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Gray and white matter volume abnormalities in generalized anxiety disorder by categorical and dimensional characterization

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Abstract

Increasing efforts have been made to investigate the underlying pathophysiology of generalized anxiety disorder (GAD), but only limited consistent information is available on gray (GM) and white matter (WM) volume changes in affected adults. Additionally, few studies employed dimensional approaches to GAD pathology. This study compares structural brain imaging data from $n = 19$ GAD subjects and $n = 24$ healthy comparison (HC) subjects, all medication-free and matched on age, sex and education. Separate categorical and dimensional models were employed using voxel-based morphometry for GM and WM. Significantly higher GM volumes were found in GAD subjects mainly in basal ganglia structures and less consistently in the superior temporal pole. For WM, GAD subjects showed significantly lower volumes in the dlPFC. Largely consistent findings in dimensional and categorical models point toward these structural alterations being reliable and of importance for GAD. While lower volume in the dlPFC could reflect impaired emotional processing and control over worry in GAD, basal ganglia alterations may be linked to disturbed gain and loss anticipation as implicated in previous functional GAD studies. As perturbations in anticipation processes are central to GAD, these areas may warrant greater attention in future studies.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.psychres.2015.10.009>.

Keywords

Generalized anxiety disorder; Worry; Intolerance of uncertainty; Neuroimaging; Structural magnetic resonance imaging; Prefrontal cortex; Putamen; Caudate nucleus; Striatum

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1. Introduction

Generalized anxiety disorder (GAD) is a prevalent (Wittchen et al., 2011; Kessler et al., 2012) and impairing anxiety disorder (Andlin-Sobocki and Wittchen, 2005; Hoffman et al., 2008). Despite growing research on neurobiology (Hilbert et al., 2014), findings on the neuroanatomical correlates of GAD remain inconsistent, possibly due to cross-study differences in methodology. Additionally, limited information is available on dimensional approaches to core GAD symptoms as emphasized in recent clinical, diagnostic and research frameworks (American Psychiatric Association, 2013; Cuthbert, 2014). The current study provides data that complement results from functional investigations and inform theories of GAD etiopathogenesis.

The overall few available studies of the neuroanatomy in GAD did not generate consistent findings. Several studies relied on a region-of-interest (ROI) approach, with different studies targeting different ROIs, limiting the chance for consistent findings. Only four studies used whole-brain approaches to assess GM volume in GAD (Schienle et al., 2011; Liao et al., 2013; Strawn et al., 2013; Moon et al., 2014), with two of these studies investigating adolescents (Liao et al., 2013; Strawn et al., 2013). The remaining two studies in adults did not report comparable findings. To remedy the current lack of replicated findings concerning GM volumes in GAD, more whole brain data are needed. A similar lack of volumetric studies is apparent for white matter (WM). Here, no whole-brain data in adults are yet available. However, investigating these volume changes in WM might prove to be complementary to studies of other indices of WM such as fractional anisotropy.

Among the regions more frequently investigated for structural changes in GAD, only higher GM volumes in the amygdala (Etkin et al., 2009; Schienle et al., 2011) and lower GM volumes in the hippocampus (Abdallah et al., 2013; Moon et al., 2014) have been successfully replicated. However, several other areas have not been investigated sufficiently, although they were prominently included in functional studies on GAD. These include ventral areas of the anterior cingulate cortex (ACC) as well as parts of the dorsomedial and dorsolateral prefrontal cortex (PFC), all of which are related to emotion processing and emotion regulation deficits in GAD (Etkin et al., 2010; Paulesu et al., 2010; Etkin and Schatzberg, 2011; Ball et al., 2013), and the basal ganglia, for which especially the putamen has shown altered responses to gain and loss anticipation in GAD (Guye et al., 2012).

Beyond comparisons of patients and healthy individuals on a categorical group level, increasing emphasis has been placed on a continuous or dimensional conceptualization of psychopathology in recent years. As a result, both the DSM-5 (American Psychiatric Association, 2013) and the Research Domain Criteria framework (e.g., Insel and Cuthbert, 2009; Cuthbert, 2014) feature dimensional approaches to mental disorders. However, research on neural correlates of GAD-related dimensions is limited, although a resting state

study by Oathes et al. (2015) already provided information from dimensional approaches to supplement diagnostic group analyses.

Taken together, the aim of the present voxel-based morphometry study was to examine GM and WM tissue in GAD subjects and healthy comparison subjects, using both a dimensional approach, as reflected in ratings of worry severity, and a categorical approach, as reflected in dichotomous GAD diagnosis. Based on the existing literature on both structural and functional changes in GAD, higher GM volumes in the amygdala and putamen and lower GM volumes in the dorsomedial and dorsolateral PFC, hippocampus and ventral ACC were expected for GAD compared with healthy controls. For the dimensional approach, a positive relationship between putamen GM volume and habitual worry severity but a negative relationship between dorsomedial and dorsolateral PFC and ventral ACC GM volumes and habitual worry severity were expected.

