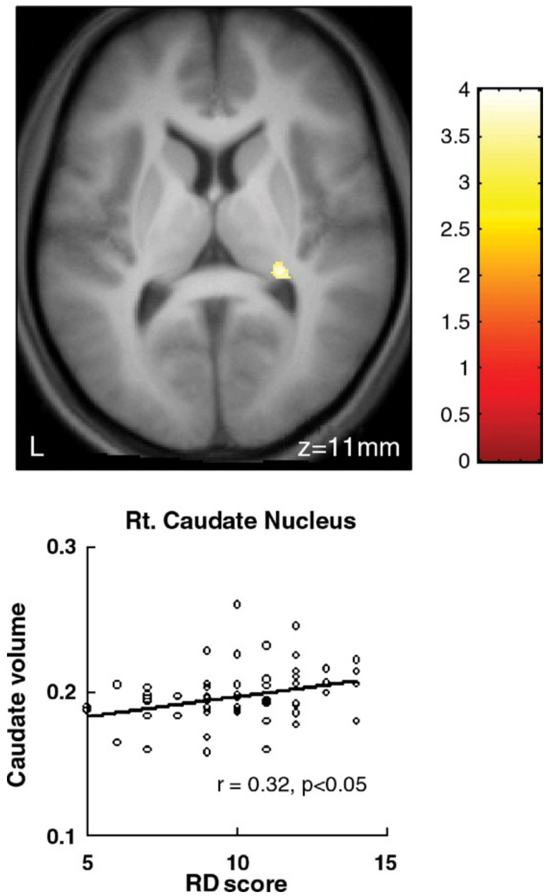

Fig. 3 – The volume of the tail of the right caudate nucleus (x, y, z=27, −31, 11, 224 voxels) and the RD score had a positive correlation that did not differ between the sexes. The cluster is superimposed on the mean T1-weighted image of 56 subjects. The correlation between the RD score (x-axis) and the mean caudate volume (y-axis) for all subjects is plotted. The regression line, Pearson's correlation coefficient, and the p-value are shown in the graph.

(Adinoff et al., 2003). These findings suggest that, as compared with male subjects, female subjects demonstrate excessive neural responses in the amygdala when they encounter aversive stimuli and that the excessive activity may lead to enlarged regional volumes.

Alternatively, in the female group, structural and/or functional changes of the amygdala may appear early in life. It is known that cortisol responses to psychological stress were lower in women than in men (Kudielka and Kirschbaum, 2005) and that the sex-related differences in the cortisol responses might be present at birth (Davis and Emory, 1995). Reduced activity in the hypothalamus–pituitary–adrenal axis during infancy and childhood may be associated with morphological changes in young female subjects. In addition, estrogen receptors are distributed in the limbic area, including the amygdala, and are thought to modulate human emotions via the serotonin system (Ostlund et al., 2003). These hormonal effects could be one of the causes for the sex-related differences observed in the present correlation analysis between HA and the amygdala volume.

It must be noted that a recent VBM study (Omura et al., 2005) reported an apparently opposite correlation between personality traits and amygdala GM concentration. The authors found a negative correlation between the neuroticism scores, which were similar to HA scores, and the right amygdala GM concentration. Several methodological differences may account for this discrepancy. First, the studies used different personality questionnaires in the experiments (the present study used TCI, while Omura et al. used NEO). Neuroticism is not equivalent to HA, but it is influenced by both HA and the character scale of self-directedness (Fruyt et al., 2000). Second, Omura et al. (2005) used unmodulated GM "concentration" for statistical analysis, but in the present study, modulated GM "volume" was used. Since the analyses of modulated data appear to be more sensitive to regionally specific structural changes than the analyses of unmodulated data (Good et al., 2001b), the present study might have a technical advantage. Third, we included the BDI score and total GM volume in the multiple regression analysis, but Omura et al. did not consider these confounding factors. Since the neuroticism score correlates positively with the depression score (Griens et al., 2002), it is critical to covariate the effects of the depressive state of the subjects from the analysis. A study by Wright et al. failed to show a significant correlation between a personality trait and whole amygdala volume (Wright et al., in press). As the authors noted in the Discussion section, whole amygdala volume measurements may obscure variations at the level of individual subnuclei that appear to have dissociable roles in emotional processing. A VBM method employed in the present study and in a study by Omura et al. (2005) was sensitive to detect volumetric changes in the subregions of the amygdala complex.

