Comprehensive neural networks for guilty feelings in young adults

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Abstract

Feelings of guilt are associated with widespread self and social cognitions, e.g., empathy, moral reasoning, and punishment. Neural correlates directly related to the degree of feelings of guilt have not been detected, probably due to the small numbers of subjects, whereas there are growing numbers of neuroimaging studies of feelings of guilt. We hypothesized that the neural networks for guilty feelings are widespread and include the insula, inferior parietal lobule (IPL), amygdala, subgenual cingulate cortex (SCC), and ventromedial prefrontal cortex (vmPFC), which are essential for cognitions of guilt. We investigated the association between regional gray matter density (rGMD) and feelings of guilt in 764 healthy young students (422 males, 342 females; 20.7 ± 1.8 years) using magnetic resonance imaging and the guilty feeling scale (GFS) for the younger generation which comprises interpersonal situation (IPS) and rule-breaking situation (RBS) scores. Both the IPS and RBS were negatively related to the rGMD in the right posterior insula (PI). The IPS scores were negatively correlated with rGMD in the left anterior insula (AI), right IPL, and vmPFC using small volume correction. A post hoc analysis performed on the significant clusters identified through these analyses revealed that rGMD activity in the right IPL showed a significant negative association with the empathy quotient. These findings at the whole-brain level are the widespread

comprehensive neural network regions for guilty feelings. Interestingly, the novel finding in this study is that the PI was implicated as a common region for feelings of guilt with interaction between the IPS and RBS. Additionally, the neural networks including the IPL were associated with empathy and with regions implicated in moral reasoning (AI and vmPFC), and punishment (AI).

Keywords:

Comprehensive neural networks; Empathy; Guilty feeling; Interpersonal situation; Posterior insula; Regional gray matter density (rGMD); Rule-breaking situation; Voxel-based morphometry (VBM).

Abbreviations

AI, anterior insula; CSF, cerebrospinal fluid; DARTEL, diffeomorphic anatomical registration through the exponentiated lie algebra; EQ, empathy quotient; GFS, guilty feeling scale; IPL, inferior parietal lobule; IPS, interpersonal situation; MNS, mirror neuron system; PI, posterior insula; RAPM, Raven's advanced progressive matrix; RBS, rule-breaking situation; rGMD, regional gray matter density; SCC, subgenual cingulate cortex; STS, superior temporal sulcus; TIV, total gray matter volume; vmPFC, ventromedial prefrontal cortex .

1. Introduction

Feelings of guilt are universal among human beings (Takahashi et al., 2004) and may mediate the relationship-enhancing effects of empathy to improve relationships (Leith and Baumeister, 1998). The ability to feel guilt is a superordinate function and generally interculturally important for living together (Ausubel, 1955). Guilty feelings are associated with the violation of moral norms. Psychologists contend that feelings of guilt and empathy assist individuals in establishing positive relationships with others (Lewis and Sullivan, 2005). Individuals with greater empathy are sensitive to feelings of guilt because empathy involves sharing another's emotional experience using the cognitive ability to take another person's perspective (Tangney et al., 2007).

We should focus on empathy because empathy is closely related to feeling guilty (Leith and Baumeister, 1998; Tangney et al., 2007). Guilt requires a feeling of attachment towards another person (Zahn et al., 2009a). Empathy is implemented by a complex network of neural regions including the insula and orbitofrontal cortices (OFC) (Decety, 2010). Empathy for others' pain, which is an important function for feeling guilt, is associated mainly with AI activity (Bernhardt and Singer, 2012; Engen and Singer,

2013).

Functional and structural neuroimaging studies of healthy subjects have investigated the neural correlates related to guilt. Functional neuroimaging studies have focused on imagining the event in which they felt the most guilt that they had ever experienced (Shin et al., 2000), intentionally or accidentally (Berthoz et al., 2006), gender difference (Michl et al., 2014), interpersonal (altruistic) guilt (Yu et al., 2013) or deontological guilt (Basile et al., 2011), compensation that might be stimulated by guilt (Yu et al., 2013), differences between guilty and embarrassment (Takahashi et al., 2004), compassion (Zahn et al., 2009a), pride (Zahn et al., 2009b), shame (Wagner et al., 2011) (Michl et al., 2014), and sadness (Wagner et al., 2011). In these functional studies, feelings of guilt were related to activation of the superior temporal/inferior parietal lobule (IPL) including the superior temporal sulcus (STS) (Takahashi et al., 2004; Michl et al., 2014), ventromedial prefrontal cortex (vmPFC) (Zahn et al., 2009b; Morey et al., 2012), insula (Shin et al., 2000; Yu et al., 2013; Michl et al., 2014), amygdala, and subgenual cingulate cortex (SCC) (Zahn et al., 2009a,b, 2013). A structural neuroimaging study dealing with healthy subjects linked proneness to guilt with individual variations in anterior brain regions (Zahn et al., 2013).

Clinical structure studies related to feelings of guilt have been conducted in patients

with antisocial disorder (Raine et al., 2000), psychopathy (de Oliveira-Souza et al., 2008), and conduct disorder (Wallace et al., 2014). Clinically, the OFC and vmPFC (Koenigs et al., 2007; Wallace et al., 2014) seem to play critical roles in feelings of guilt, as those regions are necessary for integrating outcome and belief information during moral reasoning (Ciaramelli et al., 2012) and affective processing (Wagner et al., 2011). Abnormalities in a specific amygdala–OFC limbic network might elucidate the neurobiological underpinning of psychopathy (Craig et al., 2009).