2. Methods

2.1. Subjects

The study included $n = 19$ adults with GAD and $n = 24$ healthy comparison subjects (HC). The following inclusion criteria were used: diagnosis of GAD according to DSM-IV-TR criteria, absence of any lifetime-diagnosis for the healthy comparison group. Exclusion criteria were: MRI-related exclusion, use of any psychotropic medication, a non-remitted diagnosis of substance dependence, or smoking of more than 10 cigarettes per day. All subjects were diagnosed using a standardized interview (Composite International Diagnostic Interview, CIDI; Wittchen and Pfister, 1997). Diagnoses were confirmed by clinical experts. Sixteen of the GAD subjects had at least one comorbid disorder. For detailed information about comorbidities, please refer to supplement A. Habitual worry severity was assessed using the Penn State Worry Questionnaire (PSWQ) (Meyer et al., 1990), which has been shown to possess good psychometric properties (Meyer et al., 1990; Brown et al., 1992) and sufficient sensitivity and specificity for discriminating between GAD and other anxiety disorders (Meyer et al., 1990; Fresco et al., 2003). Written informed consent was obtained from all subjects after the study procedures were explained. The study protocol was approved by the local ethics committee (EK13012009).

2.2. Analysis of demographic and clinical data

Demographic and clinical data were analyzed using chi-square tests and independent t -tests, respectively. SPSS 22 (IBM, New York, NY, USA) was used for all calculations, with the level of significance being set at $p < 0.05$.

2.3. Structural MRI data acquisition and analysis

A 3-Tesla Trio-Tim MRI whole-body scanner (Siemens, Erlangen, Germany) with a 12-channel head coil was used to acquire the structural brain imaging data. Scans were obtained using a magnetization-prepared rapid gradient echo imaging sequence (MPRAGE; 176 sagittal slices, slice thickness = 1 mm, echo time = 2.26 ms, repetition time = 1900 ms, flip angle = 9° , field of view = 256×256 mm, matrix = 256×256). For preprocessing and analysis of the data, SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>) and the

VBM8 toolbox (<<http://dbm.neuro.uni-jena.de/vbm/download/>>) were used. Anatomical data were segmented into GM, WM and cerebrospinal fluid (CSF), and normalized to Montreal Neurological Institute (MNI) space by the DARTEL algorithm (Ashburner, 2007) implemented in the VBM toolbox. In the process, the spatial resolution of the data was changed to $1.5 \times 1.5 \times 1.5 \text{ mm}^3$, and all images were modulated to account for differences in individual total tissue volume. Finally, the segmented, normalized and modulated images were smoothed with an 8 mm full-width half-maximum Gaussian kernel and checked for artifacts.

GM and WM data were analyzed separately, and an absolute image threshold of 0.15 was used for all analyses. Factorial models were used for the whole-brain analysis of group differences, and regression models for the dimensional analysis of habitual worry severity. Both approaches were completely independent from each other; diagnostic group membership was not included in the dimensional model, and PSWQ scores were not included in the categorical model. As there was considerable variance of age in the sample, age was included as a covariate in all analyses to make sure that results would not reflect age-related effects, particularly with regard to the regression analyses. To achieve an optimal balance between sensitivity and specificity and to ensure comparability to previous studies on GAD neuroanatomy, we used two different statistical thresholds for these analyses. Across the main analyses, a cluster-size based threshold for significance with 200 consecutive voxels at $p_{uncorr} < 0.001$ as employed previously (e.g. Strawn et al., 2013; Wehry et al., 2015) was used. Additionally, a second analysis with a more conservative threshold using clusterwise FWE at $p_{cFWE} = 0.05$ and a voxelwise cluster-forming threshold of $p_{uncorr} < 0.001$ was conducted on all significant clusters from the first analysis. Across all calculations, a non-stationarity correction as implemented in SPM8 was applied. As worry and GAD are also closely connected to the concept of intolerance of uncertainty (Dugas et al., 1998), additional regression models were built based on scores of the intolerance of Uncertainty Scale, short version (IUS-12; Carleton et al., 2007).

3. Results

3.1. Demographic and clinical data

Table 1 shows demographic and clinical characteristics of both groups. GAD and HC subjects were well matched regarding all sociodemographic characteristics. As expected, GAD subjects showed elevated scores compared with HC subjects in all clinical characteristics. PSWQ, BDI and IUS scores were highly correlated (PSWQ-BDI: $r = 0.778$, $p < 0.001$; PSWQ-IUS: $r = 0.809$, $p < 0.001$; BDI-IUS: $r = 0.786$, $p < 0.001$). For the subsequent regression analyses, PSWQ and IUS scores were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk, 1965). IUS scores were normally distributed across the whole sample ($p = 0.538$), which was not the case for the PSWQ ($p = 0.026$; see Fig. 1 for the distributions of these questionnaire scores within each group).

3.2. Structural MRI results: group comparison (categorical analysis)

GAD subjects showed higher GM volumes compared with HC subjects in the right striatum and right superior temporal pole, while no areas showed Significantly lower GM volumes

(see Fig. 2 and Table 2). The finding of higher GM in the right striatum remained significant after the more conservative clusterwise FWE correction. There were no GM volume differences between GAD and HC subjects in the amygdala, hippocampus or anterior ACC.

For WM, GAD subjects showed higher volumes compared with the HC subjects in the left occipital lobe, while lower volumes were found in the left dorsolateral PFC (see Table 3).