A positive correlation between the NS score and prefrontal GM volume, which showed no significant sex-related differences, corroborated our a priori hypothesis. NS is thought to be associated with the brain pathways controlling cognition, attention, and reward sensitivity and to involve the cortico-limbic circuitry, including the prefrontal cortex (Kelley et al., 2004). Prefrontal activation associated with working memory was greater in individuals having high extraversion scores than in those having low extraversion scores (Kumari et al., 2004). The frontal GM volume reduces gradually through adolescence and young adulthood, from a peak at approximately 11 to 12 years (Giedd, 2004). The findings are thought to be related to an increased myelination and declining synaptic density ("pruning"). Therefore, immature functioning of the prefrontal cortex caused by a high regional GM volume may lead to a high NS score and to risky behaviors typically seen in individuals in this age range (Kelley et al., 2004).

Animal studies have suggested that neurons in the caudate nucleus play a role in linking reward expectation to behavioral responses (Gold, 2003). In human fMRI studies, cognitive tasks involving monetary rewards activated BOLD responses in the caudate nucleus (Delgado et al., 2003, 2004; Zink et al., 2004). In particular, parametric modulation of the reward condition correlated with neural activity in the dorsal striatum (Delgado et al., 2003). In addition, the RD scores correlated positively with cerebral glucose metabolism which was measured by positron emission tomography (PET) in the caudate nucleus of normal subjects (Hakamata et al., 2005). These results support the present findings that showed that the RD score correlated positively with the volume of the dorsal part of the caudate nucleus. The reason why we could not detect a correlation between the RD score and the volume of ventral striatum is unclear. It may be attributable to the questionnaires included in the TCI because they were related to sentimentality, social attachment, and approval of others but not directly to biologically rewarding stimuli. In the present study, a significant cluster was located in the tail of the caudate nucleus. A study using lesioned monkeys (Teng et al., 2000) and one using fMRI and normal human subjects (Seger and Cincotta, 2005) suggested that the tail of the caudate nucleus plays a role in visual learning tasks. Rapid and successful learning of social rules may be related to RD because it implies the tendency toward signals of social approval.

In conclusion, we found that the temperamental traits HA, NS, and RD that were measured using TCI may be related to the morphology of the brains of normal young subjects. In particular, a positive correlation between the HA score and the volume of the dorsal amygdala might be clinically significant because a high HA score is a risk factor for depression. In addition, the correlation was significant for female subjects but not male subjects; this implies possible differences in hormonal conditions and vulnerability to socio-psychological stress that affects the development of the brain and personality. This sex-related difference could be one of the causes for the fact that women tend to suffer depression more than men. It must be noted that the present results were obtained using young subjects and cannot be generalized to include the population of middle- and old-aged subjects. Therefore, further research involving genetic, environmental, and neuropsychiatric experiments using subjects of a broad age range is required to fully elucidate the link between personality and brain structure.

4. Experimental procedures

4.1. Subjects

Fifty-six normal young subjects (Japanese, 30 males and 26 females; mean age±SD, 22.3±3.1 years) participated in the study after giving their written informed consent. These subjects were recruited from the Nagoya University community and mainly comprised undergraduate and postgraduate students. They were interviewed for the screening of their current medical status and excluded from the study if a neuropsychiatric or medical illness was revealed. The mean age and mean education years of the male and female subjects are shown in Table 1.

