Regional gray matter volume and anxiety-related traits interact to predict somatic complaints in a non-clinical sample

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Somatic complaints can be important features of an individual’s expression of anxiety. Anxiety-related traits are also risk factors for somatic symptoms. However, it is not known which neuroanatomical mechanisms may be responsible for this relationship. In this study, our first step was to use voxel-based morphometry (VBM) approaches to investigate the neuroanatomical basis underlying somatic complaints in a large sample of healthy subjects. We found a significant positive correlation between somatic complaints and parahippocampal gyrus (PHG) volume adjacent to the entorhinal cortex. Further analysis revealed that the interaction between PHG volume/entorhinal cortex and neuroticism-anxiety (N-Anx) predicted somatic complaints. Specifically, somatic complaints were associated with higher N-Anx for individuals with increased PHG volume. These findings suggest that increased PHG volume and higher trait anxiety can predict vulnerability to somatic complaints in the general population.

Keywords: anxiety; somatic complaints; somatization; neuroticism; voxel-based morphometry (VBM)

INTRODUCTION

Some somatic complaints are thought to be physical reactions that represent cognitive and emotional avoidance, which cause subjective distress and disability (Olatunji et al., 2006). Somatic complaints are common in patients with anxiety disorders, such as social anxiety (Domschke et al., 2010), posttraumatic stress disorders (Van Ommeren et al., 2002) and generalized anxiety disorder (Hoehn-Saric et al., 2004). It has been suggested that self-reported somatic complaints are strongly associated with anxiety-related traits in the general population (Røsmalen et al., 2007). Research on normal individuals also indicates that somatic complaints may contribute to the triggering and the amplification of anxiety-related personality traits (Costa and McCrae, 1985). Furthermore, individuals who have excessive somatic complaints may be more prone to misattributing somatic signals to other symptoms (Brosschot, 2002). However, why some people are more likely to report somatic complaints than others, and which neuroanatomical mechanisms are responsible for the association of somatic complaints and anxiety-related traits remains unclear.

Somatic complaints are common features of some anxiety disorders, including symptoms of fatigue, pain, heart palpitations, fear and other body sensations (Olatunji et al., 2006). These symptoms are often labeled as medically unexplained physical symptoms or psychosomatic symptoms (Mayou, 1991; van den Berg et al., 2005). Although anxiety is seen as a negative emotion that can be accompanied by distinct psychological and somatic complaints (Nabi et al., 2010), these two classes of symptoms have not received equal attention from neuroscientists. Most neuroscience research so far has focused on the neural mechanisms of the psychological components of anxiety (Barros-Loscertales et al., 2006; Bishop, 2007; Cherbuin et al., 2008), whereas few studies have examined the neural basis underlying somatic complaints. Somatic complaints may not have been understudied because they are difficult to induce in the laboratory. A growing body of evidence suggests that inter-individual variability in a wide range of human behaviors can be predicted from the structure of gray matter (GM) measured with MRI (Kanai and Rees, 2011). Thus, neuroanatomical investigations into the inter-individual variability in somatic complaints measured by self-reported questionnaires may provide a solid foundation for understanding the neural basis of somatic complaints.

Previous neuroimaging studies have revealed that the emotional aspect of somatization involves the medial temporal lobes (MTL), especially the hippocampus formation, which plays a critical role in negative emotional processing, memory formation and fear-conditioned learning (LaBar and Cabeza, 2006; Phelps, 2006). For example, pain-related emotional learning has been associated with abnormal activity in the MTL (Plooghaus et al., 2001; Baliki et al., 2010). Previous studies suggest that nociceptive processing also involves the MTL (Liu et al., 2010). More specifically, patients with chronic pain have stronger pain-evoked activity in the anterior parahippocampal gyrus (PHG) (Plooghaus et al., 2001). Furthermore, structural and functional changes in the hippocampal formation are associated with sensitivity to chronic pain (Vachon-Presseau et al., 2013), negative emotion (Roy et al., 2009), and the exacerbation of pain by anticipatory anxiety (Plooghaus et al., 2001). In summary, some clinical research indicates that structure and functional changes in the hippocampal formation are associated with symptoms of somatization. However, the relationship between individual differences in somatic complaints and the volume of regional GM in normal individuals remains unclear.