Our specific motivation in this study was to identify the widespread neural correlates of feelings of guilt at the whole-brain level with a statistically strong number of healthy subjects for the following reasons: the previous functional studies of guilt focused on some elements of feelings of guilt in small numbers (double figures) of healthy subjects. Further, the previous structural studies, apart from the one by Zahn et al. (2013), were clinical studies, in which the numbers of subjects were small and the identified regions were not directly related to feeling guilty. For example, the regions identified were found to relate to autonomic nerve activity (skin conductance and heart rate) when participants gave a videotaped speech under social stress on their lies. The prefrontal GM volumes of those with antisocial personality disorder were compared with healthy subjects (Raine et al., 2000). In other studies, the regions identified were related not to

the feeling guilt, but to the interpersonal/affective dimension of psychopathy (de Oliveira-Souza et al., 2008), and callous-unemotional traits (Wallace et al., 2014). To our knowledge, there has been only one brain-structure study of healthy subjects related directly to feelings of guilt (Zahn et al., 2013). However, that study found no specific significant relationship to the degree of feeling guilt and there were too few subjects ($N=64$) to demonstrate the neural correlates at the whole-brain level. As described in a previous report (Takeuchi et al., 2012), structural imaging studies are especially useful for investigating the anatomical correlates of personal characteristics that involve a wide range of behaviors or cognitions that occur outside the laboratory. Unlike fMRI studies, the results of structural imaging studies are not limited to the specific regions engaged in the task or responding to stimuli during scanning. Using MRI correlation studies (including studies with fMRI) to investigate the neural basis of individual differences, we established cognitive measures with proven reliability and validity to tap individual differences in cognition.

Considering the outcomes of activation and brain structural studies related to guilty feelings and the fact that empathy, moral reasoning, and punishment are essentially associated with guilty feelings (Ciaramelli et al., 2012), we hypothesized that the comprehensive neural networks for feeling guilty were widespread in regions related to

empathy, moral reasoning, and punishment, especially the insula, IPL, amygdala, SCC, and vmPFC. The purpose and novelty of this study were to elucidate the comprehensive neural networks for feelings of guilt by revealing the anatomical correlates of feeling guilt in gray matter structures in healthy young adults because no neural networks have been detected as regions significantly and directly related to the degree of feeling guilt in the whole brain, probably due to the small numbers of subjects. To assess guilty feelings in scenario-based approaches, we used the guilty feeling scale (GFS), which consists of interpersonal situation (IPS) and rule-breaking situation (RBS) scores (Ishikawa and Uchiyama, 2002). Using this classification of feelings of guilt is also a novelty of this study. To test our hypothesis, we investigated whether individual differences in IPS/RBS were associated with widespread regional gray matter densities (rGMD) using voxel-based morphometry (VBM) (Good et al., 2001) and whether these rGMD-correlated guilty feelings were associated with empathy and with rGMD of regions previously implicated in moral reasoning, and punishment. We controlled anger as a confounding variable because there is a relationship between feelings of guilt and anger (Zahn et al., 2009a; Teicher et al., 2010).

Furthermore, one brain study has investigated gender differences in guilt and demonstrated activation of the frontal cortex and amygdala in men only in the guilt

condition (Michl et al., 2014). Previous studies have shown that females experience more guilt than males (Else-Quest et al., 2012). Furthermore, Ishikawa and Uchiyama (2002) found that for male students, empathy was positively correlated with guilt feelings in the IPS, whereas empathy was positively correlated with guilt feelings in both the IPS and RBS for female students. Thus, we hypothesized the existence of sex-related differences in empathy in the RBS and rGMD. We tested this hypothesis.

2. Materials and Methods

2.1. Subjects

A total of 764 healthy, right-handed individuals (422 males and 342 females; mean age 20.7 ± 1.8 years) participated in this study as part of an ongoing project investigating associations among brain images, cognitive functions, aging, genetics, and daily habits (Takeuchi et al., 2011, 2012). Data derived from the subjects in this study will be used in other studies unrelated to the theme of this study. All subjects had normal vision and were university, college, or postgraduate students or had graduated from these institutions within 1 year before the experiment. None had a history of neurological or psychiatric illness. Handedness was evaluated using the Edinburgh Handedness Inventory (Oldfield, 1971). Written informed consent was obtained from each subject in

accordance with the Declaration of Helsinki (1991). This study was approved by the Ethics Committee of Tohoku University.

2.2. Psychological outcome measures

2.2.1. *Assessment of guilty feelings*

We used the reliable and valid GFS (Ishikawa and Uchiyama, 2002), which consists of the IPS (11 items) and RBS (10 items) tests (Ishikawa and Uchiyama, 2002).