3.3. Structural MRI results: Worry severity (dimensional analysis)

Dimensional analyses considering worry severity showed a positive relationship between habitual worry levels and GM volumes in the right striatum, left caudate nucleus and the right putamen (see Fig. 2 and Table 4). The positive relationship between right striatal volume and worry severity remained to be significant after the more conservative clusterwise FWE correction. In the right supplementary motor area (SMA), left middle cingulate gyrus (MCC) and right paracentral lobule, there was a negative relationship between GM volumes and symptom severity in the total sample. Here, this relationship between right SMA GM volume and worry severity remained significant after clusterwise FWE correction. Again, there were no associations between worry severity and amygdala, hippocampus or ACC GM volume.

For WM, no positive relationship between habitual worry levels and brain volume achieved significance, but a negative relationship between habitual worry levels and volumes in the left dorsolateral PFC, right precentral lobe and right cerebellum was found (see Table 5).

As there was little overlap between the PSWQ scores of the two groups, additional regression analyses within both groups separately were conducted (please see Supplement B).

3.4. Structural MRI results: Intolerance of uncertainty (dimensional analysis)

Dimensional analyses considering intolerance of uncertainty showed a positive relationship between intolerance of uncertainty levels and GM volumes in the right striatum, right superior temporal pole and right fusiform gyrus (see Fig. 2 and Table 6). The positive relationship between right superior temporal pole GM volume and intolerance of uncertainty remained to be significant under the more conservative clusterwise FWE correction. In the left middle cingulate gyrus (MCC) and right paracentral lobule, there was a negative relationship between GM volumes and intolerance of uncertainty. There were no associations between intolerance of uncertainty and amygdala, hippocampus or ACC GM volume.

For WM, no positive relationship between intolerance of uncertainty and brain volume became significant, but a negative relationship between intolerance of uncertainty and volumes in the right cerebellum was found (Table 7).

4. Discussion

Previous neuroimaging research demonstrated a variety of structural alterations in GAD subjects, but few findings have been successfully replicated due to a range of different

methods used and due to the common use of ROI approaches. Some areas implicated in functional studies have not been sufficiently examined in structural investigations so far. Additionally, it is unclear which of these alterations are related to chronic worry severity as a dimensional measure of core GAD psychopathology. The current study aimed to provide whole-brain data on the structural alterations in GM and WM tissue in GAD compared to HC subjects and in relation to habitual worry severity, thus expanding earlier studies. The following main results were obtained, as hypothesized, for the GAD group: (1) Higher GM volumes were found in the basal ganglia and in particular in a cluster in the right striatum including the putamen and caudate nucleus. (2) Lower WM volumes were found in the dorsolateral PFC. (3) For these key regions, the dimensional approach revealed largely comparable alterations.

Group differences in GM volume obtained in this study replicated some of the structural correlates reported for GAD subjects compared with HC subjects in earlier works and were thus partly consistent with our hypotheses. As expected, structural alterations were found in parts of the basal ganglia which were comparable in both the categorical and dimensional approaches employed in this study. Higher GM volume in the striatum in GAD has been demonstrated before in another GAD sample without comorbid depression (Liao et al., 2013) and complements functional findings of altered putamen activation related to reward processing (Guyer et al., 2012). Additionally, higher GM volumes and increased activation particularly in the caudate have been implicated as neural correlates of inhibited temperament (Guyer et al., 2006; Clauss et al., 2014), which is an important risk factor of GAD (Beesdo et al., 2010). Previous studies provided different interpretations for their findings: While Guyer and colleagues interpreted their findings from functional data as increased sensitivity for the anticipation of loss in GAD patients (Guyer et al., 2012), Liao et al. proposed for their structural data a relationship between putamen alterations and somatic complaints in GAD (Liao et al., 2013). However, the PSWQ which was used for the dimensional measurement of worry severity in the current study does not include items on putative somatic symptoms accompanying worry but focuses on cognitive aspects. Therefore, the finding that putamen and caudate nucleus alterations were also found in the dimensional approach to worry rather seems to support the former notion. Given that worry as a dysfunctional expression of loss-anticipation is the hallmark feature of GAD (American Psychiatric Association, 2000, 2013), it seems conclusive for these areas to be altered on the structural level as well. In this way, the current work provides further evidence for the putative importance of basal-ganglia related neural changes in GAD, which have been largely neglected in the past. Another mental disorder showing fronto-striatal alterations is obsessive-compulsive disorder (OCD; Piras et al., 2015). Both disorders feature repetitive thoughts and actions which affected individuals have difficulties to control, and a dysfunctional coping with negative affectivity (Comer et al., 2004; Behar et al., 2009; Bokor and Anderson, 2014). Finally, both disorders have been related to the concept of intolerance of uncertainty (Carleton, 2012; Reuther et al., 2013; Sarawgi et al., 2013). This is in line with the findings from the supplemental analyses which also showed striatal alterations in a regression based on scores of the intolerance of uncertainty scale. These commonalities between GAD and OCD might explain a set of shared neural underpinnings and should be investigated further.