4.2. Temperament and Character Inventory Japanese version

The TCI developed by Cloninger et al. (1993) was translated into Japanese along with a back-translation to ensure validity (Kijima et al., 1996, 2000). The authors confirmed the reliability and validity of the Japanese version among different Japanese groups and showed that the factor structure of the inventory was consistent with Cloninger's original theory (Kijima et al., 2000). In a study using healthy Japanese subjects, the HA score correlated positively with both the state-trait anxiety inventory (STAI) (Jiang et al., 2003) and Self-rating Depression Scale scores (Tanaka et al., 1998). In addition, the TCI is commonly used to investigate the relationship between a genetic polymorphism and personality traits in the Japanese population (Nakamura et al., 1997; Kumakiri et al., 1999). In the present study, we used the 125-item version. The advantage of the TCI and Cloninger's model over the NEO-Personality-Inventory is that the former assumes an association between personality dimensions and the activity of neurotransmitter systems (Cloninger, 1987). For example, the model in which the HA is modulated by the serotonin system is partially confirmed by several studies that employed a drug challenge test (Ruegg et al., 1997; Hansenne and Ansseau, 1999). The subjects completed the Japanese versions of the Beck Depression Inventory (BDI) (Beck and Steer, 1993). The mean and standard deviation (SD) of the four temperament scores are listed separately for the male and female groups in Table 1.

4.3. Magnetic resonance imaging and data analysis

A whole brain T1-weighted image was acquired (MPRAGE; TR=2.5 s, TE=4.38 ms, flip angle=8°, 256×256 matrix and 192 slices, voxel size=0.75×0.75×1 mm) for each subject by using the 3 T MRI system (Siemens, Allegra). A VBM analysis was conducted according to an optimized method (Good et al., 2001a). The structural images were preprocessed using the SPM2 software (Wellcome Department of Imaging Neuroscience) running under Matlab 7.0 (MathWorks, MA). First, the original images were segmented to extract the GM by using tissue signal intensity values and a priori information about the distribution of brain tissue type. Image segmentation incorporated an intensity nonuniformity correction. Subsequently, the GM partition was normalized to a T1-weighted template in the stereotactic space of the Montreal Neurological Institute (MNI) to create a customized GM template from a data set of all 56 subjects. The deformation parameters obtained by the normalization process were applied to the original images in native space to create an optimally normalized whole brain image. These images were recursively segmented and optimal GM partitions were extracted. To preserve the amount of tissue in any given anatomical region after spatial normalization, the optimal GM partitions were multiplied by the Jacobian determinants of their respective spatial transformation matrices. The aim of this modulation step was to render the final VBM statistics reflective of the local deviation of the absolute amount of tissue in the surrounding regions of the brain (Ashburner and Friston, 2000). Finally, the modulated images were spatially smoothed using a 12-mm full width at half maximum (FWHM) isotropic Gaussian kernel. After smoothing, each voxel represented the local average amount of GM in the region; the size of which was defined by the smoothing kernel.

This technique enabled us to correlate the whole brain GM volume with several parameters obtained from each participant. In contrast to the method that relied on the manual drawing of ROIs, the VBM method is fully automatic and independent of the operator's experience and subjective bias. The TCI is a self-rating questionnaire, and the score of each temperament and character is computed according to a predefined formula. Therefore, combining these two methods precludes the possibility of the subjective bias of an experimenter affecting the results. For statistical assessment, we used the multiple regression module of the SPM2, using the GM image as a dependent variable and the TCI score, sex, age, BDI score, and total GM volume as independent variables. Therefore, four regression analyses were conducted for each HA, NS, RD, and P score. As noted in the Introduction section, we examined only the positive correlation between the temperament score and the GM volume. The regions that survived the statistical threshold set at $p = 0.001$ (uncorrected) for height and at $k = 100$ voxels for extent are listed in Table 2. Furthermore, when the cluster survived this threshold, an SVC was applied to correct for multiple comparisons (family-wise error (FEW) correction) at the left amygdala, prefrontal GM, and right caudate nucleus by using a custom-made template. The custom-made template was created using the mean normalized T1-weighted images of the 56 subjects by using the MRIcro software (Brett et al., 2002). The mean GM volumes in the left amygdala, left prefrontal cortex, and right caudate nucleus were extracted using a spherical ROI ($r = 12$ mm) at the peak voxels. A correlation analysis (Pearson's correlation coefficient) between the temperament score and the GM volume extracted from these regions was conducted separately for the male and female groups. Finally, we examined whether the slope of these correlations differed between the sexes by using Fisher's z transformation (threshold was set at $p = 0.05$). Significant clusters that survived SVC are shown in Fig. 1 (left amygdala), Fig. 2 (left prefrontal cortex), and Fig. 3 (tail of the right caudate nucleus) along with a plot of the regional volume and the temperament score.

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