Accordingly, the GFS consisted of 21 statements, which were divided into two dimensions. The possible scores for the IPS range from 11 to 44 and those for the RBS from 10 to 40. This scale was constructed using an open-ended questionnaire, and 315 guilt experiences were collected and categorized into 37 situations (Kohki). Exploratory factor analysis with promax rotation was performed on 444 subjects using 37 situations, and the two factors (IPS and RBS) were derived, excluding 16 situations. The internal consistency (Cronbach's coefficient of alpha) of IPS and RBS were 0.89 and 0.86 (Ishikawa and Uchiyama, 2002), respectively. The convergent validity for the scale was confirmed by significant correlations with other measures related to feelings of guilt. Significant relationships have been found between IPS and empathy, and between RBS and social perspective taking (Ishikawa and Uchiyama, 2002). We asked the participants

to describe their anticipated feelings in situations that elicit feelings of guilt. The translation from Japanese into English was completed with permission of Dr. Ishikawa and Dr. Uchiyama (Table 1). The four-point rating scale ranged from 1 (no feeling) to 4 (extreme feeling). For each factor, the total score represented an index of guilty feelings, with higher scores indicating greater feelings of guilt.

2.2.2. Psychometric measures of general intelligence

Raven's advanced progressive matrix (RAPM; Raven, 1998) was used to assess intelligence (Raven, 1998) and adjust for the effect of general intelligence on brain structures (Haier et al., 2004). Each item consisted of a 3×3 matrix with a missing piece to be completed by selecting the best of eight alternatives. The score on this test (number of correct answers in 30 min) was used as an index of the individual's intelligence.

2.2.3. Empathy quotient (EQ)

The Japanese versions of the EQ questionnaires (Baron-Cohen and Wheelwright, 2004; Wakabayashi et al., 2007) were administered. EQ score was used as an index of empathy, which is defined as the drive to identify the mental state of another individual and to respond with an appropriate emotion, with the aim of predicting and responding to the behavior of another person (Baron-Cohen et al., 2005). These tests consisted of

40 items, plus 20 filler items that were not scored. The scales consisted of self-descriptive statements scored using a four-point scale ranging from “strongly disagree” to “strongly agree” responses. Half of the items were worded to produce an “agree” response, and the rest were designed to elicit a “disagree” response. Items were randomized to avoid a response bias. Each strong empathizing response was awarded 2 points, and each slightly empathizing response was awarded 1 point (i.e., each item was scored as 2, 1, or 0), resulting in a total score ranging from 0 to 80.

2.2.4. State-Trait Anger Expression Inventory

The State-Trait Anger Expression Inventory (STAXI) is a self-report questionnaire that consists of 44 items (Forgays et al., 1997). Trait subscale of the STAXI had high internal consistency and test-retest reliability in an Asian population (Bishop and Quah, 1998).

2.3. Behavioral data analysis

Behavioral data were analyzed using IBM SPSS Statistics 22 software. Differences in scores on cognitive measure (RAPM, IPS/RBS, EQ, and trait subscale of the STAXI) and an additional covariate (age) were analyzed between the sexes using analysis of variance (ANOVA). We analyzed Pearson’s correlations among the IPS, RBS, EQ, and

trait subscale of the STAXI scores. Results with $P < 0.05$ were considered statistically significant.

2.4. Image acquisition and analysis

2.4.1. Image acquisition

MRI data acquisition was performed using a 3-T Philips Achieva scanner (Philips Medical Systems, Best, The Netherlands). High-resolution T1-weighted structural images (T1WIs: 240×240 matrix, TR = 6.5 ms, TE = 3 ms, FOV = 24 cm, slices = 162, slice thickness = 1.0 mm) were collected using a magnetization-prepared rapid gradient-echo sequence.

2.4.2. Preprocessing of T1-weighted structural data

Potential correlates of gray matter in VBM may include the number and size of neurons and glial cells, the level of synaptic bulk, and the number of neurites (May and Gaser, 2006), although this notion has yet to be confirmed by histological studies. Gray matter and structures are known to be associated with various cognitive abilities, and investigations of these associations have been used to identify the brain regions associated with specific cognitive abilities or characteristics (Haier et al., 2004).

Structural imaging provides unique and distinctive information about the neural origin

of individual cognitive characteristics.

Preprocessing of the structural data was performed using statistical parametric mapping software (SPM8; Wellcome Department of Cognitive Neurology, London, UK)

implemented in Matlab (Mathworks, Inc., Natick, MA, USA). Using the new

segmentation algorithm implemented in SPM8, T1-weighted structural images of each

individual were segmented into six tissues. In this process, the gray matter tissue

probability map (TPM) was manipulated from maps implemented using the software so

that the voxel signal intensities (the gray matter tissue probability of the default tissue

gray matter TPM + white matter tissue probability of the default TPM) > 0.25 were set at

0. Using this manipulated gray matter TPM, the dura matter was less likely to be

classified as gray matter (compared with using the default gray matter TPM) without

other substantial segmentation problems. In this new segmentation process, default

parameters were used, with the exception that affine regularization was performed using

the International Consortium for Brain Mapping template for East Asian brains. We then

used the diffeomorphic anatomical registration through the exponentiated lie algebra

(DARTEL) registration process implemented in SPM8. In this process, we used

DARTEL-imported images of the six gray matter TPMs from the abovementioned new

segmentation process. First, the template for the DARTEL procedures was created using

imaging data from 63 subjects who participated in an experiment in our laboratory (Takeuchi et al., 2011). Next, using this existing template, the DARTEL procedures were performed for all subjects in the present study. In these procedures, default parameter settings were used. The resulting images were spatially normalized to the Montreal Neurological Institute (MNI) space to produce images with 1.5 × 1.5 × 1.5-mm³ voxels. Subsequently, all images were smoothed by convolving them with an isotropic Gaussian kernel of 12-mm full-width at half-maximum (FWHM).