Additionally, there were also higher GM volumes in the superior temporal pole in GAD subjects compared with HC subjects. These were also found for the dimensional approach to intolerance of uncertainty. While largely neglected in GAD research so far, structural alterations in the temporal gyri have been repeatedly reported in the GAD literature (De Bellis et al., 2002; Moon et al., 2014; Strawn et al., 2014). Nonclinical studies have related the temporal poles to a number of tasks including affective face processing and empathy (Olson et al., 2007; Jankowiak-Siuda et al., 2011), but more precise conclusions should not be drawn without more replication and inclusion of task-based investigations in GAD.

Significant volume changes in the PFC were only detected for WM but not for GM, contrary to hypotheses. Previous structural (e.g., Schienle et al., 2011; Strawn et al., 2013; Strawn et al., 2014) and functional (e.g., McClure et al., 2007; Strawn et al., 2012; Ball et al., 2013) MRI studies have reported abnormal GM volume in various parts of the PFC, although not in all studies (Liao et al., 2014; Moon et al., 2014). Additionally, structural PFC alterations are present in disorders related to GAD such as major depression (Kempton et al., 2011; Du et al., 2012; Sacher et al., 2012). In GAD, dorsolateral PFC function has mainly been related to emotion regulation (Ball et al., 2013) and top-down inhibition (Price et al., 2011). In this sample, however, lower PFC volume in the GAD group compared to the HC group was only detected for WM. There was also a negative relationship between PSWQ scores and WM volume in this area. The interpretation of these WM differences is limited as only few studies so far reported WM volume in GAD (De Bellis et al., 2002; Strawn et al., 2013). However, while relatively little WM volume data in GAD are available, a range of diffusion tensor imaging studies already demonstrated the importance of pathological changes in WM tracts and microstructure for GAD (Brambilla et al., 2012; Tromp et al., 2012; Zhang et al., 2013), with some of these findings being related to the PFC as well (Tromp et al., 2012). The results reported here might provide another target for such investigations in the future.

No volumetric differences were detected for other regions such as the amygdala. While not all investigations of GM volume differences in GAD report higher amygdala volumes, these were nevertheless found in a number of studies (De Bellis et al., 2000; Etkin et al., 2009; Schienle et al., 2011). On the contrary, amygdala GM decreases were shown for major depression in a number of meta-analyses (Hamilton et al., 2008; Sacher et al., 2012; Bora et al., 2012a). Since there was a considerable amount of depressive comorbidity in this current sample, this may have contributed to the non-significance of results for the whole sample. An earlier study testing for GM differences between subjects with major depression and a comorbid anxiety disorder against HC subjects did not find significant differences in the amygdala as well (van Tol et al., 2010).

The current results must be viewed in light of the limitations of this study: The sample size was relatively small, possibly limiting the power of the statistical analyses and thereby increasing type II error rate. As it is necessary to balance type I and type II error risk (Lieberman and Cunningham, 2009), we used a more liberal threshold that has been used before by structural MRI research in GAD (Strawn et al., 2013) while also indicating which results still remain significant under a more conservative FWE correction as recommended by Bennett et al. (2009). Those results that failed to stay significant with this correction should be viewed with more caution and should be replicated by future studies, ideally

employing larger sample sizes. With both types of correction, we effectively used a cluster-based threshold to correct for multiple comparisons. As recently emphasized by Woo et al. (2014), it should be noted that the spatial precision of results acquired this way is inferior to results acquired using voxel-wise inference. Additionally, the GAD group allowed for comorbid depression and generally showed relatively high scores in depressiveness as indicated by the BDI, which might have influenced our results. Also, high correlations between PSWQ, BDI and IUS scores limited the ability to investigate the relationship of certain structural alterations to specific GAD symptoms. However, by taking this approach an ecologically-valid sample was ensured. Still, future studies might benefit from adding measures that are tailored to specific aspects of GAD symptomatology. Additionally, the main finding of structural alterations in basal-ganglia regions has been shown before in another GAD sample without comorbid depression (Liao et al., 2013). Also, while there was higher GM volume in the putamen and caudate nucleus in this study, the literature reports consistently lower volumes in these structures for major depression (Kempton et al., 2011; Bora et al., 2012a, 2012b). Therefore, it seems unlikely that this result was driven by influence from comorbid depression. There are already studies trying to elucidate the effect of comorbid anxiety and depression, mainly by comparing anxious depression with nonanxious depression (van Tol et al., 2010; Inkster et al., 2011; Wehry et al., 2015). In addition, future studies should employ an explicit comparison between pure (non-comorbid) GAD patients and pure MD patients to further disentangle the neurostructural correlates of both disorders. Lastly, we used a dimensional approach to worry although worry scores showed a relatively bivariate distribution. Consequently, the results of this analysis should be viewed with caution. However, these results were comparable to the results of the analysis of intolerance of uncertainty scores, which were normally distributed and showed considerable overlap between groups. As worry and intolerance of uncertainty are also theoretically related (Starcevic and Berle, 2006), this seems to support the associations that were obtained for worry on the complete sample.