Research indicates that the frequency of somatic complaints is strongly associated with anxiety-related personality traits (Mathews and Mackintosh, 1998; Røsmalen et al., 2007). Psychosomatic diseases are thought to be particularly prone to being exacerbated by psychological factors such as, worry, fear and tension (Pennebaker, 2000).
Moreover, individuals with high levels of neuroticism are more vulnerable to physical symptoms and tend to report more somatic complaints (Kangas and Montgomery, 2011). Furthermore, physically vulnerable to physical symptoms and tend to report more somatic complaints (Kangas and Montgomery, 2011). Moreover, individuals with high levels of neuroticism are more vulnerable to physical symptoms and tend to report more somatic complaints (Kangas and Montgomery, 2011). Therefore, we expected that individuals with smaller rGMV would report stable somatic complaints regardless of their anxiety-related traits.

**METHOD**

**Participants**
A total of 288 right-handed, healthy volunteers (134 women and 154 men; mean age = 19.9 years, s.d. = 1.3; age range: 17–27 years) participated in the study as part of our ongoing project to examine the associations among brain imaging, creativity and mental health. All participants were university students from the local community of Southwest University, China. Participants were screened to confirm healthy development by a self-report questionnaire before the scan. We excluded participants who had a history of psychiatric or neurological disorders, received mental health treatment or had taken psychiatric medications were excluded. All participants gave their informed written consent and we also obtained informed written consent from the two youngest participants (aged 17 years old) guardians who were their college instructors. The Brain Imaging Center Institutional Review Board of Southwest China University approved this study and the experiment procedure which in accordance with the standards of the Declaration of Helsinki (1991).

**Measuring the level of somatic complaints**
The self-rating anxiety scale (SAS) is a 20-item measure of the frequency of anxiety symptoms and was developed primarily as a measure of somatic complaints associated with anxiety reaction (Zung, 1971). It consists of 15 somatic and 5 affective symptoms that are related to anxiety and has demonstrated adequate internal consistency and test–retest reliability. The initial psychometric evaluation of the measure revealed adequate split-half reliability ($r = 0.71$; Zung, 1971). A subsequent evaluation reported adequate internal consistency in normal college students ($\alpha = 0.81$; Olatunji et al., 2006). The SAS also had good test-retest reliability in a clinical sample of agoraphobics over a period ranging from 1 to 16 weeks ($r = 0.81–0.84$) (Michelson and Mavissakalian, 1983). In our study, the Cronbach’s alpha coefficient for internal consistency in this sample was 0.73. The mean SAS total score was 35.14 (S.D. = 6.46), which is consistent with the prior work (mean score = 33.09, S.D. = 6.88) (Olatunji et al., 2006). The 20-item SAS is also a self-report assessment tool to measure somatic complaints, with each response using a 4-point scale from ‘none of the time’ to ‘most of the time’. It contains items that mainly assess physiological symptoms commonly associated with anxiety. Examples of SAS items are as follows: ‘My arms and legs shake and tremble’ (Somatic arousal); ‘I feel more nervous and anxious than usual’ (Emotional arousal); ‘I feel weak and get tired easily’ (Fatigue); ‘I am bothered by headaches, neck and back pain’ (Pain); ‘I have nightmares’ (Sleep). The SAS is considered a sensitive and ecologically valid measure of somatic complaint levels in patients as well as in non-clinical participants (Olatunji et al., 2006).