2.4.3. Statistical analyses

We investigated whether rGMD was associated with individual differences in the IPS/RBS scores. Statistical analyses of the morphological data were performed using VBM5 software, an extension of SPM5.

In the analyses, we included only voxels that showed rGMD values >0.05 in all subjects.

The primary purpose for using gray matter thresholds was to cut the periphery of the gray matter areas and to effectively limit the areas for analysis. We performed this procedure by limiting the areas for analysis to those likely to be gray matter. The voxels outside the brain areas were more likely to be affected by signals outside the brain through smoothing. Masking the analysis to brain areas was performed in fMRI analyses using SPM5 by default.

We performed separate multiple regression analyses for IPS and RBS, as the two subscores may have been partly of the same fundamental nature, and correcting one subscore with the other in multiple regression analysis may have been inappropriate. The analyses were performed with sex, age, RAPM score, and total intracranial volume (TIV; total gray matter volume + total white matter volume + total CSF; cerebrospinal fluid volume) as additional covariates, resulting in five covariates in total. When the total brain volume was included as a covariate in the analyses of density measurements, the analyses assessed the density of tissues that could not be explained by the total brain volume.

Next, we investigated whether the relationships between rGMD and IPS/RBS scores differed between males and females (i.e., whether the interaction between sex and IPS/RBS scores affected rGMD). In the two whole-brain analyses, we used voxel-wise analyses of covariance (ANCOVA) in which sex difference was a group factor (using the full factorial option of SPM5). Age, RAPM score, IPS score, and TIV were covariates in one analysis. In another analysis, age, RAPM score, RBS score, and TIV were covariates. All of these covariates, except TIV, were modeled so that the unique relationship of each covariate with rGMD could be seen in each sex (using the interactions option in SPM5), which would allow investigation of the interaction effects

of sex and the covariates. The TIV was modeled so that this covariate had a common relationship with rGMD between the sexes. The interaction effect between sex and IPS/RBS scores on rGMD was assessed using t -contrasts. The statistical significance level was set at $P < 0.05$ and corrected at the non-isotropic adjusted cluster level (Hayasaka et al., 2004) with an underlying voxel level of $P < 0.0025$. In this non-isotropic cluster-size test of random field theory, a relatively higher cluster-determining threshold combined with high smoothing values of more than 6 voxels led to appropriate conservativeness in real data. With high smoothing values, an uncorrected threshold of $P < 0.01$ seemed to lead to anticonservativeness, whereas that of $P < 0.001$ seemed to lead to slight conservativeness (Silver et al., 2010). We used the VBM5/SPM5 version of this test. A previous validation study of this test using a real dataset (Silver et al., 2010) showed that the conditions of this cluster-size test were limited and were dependent on the smoothness of the data, as described above. However, SPM8 and SPM5 estimated that the actual FWHM differed substantially in the areas analyzed, which can directly affect the cluster test threshold. Therefore, regardless of the version (SPM5 or SPM8), our view is that the conditions shown in the previous study (Silver et al., 2010) were no longer guaranteed in SPM8 because different analyses were performed with different return results.

For areas with a strong *a priori* hypothesis, namely the bilateral insula, IPL, amygdala, SCC, and vmPFC, the statistical significance level was set at $P < 0.05$ with small volume correction (SVC) in regions of interests (ROIs), corrected at false discovery rate (FDR). All ROIs were constructed using the WFU PickAtlas Tool (<http://www.fmri.wfubmc.edu/cms/software#PickAtlas>) (Maldjian et al., 2004; Maldjian et al., 2003) and were based on the AAL option of the PickAtlas. First, we combined ‘Insula_L,’ ‘Insula_R,’ ‘Parietal_Inf_L,’ ‘Parietal_Inf_R,’ ‘Amygdala_L,’ ‘Amygdala_R,’ ‘Cingulate_Front_L,’ ‘Cingulate_Front_R,’ ‘Frontal_Sup_Medial_L,’ ‘Frontal_Sup_Medial_R,’ ‘Frontal_Med_Orb_L,’ and ‘Frontal_Med_Orb_R’ and generated the ROI. We used the ROI with the SVC.

2.4.4. Associations between rGMD and psychological scores on the EQ

The psychological data were analyzed using the IBM SPSS Statistics 22. Results with $P < 0.05$ (uncorrected) were considered statistically significant in these analyses. The post hoc analyses using the mean rGMD value within the significant clusters identified through abovementioned analyses and associated psychological variables were also performed using this software.

3. Results

3.1. Behavioral data

Fig. 1 shows the distribution of IPS (a) and RBS (b) scores, and Table 2 shows the age, RAPM, IPS, RBS, EQ, and trait subscale of the STAXI scores in male and female study participants. ANOVA revealed that the RAPM was significantly higher in males than in females ($P < 0.05$), whereas the IPS, RBS, and EQ scores were significantly higher in females than in males ($P < 0.001$). Table 3 shows the correlations among the IPS, RBS, EQ and trait subscale of the STAXI scores. The IPS and RBS scores showed significant positive correlations with the EQ scores ($P < 0.001$).