In conclusion, this study aimed at replicating earlier results on structural brain alterations in GAD and at extending these findings by employing a whole brain analysis and including an additional dimensional approach. It provides further evidence for neurostructural changes in the prefrontal and temporal cortex and particularly in basal-ganglia areas. Structural alterations in the putamen and caudate nucleus were not investigated before but previous functional MRI research has noted its relevance for the understanding of anticipation of loss in GAD. The results reported here expand the available knowledge on GAD neurobiology and provide further information for the better understanding of potential mechanisms of the disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- Abdallah CG, Coplan JD, Jackowski A, Sato JR, Mao X, Shungu DC, Mathew SJ. A pilot study of hippocampal volume and N-acetylaspartate (NAA) as response biomarkers in riluzole-treated patients with GAD. *Eur. Neuropsychopharmacol.* 2013; 23:276–284. [PubMed: 22739126]
- American Psychiatric Association. *Diagnostic and Statistical Manual Of Mental Disorders, Text Revision (DSM-IV-TR)*. 4th. Washington, DC: Author; 2000.
- American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders*. 5th. Washington, DC: Author; 2013.
- Andlin-Sobocki P, Wittchen HU. Cost of anxiety disorders in Europe. *Eur. J. Neurol.* 2005; 12:39–44. [PubMed: 15877777]
- Ashburner J. A fast diffeomorphic image registration algorithm. *Neuroimage.* 2007; 38:95–113. [PubMed: 17761438]
- Ball TM, Ramsawh HJ, Campbell-Sills L, Paulus MP, Stein MB. Prefrontal dysfunction during emotion regulation in generalized anxiety and panic disorders. *Psychol. Med.* 2013; 43:1475–1486. [PubMed: 23111120]
- Beesdo K, Pine DS, Lieb R, Wittchen HU. Incidence and risk patterns of anxiety and depressive disorders and categorization of generalized anxiety disorder. *Arch. Gen. Psychiatry.* 2010; 67:47–57. [PubMed: 20048222]
- Behar E, DiMarco ID, Hekler EB, Mohlman J, Staples AM. Current theoretical models of generalized anxiety disorder (GAD): conceptual review and treatment implications. *J. Anxiety Disord.* 2009; 23:1011–1023. [PubMed: 19700258]
- Bennett CM, Wolford GL, Miller MB. The principled control of false positives in neuroimaging. *Soc. Cogn. Affect. Neurosci.* 2009; 4:417–422. [PubMed: 20042432]
- Bokor G, Anderson PD. Obsessive-compulsive disorder. *J. Pharm. Pract.* 2014; 27:116–130. [PubMed: 24576790]
- Bora E, Fornito A, Pantelis C, Yucel M. Gray matter abnormalities in major depressive disorder: a meta-analysis of voxel based morphometry studies. *J. Affect. Disord.* 2012a; 138:9–18. [PubMed: 21511342]
- Bora E, Harrison BJ, Davey CG, Yucel M, Pantelis C. Meta-analysis of volumetric abnormalities in cortico-striatal-pallidal-thalamic circuits in major depressive disorder. *Psychol. Med.* 2012b; 42:671–681. [PubMed: 21910935]
- Brambilla P, Como G, Isola M, Taboga F, Zuliani R, Goljevscek S, Ragogna M, Brondani G, Baiano M, Perini L, Ferro A, Bazzocchi M, Zuiani C, Balestrieri M. White-matter abnormalities in the right posterior hemisphere in generalized anxiety disorder: a diffusion imaging study. *Psychol. Med.* 2012; 42:427–434. [PubMed: 21781374]
- Brown TA, Antony MM, Barlow DH. Psychometric properties of the Penn State Worry Questionnaire in a clinical anxiety disorders sample. *Behav. Res. Therapy.* 1992; 30:33–37.
- Carleton RN. The intolerance of uncertainty construct in the context of anxiety disorders: theoretical and practical perspectives. *Exp. Rev. Neurother.* 2012; 12:937–947.
- Carleton RN, Norton MA, Asmundson GJ. Fearing the unknown: a short version of the intolerance of uncertainty scale. *J. Anxiety Disord.* 2007; 21:105–117. [PubMed: 16647833]
- Clauss JA, Seay AL, VanDerKlok RM, Avery SN, Cao A, Cowan RL, Benningfield MM, Blackford JU. Structural and functional bases of inhibited temperament. *Soc. Cogn. Affect. Neurosci.* 2014; 9:2049–2058. [PubMed: 24493850]
- Comer JS, Kendall PC, Franklin ME, Hudson JL, Pimentel SS. Obsessing/worrying about the overlap between obsessive-compulsive disorder and generalized anxiety disorder in youth. *Clin. Psychol. Rev.* 2004; 24:663–683. [PubMed: 15385093]
- Cuthbert BN. The RDoC framework: facilitating transition from ICD/DSM to dimensional approaches that integrate neuroscience and psychopathology. *World Psychiatry.* 2014; 13:28–35. [PubMed: 24497240]
- De Bellis MD, Casey BJ, Dahl RE, Birmaher B, Williamson DE, Thomas KM, Axelson DA, Frustaci K, Boring AM, Hall J, Ryan ND. A pilot study of amygdala volumes in pediatric generalized anxiety disorder. *Biol. Psychiatry.* 2000; 48:51–57. [PubMed: 10913507]