**MRI data acquisition**
MR images were acquired on a 3.0-T Siemens Trio MRI scanner (Siemens Medical, Erlangen, Germany). High-resolution T1-weighted anatomical images were acquired using a magnetization-prepared rapid gradient echo sequence (repetition time $= 1900$ ms; echo time $= 2.52$ ms; inversion time $= 900$ ms; flip angle $= 9$ degrees; resolution matrix $= 256 \times 256$; slices $= 176$; thickness $= 1.0$ mm; voxel size $= 1 \times 1 \times 1$ mm).

**VBM**
The MR images were processed using the SPM8 program (Wellcome Department of Cognitive Neurology, London, UK; www.fil.ion.ucl.ac.uk/spm) implemented in Matlab 7.8 (MathWorks Inc., Natick, MA, USA). Each MR image was first displayed in SPM8 to screen for artifacts or gross anatomical abnormalities. For better registration, the reorientation of the images was manually set to the anterior commissure. The images were segmented into GM and white matter (WM) by using the new segmentation in SPM8. Subsequently, we perform Diffeomorphic Anatomical Registration through Exponentiated Lie algebra in SPM8 for registration, normalization, and modulation. To ensure that regional differences in the absolute amount of GM were conserved, the image intensity of each voxel was modulated by the Jacobian determinants. Then, registered images were transformed to Montreal Neurological Institute (MNI) space. Finally, the normalized modulated images (GM and WM images) were smoothed with a 12-mm full-width at half-maximum Gaussian kernel to increase their signal to noise ratio.

**Statistical analysis**
Statistical analyses of rGMV data were performed using SPM8. In the whole-brain analyses, we used a multiple linear regression to identify regions where rGMV was associated with individual differences in the level of somatic complaints. In the multiple linear regression analyses,
the scores of somatic complaints were used as the variable of interest. To control for possible confounding variables, age, sex, N-Anx and global volumes of GM were entered as covariates into the regression model. To avoid edge effects around the borders between GM and WM, an absolute threshold masking of 0.2 was used, meaning that voxels with GM values lower than 0.2 were excluded from the analyses.

We also examined the association between rGMV and somatic complaints and whether these associations differed between sexes. In whole-brain analysis, we used a voxel-wise ANCOVA in which sex difference was a group factor (using the full factorial option of SPM8). These methods have successfully employed in the previous study (Blankstein et al., 2009; Takeuchi et al., 2013b). In this analysis, age, somatic complaints and global GMV were covariates. The centering option was used to center these interactions. The main effects of somatic complaints and the interactions between sex and somatic complaints were assessed using t-contrasts.

For all analyses, the cluster-level statistical threshold was set at $P < 0.05$, and corrected at the non-stationary cluster correction (Hayasaka et al., 2004) with an underlying voxel level of $P < 0.001$.

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 six voxels leads to appropriate conservativeness in real data. With high smoothing values, an uncorrected threshold of $P < 0.01$ seems to lead to anti-conservativeness, whereas that of $P < 0.001$ seems to lead to slight conservativeness (Silber et al., 2011). Non-stationary cluster size tests can be safely applied to data known to be non-stationary (e.g. not uniformly smooth), such as VBM data (Hayasaka et al., 2004; Takeuchi et al., 2013a).

Moderation and mediation analysis

A moderator variable is a variable that affects the direction and/or strength of the relationship between an independent variable and a dependent variable. Moderation studies address questions like ‘when (under what conditions/situations)’ or ‘for whom’ does X have a stronger/weaker (positive/negative) relation with or effect on Y. Moderation analyses were conducted using the interaction effect MODPROBE macro designed for SPSS and SAS (Hayes and Matthes, 2009). To test whether the strength of the relationship between anxiety-related traits and somatic complaints were affected by the volume size of regional GM, we performed a moderation analysis. According to the Neyman technique to examine the interaction effect MODPROBE macro designed for SPSS (Preacher and Hayes, 2008). This macro uses bootstrapped sampling to estimate the indirect mediation effect. In this analysis, 2000 bootstrapped samples were drawn and bias corrected 95% bootstrap confidence intervals (CI) were reported. CIs that do not include zero indicate a significant indirect effect of the independent variable on the dependent variable through the mediators (Preacher and Hayes, 2008).