3.2. MRI data

3.2.1. *Correlation between rGMD and IPS/RBS scores in all subjects*

Multiple regression analysis was performed with rGMD as the dependent variable and age, sex, RAPM, and IPS score as the independent variables. We identified significant negative correlations between the IPS score and rGMD in the right posterior insula (PI) (Fig. 2 a1, b1; Table 4), the left AI (Fig. 2 a2, b2; Table 4), and the right IPL (Fig. 2 a3, b3; Table 4). Multiple regression analysis performed with rGMD as the dependent variable and age, sex, RAPM, and RBS as the independent variables revealed a significant negative correlation of RBS scores with rGMD in the right cluster that

mainly spread in the PI (Fig. 2 a4, b4; Table 4). Since the right PI was correlated with both IPS and RBS scores, we checked whether there was statistical overlap between the regions. We performed multiple regression analysis for IPS with sex, age, RAPM score, and TIV as covariates at $P < 0.05$ with SVC in the right PI (the cluster formed by the whole-brain analysis related to the RBS scores), corrected at the non-isotropic adjusted cluster level with an underlying voxel level of $P < 0.0025$. The right PI overlapped significantly (peak voxel of MNI: $x = 39$, $y = -6$, $z = -8$, t score 3.85, corrected p value with SVC [cluster] 0.001, cluster size 473 voxels). A conjunction analysis was also performed for IPS and RBS with sex, age, RAPM score, and TIV as covariates at $P < 0.05$ with SVC in the right PI (the 'Insula_R' formed by AAL option of the PickAtlas), corrected at the non-isotropic adjusted cluster level with an underlying voxel level of $P < 0.0025$ because the conjunction analysis is the most statistically robust procedure to look for commonalities and differences between different data sets due to without interactional effect (Price and Friston, 1997). However, there was no significant correlations between rGMD and the conjunction of IPS and RBS scores.

In these analyses, we found no significant positive correlations between rGMD and the IPS/RBS scores.

In areas with *a priori* hypothesis, ROI analyses were performed, and SVC was applied.

After correcting for the effects of age, sex, RAPM, and TIV, this analysis revealed a significant negative correlation of IPS score with rGMD in the vmPFC (Fig. 3a, b) at $P < 0.05$, corrected for false discovery rate (FDR) at the voxel level within the ROI for areas with *a priori* hypothesis. However, the analysis failed to reveal a significant correlation of IPS and RBS scores with rGMD in the amygdala and SCC.

Furthermore, because there seems to be clear outliers (people scoring less than 20) in IPS scores, some might question whether these affected the slope of the regression lines.

We repeated the analyses with the 753 subjects after excluding the 11 subjects scoring less than 20 as clear outliers in the IPS scores. There was a significant tendency between the right IPL and IPS scores with SVC in the ROIs (FDR) (peak voxel of MNI: $x = 35$, $y = -39$, $z = 49$, t score 3.95, corrected p -value with SVC [cluster] 0.091). No other significant results were observed between the IPS scores and rGMD in this analysis.

However, there were subjects with very low feelings of guilt in the IPS who diverged from normal distribution. Although no other significant results were observed between the scores of the IPS and rGMD in this analysis, the t scores did not change greatly (peak voxel of MNI: $x = 38$, $y = -9$, $z = -11$, t score 2.95, corrected p value with SVC 0.221; peak voxel of MNI: $x = -27$, $y = 18$, $z = 9$, t score 3.42, corrected p -value with SVC 0.117; peak voxel of MNI: $x = 26$, $y = 59$, $z = -2$, t score 2.84, corrected p -value

with SVC 0.252). Moreover, we performed the same analyses with the 753 subjects, using the significant regions with an underlying voxel level of $P < 0.0025$ with the 753 subjects without outliers as a ROI. Then, we identified significant negative correlations between the IPS score and rGMD in the right PI (peak voxel of MNI: $x = 38, y = -13, z = -12$, t score 3.73, corrected p -value with SVC 0.002), left AI (peak voxel of MNI: $x = -27, y = 17, z = 10$, t score 3.61, corrected p -value with SVC 0.002), right IPL (peak voxel of MNI: $x = 35, y = -39, z = 49$, t score 3.95, corrected p -value with SVC 0.002), and left vmPFC (peak voxel of MNI: $x = 26, y = 59, z = 4$, t score 3.74, corrected p -value with SVC 0.002) in the ROI (FDR).

3.2.2. Interaction effects of sex and IPS/RBS on rGMD

Using data from both sexes with respect to the covariates of age, RAPM, TIV, and IPS score, ANCOVA revealed no significant effect of the interaction between IPS scores and sex on rGMD. Similarly, ANCOVA revealed no interaction effect between RBS score and sex on rGMD. Moreover, the ANCOVA revealed no interaction between the trait subscale of the STAXI score and sex on the rGMD.

3.2.3. Post hoc analyses of the associations between rGMD of the identified significant clusters and psychological correlates of the EQ score

After correcting for the effects of age, sex, RAPM, and TIV, multiple regression analyses revealed a significant negative relation of the EQ score with the mean rGMD

of the abovementioned significant cluster in the right IPL based on *a priori* hypothesis ($t = -2.08, P = 0.038, \beta = -0.078$) (Fig.4).