- De Bellis MD, Keshavan MS, Shifflett H, Iyengar S, Dahl RE, Axelson DA, Birmaher B, Hall J, Moritz G, Ryan ND. Superior temporal gyrus volumes in pediatric generalized anxiety disorder. *Biol. Psychiatry*. 2002; 51:553–562. [PubMed: 11950457]
- Du MY, Wu QZ, Yue Q, Li J, Liao Y, Kuang WH, Huang XQ, Chan RCK, Mechelli A, Gong QY. Voxelwise meta-analysis of gray matter reduction in major depressive disorder. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*. 2012; 36:11–16.
- Dugas MJ, Gagnon F, Ladouceur R, Freeston MH. Generalized anxiety disorder: a preliminary test of a conceptual model. *Behav. Res. Ther.* 1998; 36:215–226. [PubMed: 9613027]
- Etkin A, Prater KE, Hoefl F, Menon V, Schatzberg AF. Failure of anterior cingulate activation and connectivity with the amygdala during implicit regulation of emotional processing in generalized anxiety disorder. *Am. J. Psychiatry*. 2010; 167:545–554. [PubMed: 20123913]
- Etkin A, Prater KE, Schatzberg AF, Menon V, Greicius MD. Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. *Arch. Gen. Psychiatry*. 2009; 66:1361–1372. [PubMed: 19996041]
- Etkin A, Schatzberg AF. Common abnormalities and disorder-specific compensation during implicit regulation of emotional processing in generalized anxiety and major depressive disorders. *Am. J. Psychiatry*. 2011; 168:968–978. [PubMed: 21632648]
- Fresco DM, Mennin DS, Heimberg RG, Turk CL. Using the Penn State Worry Questionnaire to identify individuals with generalized anxiety disorder: a receiver operating characteristic analysis. *J. Behav. Ther. Exp. Psychiatry*. 2003; 34:283–291. [PubMed: 14972674]
- Guyer AE, Choate VR, Detloff A, Benson B, Nelson EE, Perez-Edgar K, Fox NA, Pine DS, Ernst M. Striatal functional alteration during incentive anticipation in pediatric anxiety disorders. *Am. J. Psychiatry*. 2012; 169:205–212. [PubMed: 22423352]
- Guyer AE, Nelson EE, Perez-Edgar K, Hardin MG, Roberson-Nay R, Monk CS, Bjork JM, Henderson HA, Pine DS, Fox NA, Ernst M. Striatal functional alteration in adolescents characterized by early childhood behavioral inhibition. *J. Neurosci.* 2006; 26:6399–6405. [PubMed: 16775126]
- Hamilton JP, Siemer M, Gotlib IH. Amygdala volume in major depressive disorder: a meta-analysis of magnetic resonance imaging studies. *Mol. Psychiatry*. 2008; 13:993–1000. [PubMed: 18504424]
- Hilbert K, Lueken U, Beesdo-Baum K. Neural structures, functioning and connectivity in generalized anxiety disorder and interaction with neuroendocrine systems: a systematic review. *J. Affect. Disord.* 2014; 158:114–126. [PubMed: 24655775]
- Hoffman D, Dukes E, Wittchen H-U. Human and economic burden of generalized anxiety disorder. *Depression Anxiety*. 2008; 25:72–90. [PubMed: 17146763]
- Inkster B, Rao AW, Ridler K, Nichols TE, Saemann PG, Auer DP, Holsboer F, Tozzi F, Muglia P, Merlo-Pich E, Matthews PM. Structural brain changes in patients with recurrent major depressive disorder presenting with anxiety symptoms. *J. Neuroimaging*. 2011; 21:375–382. [PubMed: 20977527]
- Insel TR, Cuthbert BN. Endophenotypes: bridging genomic complexity and disorder heterogeneity. *Biol. Psychiatry*. 2009; 66:988–989. [PubMed: 19900610]
- Jankowiak-Siuda K, Rymarczyk K, Grabowska A. How we empathize with others: a neurobiological perspective. *Med. Sci. Monit.* 2011; 17:Ra18–Ra24. [PubMed: 21169921]
- Kempton MJ, Salvador Z, Munafo MR, Geddes JR, Simmons A, Frangou S, Williams SCR. Structural neuroimaging studies in major depressive disorder meta-analysis and comparison with bipolar disorder. *Arch. Gen. Psychiatry*. 2011; 68:675–690. [PubMed: 21727252]
- Kessler RC, Petukhova M, Sampson NA, Zaslavsky AM, Wittchen HU. Twelve-month and lifetime prevalence and lifetime morbid risk of anxiety and mood disorders in the United States. *Int. J. Methods Psychiatr. Res.* 2012; 21:169–184. [PubMed: 22865617]
- Liao M, Yang F, Zhang Y, He Z, Song M, Jiang TZ, Li ZX, Lu SJ, Wu WW, Su LY, Li LJ. Childhood maltreatment is associated with larger left thalamic gray matter volume in adolescents with generalized anxiety disorder. *PLoS One*. 2013; 8
- Liao M, Yang F, Zhang Y, He Z, Su L, Li L. Lack of gender effects on gray matter volumes in adolescent generalized anxiety disorder. *J. Affect. Disord.* 2014; 155:278–282. [PubMed: 24262640]