RESULTS

Sample descriptive statistics

The summed score for the SAS scale was used as an index of somatic complaints, whereby a higher score indicated more somatic complaints. Table 1 lists the characteristics of demographics of the total sample. As indicated in Table 1, somatic complaints were positively correlated with N-Anx ($r = 0.44$, $P < 0.001$), indicating that the SAS has good discriminate validity.

VBM results

Somatic complaints scores were positively correlated with the GM volume in a cluster that mainly included areas in the anterior part of bilateral PHG adjacent to the entorhinal cortex (right): cluster size $= 1587$, $t = 5.71$, $P$ (corr) $= 0.006$, $1 - \beta = 0.99$; left: cluster size $= 783$, $t = 4.36$, $P$ (corr) $= 0.034$, $1 - \beta = 0.98$; Figure 1 and Table 2. Furthermore, we also found that SAS scores were negatively correlated with the GM volume of the left postcentral gyrus (cluster size $= 108$, $t = 4.28$, $P$ (corr) $= 0.037$, $1 - \beta = 0.98$; Figure 2 and Table 2). Age, gender, N-Anx and global GM volumes were included as covariates in all analyses. Meanwhile, to examine the correlation between somatic complaints or anxiety and regional GMV affected by the global GM volume, we further examined the relationship between the global GM volume and the level of somatic complaints or anxiety. There was no significance correlation between the global GM volume and the level of somatic complaints ($r = 0.14$, $P = 0.81$) or anxiety ($r = -0.04$, $P = 0.5$).

We also examined the association between rGMV and somatic and whether these associations differed between sexes. The analysis of the interaction between sex and somatic complaints on rGMV did not reveal any significant results.

Moderation analysis

Based on previous studies, we hypothesized that individual differences in PHG volume would moderate the relationship between anxiety-related traits and somatic complaints. There was a significant interaction between N-Anx and PHG volume (Table 2). ($\Delta R^2 = 0.011$, $b = 2.55$, $t = 2.06$, $P = 0.038$), such that higher somatic complaints were associated with high N-Anx for participants with relatively increased PHG volume (up 89.9%, $n = 259$) but not for those with decreased PHG volume (remaining 10.1%, $n = 29$; Figure 3).

Mediation analysis

Indirect mediation effects can be interpreted as the strength of the relationship between somatic complaints and anxiety-related traits when accounting for mediating pathways (Hayes, 2009). Anxiety-related traits were positively associated with somatic complaints ($r = 0.44$, $P < 0.001$). Somatic complaints were positively related to PHG volume ($r = 0.32$, $P < 0.001$). Anxiety-related traits were not significantly correlated with PHG volume ($r = 0.033$, $P > 0.05$). To test the significance of the indirect effect between N-Anx and somatic complaints, bootstrap resampling was used. Results showed no significant indirect effect between somatic complaints and anxiety-related traits [CI: $-0.02$, $0.052$] through PHG volume.
DISCUSSION
In this study, we found that somatic complaints were associated with a significant increase in volume in a cluster that included areas in the anterior part of the bilateral PHG adjacent to the entorhinal cortex, and a reduction in left postcentral volume. Consistent with our hypothesis, we further found that the volume of the PHG/entorhinal cortex moderated the relationship between somatic complaints and N-Anx. Specifically, we found that higher somatic complaints were correlated with higher neuroticism-anxiety scores in individuals with higher PHG/entorhinal cortex volume, but not in individuals with lower PHG/entorhinal cortex volume.