3.2.4. Post hoc analyses of the associations between rGMD of the identified significant clusters and psychological correlates of the trait subscale of the STAXI score

After correcting for the effects of age, sex, RAPM, and TIV, multiple regression analyses revealed no significant relationship between trait subscale of the STAXI score and the mean rGMD of the abovementioned significant cluster in the right PI (MNI: 39 -6 -8, $t = -0.404, P = 0.687, \beta = -0.015$; MNI: 41 0 -8, $t = -1.136, P = 0.2567, \beta = -0.041$), left AI ($t = -0.173, P = 0.862, \beta = -0.006$), right IPL ($t = 0.258, P = 0.796, \beta = 0.009$), and vmPFC, ($t = 1.263, P = 0.207, \beta = 0.045$).

4. Discussion

The novel finding in this study is that it identified the PI as an important region underpinning feelings of guilt using brain structures at the whole-brain level in a statistically powerful study with a large number of subjects, while previous research highlighted the role of the AI, not PI (Shin et al., 2000; Yu et al., 2013; Michl et al.,

2014).

The result that the IPS and RBS scores measuring feelings of guilt were related to clusters located mainly in the right PI, suggesting that this area is the common neurological correlate of feeling guilty with interaction between the IPS and RBS, affords new perspectives on the neural correlates of feelings of guilt. We should discuss the overlapping psychological features of the IBS and RBS, which might have led to this overlapping localization in the PI. The two features of feeling guilty are the same point that induce emotional distress over the deviation from norms and lead to urge-compensation behavior (Ohnishi, 2008). Regarding the IBS, guilt serves various relationship-enhancing functions, including motivating people to treat partners well, minimizing inequities, enabling less powerful partners to get their way, and redistributing emotional distress (compensation behavior) (Baumeister et al., 1994). Regarding the RBS, guilt leads to psychopathology such as depression, because guilt is accompanied by angry at ourselves, loneliness from surrounding people, and self-negative emotion about deviation from norms (Freud, 1917). We believe that the PI might have functions related to distressing emotions that urge a certain behavior because the insula is involved in some aspects of emotions. This region is active in response to hatred (Zeki and Romaya, 2008), romantic love (Bartels and Zeki, 2004),

disgust (Phillips et al., 1997) and anger (Schultheiss et al., 2008; Paulus et al., 2010).

These emotions, including romantic love, are all distressing and such emotions strongly motivate one to take certain action (Zeki and Romaya, 2008). Interestingly, the recognition and experience of disgust is lost with damage to the PI (Phillips et al., 1997). Moreover, greater activation of the PI was related to a perception of fairness associated with equal allocation of resources (Wright et al., 2011). And individuals who perceived their group as relatively advantaged felt guilty, as they perceived this inequality as unfair in terms of social norms (Leach et al., 2006). Accordingly, the PI plays a critical role in feelings of guilt.

The IPS was associated with the left AI, and right IPL, and mPFC (located in vmPFC), which are involved in social and emotional cognition. The IPS scale might be more sensitive to subjects in this study because feelings of guilt were most intense in the IPS among college students and in the RBS among junior high school students (Ishikawa and Uchiyama, 2002). Furthermore, it is natural that there is a much stronger relation between empathy and the IPS, which directly includes an inter-personal aspect, than between empathy and the RBS, which is indirectly related to empathy thorough perspective taking. In fact, stronger significant relationships have been found between IPS and EQ than between RBS and EQ (Table 3). Moreover, there was a significant ($P <$

0.05, uncorrected) negative relation between EQ and IPL. These might be reasons why IPS scores, and not RBS scores, were associated with rGMD reductions within the AI, vmPFC, and IPL. Regarding negative emotions, the AI is associated with empathy for pain (Lamm et al., 2011) and plays a pivotal emotional role during pain and disgust in the self and others (Frith and Frith, 2007). The AI plays a crucial role in the emergence of social emotions related to others (Singer, 2007) and especially mediates negative emotional states including feelings of guilt (Shin et al., 2000). In support of the relationship between the AI and feelings of guilt, increased regional cerebral blood flow in the anterior para-limbic regions including the AI was observed during feelings of guilt (Shin et al., 2000). Additionally, unfair punishment was associated with activation in the AI (White et al., 2014), and activation in the insula was elicited by guilt-inducing stimuli in an fMRI study (Michl et al., 2014). By contrast, the IPL plays an integral role in social processing, especially during feelings of empathy (Janowski et al., 2013); additionally, the mirror neuron system (MNS), which functions in understanding the intentions of others and may be a neural substrate of empathy, is associated with activity in the IPL (Cattaneo and Rizzolatti, 2009). In particular, the right IPL is believed to play an important role in ascribing intention to others (Fogassi et al., 2005). Thus, the IPL has a critical role in empathy using this function of the MNS. Empathy is essential for

feelings of guilt (Leith and Baumeister, 1998); hence, the IPL is related to such feelings.

Consistent with this notion, the rGMD of the IPL was negatively correlated with EQ scores. Furthermore, the vmPFC has been implicated in higher social brain regions in the regulation of moral behavior (Moll and de Oliveira-Souza, 2007). Somatic marker circuitry (SMC), which posits that emotion-based biasing signals arise from the body (somatic markers), is integrated in higher brain regions (the vmPFC, in particular) (Damasio, 1996). These decision may be related to feelings of guilt, as guilt is a core emotion governing decisions in personal and social realms by promoting compliance with social norms and self-imposed standards (Morey et al., 2012). Accordingly, based on the functions of these regions, the AI, and IPL, and vmPFC are presumed to be the main neural correlates of guilty feelings in IPS.

Our VBM findings that rGMD was negatively related to IPS and RBS in healthy subjects are incongruent with the assumption that a larger gray matter region is associated with greater efficacy (Kanai and Rees, 2011). Previous clinical VBM studies reported a negative association of emotional and social competence with rGMD in the mPFC in the nodes of the social cognition network or somatic marker circuitry among patients with schizophrenia (Yamada et al., 2007). The incongruence between clinical VBM studies and our study described herein may be due to the following mechanisms.

Among certain clinical samples, neuronal degeneration and neuron loss lead to reduced social or emotional competency and reduced gray matter signaling in VBM (Seeley, 2008). Furthermore, potential correlates of the amount of rGMD may include the number and size of neurons and glia and the level of synaptic bulk; neuritis (Draganski et al., 2004; May and Gaser, 2006) and VBM cannot differentiate between these possibilities. In neurodegeneration, it is widely assumed that less gray matter in VBM corresponds to neural loss (Baron et al., 2001; Thieben et al., 2002). On the other hand, our VBM finding is congruent with the cases in which lower gray matter was associated with better task performance (Kanai and Rees, 2011). Among healthy young adults, adaptive development supported by increased synaptic pruning may lead to heightened feelings of guilt and reduced rGMD signaling (Kanai and Rees, 2011).

We should discuss controlling for possible confounding variables such as empathy, social emotion, agency, and valence. We controlled for anger as a confounding variable. However, there was no significant relationship between them. Empathy and feelings of guilt are related because empathy is likely to play an important role in the guilt-processing underlying moral cognition and social emotion-processing, and because guilt motivates other-oriented empathy (Jankowski and Takahashi, 2014).

This study had several limitations. First, as with our previous studies using college

student cohorts (Song et al., 2008; Jung et al., 2010; Takeuchi et al., 2011, 2012), we used young healthy subjects with a high educational background. Limited sampling of the full range of intellectual abilities is a common hazard when sampling from college cohorts (Jung et al., 2010). This limited sampling diminishes our ability to rule out the effects of age or educational level that could strongly impact the brain structures and affect the sensitivity of the analyses. Nevertheless, IPS/RBS scores are associated with education level as well as age (across a wide age range). This selection bias may explain our failure to find a significant association between IPS/RBS scores and amygdala and SCC activity, which was against our *a priori* hypothesis, because the magnitude of amygdala-SCC interaction was a strong predictor of variations in temperamental anxiety and risk of depression (Pezawas et al., 2005), the subjects with anxiety and depression could not participate in the experiment. Regarding the threshold of the statistical analyses, we used an uncorrected $P < 0.05$ based on a strong *a priori* hypothesis regarding the behavioral results. Furthermore, because there was a significant tendency between only the right IPL and the IPS scores at $P < 0.05$ with SVC in ROI, corrected at FDR without outliers subjects ($N = 11$), the association among insula, vmPFC and IPS might be specific to subclinical people with low feelings of guilt in the IPS. However, using the result without outliers is controversial because ~~the~~

removal of outliers to acquire a significant result is usually a questionable research practice in psychology (Bakker and Wicherts, 2014a). In addition, one issue with outliers in psychological data might be that removing outliers increases the Type I error rate (Bakker and Wicherts, 2014b).

5. Conclusion

A novel finding of this study is that the PI was implicated as a common important region for feelings of guilt with interaction between the IPS and RBS based on the direct association with the degree of the feeling guilty. The PI seems to be related to emotional distress over the deviation from norms and leads to urge-compensation behavior. The neural networks for feelings of guilt include the broad regions associated with empathy (IPL) and regions previously implicated in moral reasoning (AI), and punishment (AI). Elucidating the comprehensive neural networks for feelings of guilt at the whole-brain level might provide clues for improving human relationships.

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Figure legends

Fig. 1a. Distribution of interpersonal situation (IPS) scores in males and females.

1b. Distribution of rule-breaking situation (RBS) scores in males and females.

Histograms show the distributions of IPS/RBS scores in males and females.

Fig. 2. Regions showing a correlation between regional grey matter density (rGMD) and IPS/RBS scores.

The red-to-yellow color scale indicates the t -score for the negative correlation between rGMD and IPS/RBS scores ($P < 0.0025$, uncorrected; $k > 100$ for visualization purposes). Regions showing correlations were overlaid on a single T1 image in the SPM5 toolbox. Areas of significant correlations are shown in the right posterior insula (PI) (a1), the left anterior insula (AI) (a2), and right inferior parietal lobule (IPL) (a3). A scatterplot of the IPS scores and mean rGMD values in the significant clusters in the right PI (b1), left AI (b2), and right IPL (b3) are shown. Regions of significant correlations in the right PI are shown (a4).

A scatterplot of the RBS scores and mean rGMD values in the significant clusters in the right PI is shown (b4).