Increased volume in the cluster included the anterior PHG/entorhinal cortex, which was associated with anxiety-related somatic complaints. The PHG is an important connecting pathway of the limbic system and it connects the amygdala and the hippocampus (Stefanacci et al., 1996). It has been suggested that the PHG and hippocampal connections with others subcortical structures may play a key role in the regulation of stress (Ulrich-Lai and Herman, 2009), consolidation of memory (Van Strien et al., 2009; Wang and Morris, 2010), and emotional learning (LaBar and Cabeza, 2006). For example, somatoform pain disorders show increased neural response to pain stimulation in the PHG (Gündel et al., 2008), and pain-related activity in the hippocampus of these patients has been associated with daily physical complaints (Gondo et al., 2012). PHG dysfunctions may be a risk factor for anxiety-related disorders, such as social anxiety disorder (Etkin and Wager, 2007; Goldin et al., 2009), specific phobias (Veltman et al., 2004), and posttraumatic stress disorders (Etkin and Wager, 2007). In particular, PHG hyperactivity has been found in individuals with social phobia during conditions of social threat (Phan et al., 2006). Also, a recent VBM study reported that individuals suffering from social anxiety disorder have increased GMV in the PHG compared with individuals suffering from panic disorder or controls (Talati et al., 2013). Moreover, the adjacent entorhinal cortex, which is part of the anterior PHG (Bernasconi et al., 1999; van Hoesen et al., 2004), provides sensory information to the hippocampus in memory.

Table 1 Demographic and psychometric measures (n = 288)

<table>
<thead>
<tr>
<th></th>
<th>Mean (s.d.)</th>
<th>Range</th>
<th>Association with SASa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>19.94 (1.32)</td>
<td>17–27</td>
<td>—</td>
</tr>
<tr>
<td>SAS</td>
<td>35.14 (6.46)</td>
<td>20–54</td>
<td>—</td>
</tr>
<tr>
<td>N-Anx</td>
<td>24.17 (4.26)</td>
<td>13–35</td>
<td>0.44**</td>
</tr>
</tbody>
</table>

*aPearson bivariate correlations, shown are r-values. **P < 0.001.

Fig. 1 Regional gray matter volume correlated with Self-Rating Anxiety Scale (SAS). The parahippocampal (PHG) volume adjacent to entorhinal cortex exhibited significant positive correlation with SAS. A scatterplot between SAS and gray matter volume adjusted for age, gender, and total gray matter volume is shown for illustration purpose only.
Results are significant after multiple comparisons correction. Pearson bivariate correlations with somatic complaints, shown are r-values.

Table 2 Summary of the GMV associations with somatic complaints

<table>
<thead>
<tr>
<th>Brain region</th>
<th>MNI coordinates</th>
<th>Voxels size</th>
<th>Peak T value</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parahippocampal/entorhinal</td>
<td>30 -3 -34</td>
<td>1587</td>
<td>5.71</td>
<td>0.323**</td>
</tr>
<tr>
<td>Negative correlation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parahippocampal/entorhinal</td>
<td>-32 0 36</td>
<td>783</td>
<td>4.36</td>
<td>0.25**</td>
</tr>
<tr>
<td>Postcentral gyrus</td>
<td>-48 -20 48</td>
<td>108</td>
<td>4.28</td>
<td>-0.249**</td>
</tr>
</tbody>
</table>

Results are P < 0.05, corrected for multiple comparisons at a cluster level with non-stationary correction, with an underlying voxel level of P < 0.001, uncorrected.

**P < 0.001. Pearson bivariate correlations with somatic complaints, shown are r-values.

and learning. The entorhinal cortex also is involved in nociceptive processing and the generation of pain perception (Ploghaus et al., 2001; Liu and Chen, 2009), and it plays an important role in fear and anxiety (Barkus et al., 2010). Damage to the ventral hippocampus (the entorhinal cortex) in rats makes them less susceptible to contextual fear conditioning (Bannerman et al., 2003) and reduces their expression of fear (Kjelstrup et al., 2002). In humans, Ploghaus et al. (2001) found that anxiety-induced hyperalgesia is associated with activation of the entorhinal cortex during a Pavlovian delay-conditioning task. Thus, our data provide direct evidence of the role of the parahippocampal/entorhinal cortex in anxiety, especially in anxiety-related somatic complaints. This suggests that increased parahippocampal/entorhinal cortex volume may be associated with increased vulnerability to somatic complaints in the general population.

In this study, the relationship between somatic complaints and individual neuroticism-anxiety were moderated by increased PHG/entorhinal cortex volume. Previous studies have revealed that the anterior hippocampal formation is associated with neuroendocrine stress responses and that it plays a critical role in anxiety-related behaviors that could become more prominent in somatoform disorders (Vachon-Presseau et al., 2013). Interestingly, Gray and McNaughton propose that the septohippocampal system acts as a comparator contrasting approaching fear with predicted perceptual information (Gray and McNaughton, 1982; McNaughton, 2003). This process is accompanied by anxiety. Additionally, it has been suggested that high trait anxiety or neuroticism are vulnerability factors for the development of anxiety disorders (Sandi and Richter-Levin, 2009). Moreover, VBM studies also have shown that trait anxiety is positively correlated with hippocampal volumes in both patients with clinical anxiety and healthy subjects (Rusch et al., 2001; Baur et al., 2012). Based on the results of our study, we propose that increased PHG volume may further interact with N-Anx to modulate somatic complaints and the potential risk for anxiety.

We also found that somatic complaints were negatively associated with GM volume in the left postcentral gyrus. The postcentral gyrus is the location of the primary somatosensory cortex, which plays a role in general somatic sensation. It has been suggested that the primary somatosensory cortex is involved in pain processing (Kanda et al., 2000; Inui et al., 2003; Mancini et al., 2012). The reduced postcentral volume may be a sensitivity index for pain perception, because it is associated with an increased pain threshold in individuals with a relatively high rate of somatic complaints. It is generally accepted that the PHG and postcentral gyrus are involved in sensory-limbic projection pathways that may be associated with nociceptive responses (Treede et al., 2000). The early processing of somatic sensory input occurs in the somatosensory cortices, and the output is directed towards the insular cortex and PHG (Friedman et al., 1986; Karhu and Tesche, 1999). The postcentral gyrus is involved in the somatosensory cortices, and may be associated with the processing of the sensory intensity of somatic complaints (Oertel et al., 2007), whereas, the PHG is part of the limbic system that is known to process the affective dimension of somatic complaints (Oertel et al., 2007).

Our findings are limited to the instrument we used to assess somatic complaints. Specifically, we used a self-report questionnaire measure of
somatic complaints. Self-reports reflect a range of cognitive biases, such as overestimation, known as the Kruger-Dunning effect (Kanai and Rees, 2011). Also, our sample consisted of highly educated, normal, young adults, who may be less physically responsive than the general population. Therefore, it is not known whether our results would be consistent with clinical samples. Given the relationship between somatic complaints and individual differences in hippocampal formation volume, studying somatic complaints in a clinical sample may be helpful to understand how the hippocampal formation affects somatic complaints. We know that VBM analysis is a more comprehensive measure that integrates changes in cortical folding and thickness. So, other direct measures of the brain structure (such as cortical thickness, surface area or local gyriﬁcation index, etc.) should be used in further studies to better understand these relationships (Wei et al., 2013). Additionally, the image denoising method needs to be improved for a large sample in a future study (e.g. a denoising filter based on Spatial Adaptive Non-Local Means).

In summary, we have found that somatic complaints are intimately associated with individual differences in the volume of the parahippocampal/entorhinal cortex. Moreover, the relationship between somatic complaints and anxiety-related traits were moderated by parahippocampal/entorhinal cortex volume. These ﬁndings indicate that increased parahippocampal/entorhinal volume might be a key indicator of somatic complaints. Further studies are needed to investigate the longitudinal relationship among somatic complaints, anxiety disorders and GMV change.

REFERENCES