Fig. 3. Regions showing a correlation between rGMD and IPS scores over *a priori* regions of interest.

The red-to-yellow color scale indicates the t -score for the negative correlation between rGMD and IPS score ($P < 0.0025$, uncorrected for visualization purposes). Regions showing correlations were overlaid on a single T1 image in the SPM5 toolbox. Regions of significant correlations are shown in the left ventromedial prefrontal cortex (vmPFC) (a). A scatterplot of the IPS scores and mean rGMD values in the significant clusters in the left vmPFC is shown (b).

Fig.4. A correlation between rGMD in the IPL and EQ scores.

A scatterplot of the EQ scores and mean rGMD values in the significant clusters in the right IPL is shown.

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Table 1

Guilty Feelings Scale for the younger generation

Interpersonal situation (IPS)

- 1 I deceived my friend.
- 2 I hurt my friend.
- 3 I uncovered my friend's secret.
- 4 I broke a promise.
- 5 I told a lie.
- 6 I was hard on my friend.
- 7 I said bad things about my friend.
- 8 I caused problems for my colleagues because of my actions.
- 9 I ignored my friend.
- 10 I stole something.
- 11 I broke something belonging to my friend.

Rule-breaking situation (RBS)

- 1 I did something in my class that had nothing to do with the lesson.
- 2 I broke the rules and rode a bicycle with two people on it.
- 3 I crossed the road when the traffic signal was red.
- 4 I broke one of the school rules.
- 5 I crossed railway tracks at a crossing when the signal was ringing.
- 6 I did not pay the full bus or train fare.
- 7 I made noise while on the train.
- 8 I defied my parents.
- 9 I played until midnight without my parents' permission.
- 10 I threw trash on the ground, not in a garbage can.

Table 2**Differences in age, RAPM, IPS, RBS, EQ, and Trait STAXI scores, and ANOVA results in males and females.**

	Total		Males (<i>N</i> = 422)		Females (<i>N</i> = 342)		<i>P</i>	<i>F</i>
Measure	Mean	SD	Mean	SD	Mean	SD		
Age	20.7	1.8	20.8	2.0	20.6	1.7	0.093	2.8
RAPM	28.7	3.7	29.0	3.8	28.3	3.7	0.015 [*]	5.9
IPS	34.0	5.3	32.6	5.2	35.7	5.0	< 0.001 ^{**}	67.5
RBS	20.9	4.9	19.9	4.6	22.2	4.9	< 0.001 ^{**}	44.8
EQ	31.1	10.0	28.9	9.6	33.8	9.9	< 0.001 ^{**}	46.7
Trait STAXI	19.9	5.6	19.7	5.4	20.1	5.9	0.276	1.2

^{*}*P* < 0.05, ^{**}*P* < 0.001.

Abbreviations: ANOVA, analysis of variance; EQ, empathising quotient; IPS, interpersonal situation; RAPM, Raven's Advanced

Progressive Matrix; RBS, rule-breaking situation; SD, standard deviation, Trait STAXI, trait subscale of the state-trait anger expression inventory.

Table 3

Pearson's correlations among the IPS, RBS, EQ, and Trait STAXI scores.

	IPS	RBS	EQ	Trait STAXI
IPS	—			
RBS	0.476 [*]	—		
EQ	0.329 [*]	0.169 [*]	—	
Trait STAXI	-0.142 [*]	-0.105 [*]	-0.145 [*]	—

^{*} $P < 0.001$

Abbreviations: EQ, Empathising quotient; IPS, interpersonal situation; RBS, rule-breaking situation, Trait STAXI, trait subscale of the state-trait anger expression inventory.

Table 4

Brain regions with significant correlations between regional gray matter density (rGMD) and IPS/RBS scores.

Brain region	R/L	x	y	z	t score	Corrected	Cluster size	Corrected
						<i>P value</i>	(k _E)	<i>P value</i> with
						(cluster)		SVC
						(FWE)		(FDR)
Negative correlation between rGMD and the IPS score								
PI	R	39	-6	-8	3.85	0.001***	1003	0.018*
PI		38	-13	-12	3.75			
PI		35	9	13	3.54			
AI	L	-27	17	10	3.85	0.001**	808	0.023*
IPL	R	33	-39	48	4.54	0.044**	956	0.010*
vmPFC	L	21	56	-2	3.35	0.305	349	0.036*
Negative correlation between rGMD and the RBS score								
PI	R	41	0	-8	3.83	< 0.001***	2022	0.073
STG		60	-15	1	3.82			

* $P < 0.05$ corrected for multiple comparisons at the cluster level for areas with strong *a priori* hypothesis.

** $P < 0.05$ corrected for multiple comparisons at the cluster level.

*** $P < 0.01$ corrected for multiple comparisons at the cluster level.

No regions showed significant positive correlations between rGMD and IPS/RBS scores.

Abbreviations: AI, anterior insula; EQ, Empathising quotient; FDR, false discovery rate; FWE, family wise error; IPL, inferior parietal lobule; IPS, interpersonal situation; L, left; mPFC, medial prefrontal cortex; PI, posterior insula; R, right; RBS, rule-breaking situation; rGMD, regional gray matter density; SVC, small volume correction.

Figure1

Figure2

Figure3

Figure4