- Lieberman MD, Cunningham WA. Type I and Type II error concerns in fMRI research: re-balancing the scale. *Soc. Cogn. Affect. Neurosci.* 2009; 4:423–428. [PubMed: 20035017]
- McClure EB, Monk CS, Nelson EE, Parrish JM, Adler A, Blair RJR, Fromm S, Charney DS, Leibenluft E, Ernst M, Pine DS. Abnormal attention modulation of fear circuit function in pediatric generalized anxiety disorder. *Arch. Gen. Psychiatry.* 2007; 64:97–106. [PubMed: 17199059]
- Meyer TJ, Miller ML, Metzger RL, Borkovec TD. Development and validation of the Penn State Worry Questionnaire. *Behav. Res. Ther.* 1990; 28:487–495. [PubMed: 2076086]
- Moon CM, Kim GW, Jeong GW. Whole-brain gray matter volume abnormalities in patients with generalized anxiety disorder: voxel-based morphometry. *Neuroreport.* 2014; 25:184–189. [PubMed: 24476839]
- Oathes DJ, Patenaude B, Schatzberg AF, Etkin A. Neurobiological signatures of anxiety and depression in resting-state functional magnetic resonance imaging. *Biol. Psychiatry.* 2015; 77:385–393. [PubMed: 25444162]
- Olson IR, Ploaker A, Ezzyat Y. The Enigmatic temporal pole: a review of findings on social and emotional processing. *Brain.* 2007; 130:1718–1731. [PubMed: 17392317]
- Paulesu E, Sambugaro E, Torti T, Danelli L, Ferri F, Scialfa G, Sberna M, Ruggiero GM, Bottini G, Sassaroli S. Neural correlates of worry in generalized anxiety disorder and in normal controls: a functional MRI study. *Psychol. Med.* 2010; 40:117–124. [PubMed: 19419593]
- Piras F, Chiapponi C, Girardi P, Caltagirone C, Spalletta G. Widespread structural brain changes in OCD: a systematic review of voxel-based morphometry studies. *Cortex.* 2015; 62C:89–108.
- Price RB, Eldreth DA, Mohlman J. Deficient prefrontal attentional control in late-life generalized anxiety disorder: an fMRI investigation. *Transl. Psychiatry.* 2011; 1:e46. [PubMed: 22833192]
- Reuther ET, Davis TE, Rudy BM, Jenkins WS, Whiting SE, May AC. Intolerance of uncertainty as a mediator of the relationship between perfectionism and obsessive-compulsive symptom severity. *Depression Anxiety.* 2013; 30:773–777. [PubMed: 23609998]
- Sacher J, Neumann J, Funfstuck T, Soliman A, Villringer A, Schroeter ML. Mapping the depressed brain: a meta-analysis of structural and functional alterations in major depressive disorder. *J. Affect. Disord.* 2012; 140:142–148. [PubMed: 21890211]
- Sarawgi S, Oglesby ME, Cogle JR. Intolerance of uncertainty and obsessive-compulsive symptom expression. *J. Behav. Ther. Exp. Psychiatry.* 2013; 44:456–462. [PubMed: 23832229]
- Schienze A, Ebner F, Schafer A. Localized gray matter volume abnormalities in generalized anxiety disorder. *Eur. Arch. Psychiatry and Clin. Neurosci.* 2011; 261:303–307. [PubMed: 20820793]
- Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika.* 1965; 52:591.
- Starcevic V, Berle D. Cognitive specificity of anxiety disorders: a review of selected key constructs. *Depression Anxiety.* 2006; 23:51–61. [PubMed: 16402368]
- Strawn JR, Wegman CJ, Dominick KC, Swartz MS, Wehry AM, Patino LR, Strakowski SM, Adler CM, Eliassen JC, DelBello MP. Cortical surface anatomy in pediatric patients with generalized anxiety disorder. *J. Anxiety Disord.* 2014; 28:717–723. [PubMed: 25155256]
- Strawn JR, Wehry AM, Chu WJ, Adler CM, Eliassen JC, Cerullo MA, Strakowski SM, Delbello MP. Neuroanatomic abnormalities in adolescents with generalized anxiety disorder: a voxel-based morphometry study. *Depression Anxiety.* 2013; 30:842–848. [PubMed: 23495075]
- Strawn JR, Wehry AM, DelBello MP, Rynn MA, Strakowski S. Establishing the neurobiologic basis of treatment in children and adolescents with generalized anxiety disorder. *Depression Anxiety.* 2012; 29:328–339. [PubMed: 22511364]
- Tromp DP, Grupe DW, Oathes DJ, McFarlin DR, Hernandez PJ, Kral TR, Lee JE, Adams M, Alexander AL, Nitschke JB. Reduced structural connectivity of a major frontolimbic pathway in generalized anxiety disorder. *Arch. Gen. Psychiatry.* 2012; 69:925–934. [PubMed: 22945621]
- van Tol MJ, van der Wee NJ, van den Heuvel OA, Nielen MM, Demenescu LR, Aleman A, Renken R, van Buchem MA, Zitman FG, Veltman DJ. Regional brain volume in depression and anxiety disorders. *Arch. Gen. Psychiatry.* 2010; 67:1002–1011. [PubMed: 20921116]
- Wehry AM, McNamara RK, Adler CM, Eliassen JC, Croarkin P, Cerullo MA, DelBello MP, Strawn JR. Neurostructural impact of co-occurring anxiety in pediatric patients with major depressive

disorder: a voxel-based morphometry study. *J. Affect. Disord.* 2015; 171:54–59. [PubMed: 25285899]

Wittchen, H-U.; Pfister, H. DIA-X Interview. Frankfurt: Swets & Zeitlinger; 1997.

Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jonsson B, Olesen J, Allgulander C, Alonso J, Faravelli C, Fratiglioni L, Jennum P, Lieb R, Maercker A, van Os J, Preisig M, Salvador-Carulla L, Simon R, Steinhausen HC. The size and burden of mental disorders and other disorders of the brain in Europe 2010. *Eur. Neuropsychopharmacol.* 2011; 21:655–679. [PubMed: 21896369]

Woo CW, Krishnan A, Wager TD. Cluster-extent based thresholding in fMRI analyses: Pitfalls and recommendations. *Neuroimage.* 2014; 91:412–419. [PubMed: 24412399]

Zhang Y, Li L, Yu R, Liu J, Tang J, Tan L, Liao M, Yang F, Shan B. White matter integrity alterations in first episode, treatment-naïve generalized anxiety disorder. *J. Affect. Disord.* 2013; 148:196–201. [PubMed: 23305653]

Fig. 1. Distribution of PSWQ and IUS-12 scores across both groups. HC: healthy control group; GAD: generalized anxiety disorder group; PSWQ: Penn State Worry Questionnaire; IUS-12: Intolerance of Uncertainty Scale 12.

Fig. 2.

Altered gray matter volumes in the right striatum. For better visual comparison, all images are presented at $z = -9$. Bars represent the mean proportion of gray matter volume in the whole significant cluster; error bars represent the standard error. Upper image: significant region and group comparison of gray matter values. Middle image: significant region and regression plot of gray matter values and PSWQ scores. Lower image: significant region and regression plot of gray matter values and IUS-12 scores. Blue triangles represent healthy subjects and red squares represent GAD subjects in the regression plots. HC: healthy control

group; GAD: generalized anxiety disorder group; R: right side; PSWQ: Penn State Worry Questionnaire; IUS-12: Intolerance of Uncertainty Scale 12; analysis: minimum cluster size = 200; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

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Table 1

Sample characteristics.

	HC (<i>n</i> = 24)	GAD (<i>n</i> = 19)	<i>chi</i> ² / <i>df</i>	<i>p</i>
<i>Sociodemographic characteristics</i>				
Female sex (<i>n</i> , %)	17 (70.8)	16 (84.2)	1.063 (1)	0.302
Age (years; mean, sd)	32.25 (9.33)	33.47 (8.90)	0.436 (41)	0.665
Right-handed (<i>n</i> , %)	21 (87.5)	17 (89.5)	0.040 (1)	0.841
Secondary school (<i>n</i> , %)	18 (75.0)	12 (63.2)	1.641 (2)	0.440
Non-smoker (<i>n</i> , %)	22 (95.7)	19 (100.0)	0.846 (1)	0.358
<i>Clinical characteristics</i>				
PSWQ (mean, sd)	36.00 (9.98)	62.00 (6.22)	9.921 (41)	< 0.001
BDI (mean, sd)	4.50 (4.82)	21.16 (7.37)	8.931 (41)	< 0.001
IUS-12 (mean, sd)	25.75 (6.72)	41.00 (7.03)	7.241 (41)	< 0.001
STAI-T (mean, sd)	33.58 (6.77)	56.16 (7.11)	10.621 (41)	< 0.001

HC: healthy control group; GAD: Generalized Anxiety Disorder group; PSWQ: Penn State Worry Questionnaire; BDI: Beck Depression Inventory-II; IUS-12: Intolerance of Uncertainty Scale-12; STAI-T: State-Trait-Anxiety-Index, Trait version.

Table 2

Whole brain analysis on group differences in gray matter volume.

Contrast	Region	Side	Voxels	t	x	y	z	cFWE
GAD > HC								
Striatum		R	2507	4.95	33	-10	-9	0.002
Superior temporal pole		R	294	4.76	35	14	-23	n.s.
HC > GAD: no significant differences								

HC: healthy comparison group; GAD: generalized anxiety disorder group; R: right side; L: left side; Voxels: number of voxels per cluster; x, y, z: MNI coordinates of peak voxel; n.s.: non-significant; main analysis: minimum cluster size = 200; $p < 0.001$, corrected for non-stationarity. Additionally, the level of significance using clusterwise FWE (p_{cFWE}) is given.

Table 3

Whole brain analysis on group differences in white matter volume.

Contrast	Region	Side	Voxels	t	x	y	z	cFWE
GAD > HC								
Occipital lobe		L	254	4.34	-27	-76	4	n.s.
HC > GAD								
Middle frontal lobe		L	261	4.94	-33	14	46	n.s.

HC: healthy control group; GAD: generalized anxiety disorder group; L: left side; Voxels: number of voxels per cluster; x, y, z: MNI coordinates of peak voxel; n.s.: non-significant; main analysis: minimum cluster size = 200; $p < 0.001$, corrected for non-stationarity. Additionally, the level of significance using clusterwise FWE (p_{cFWE}) is given.

Table 4

Whole brain analysis: Associations between dimensional worry severity and gray matter volume.

Contrast	Region	Side	Voxels	t	x	y	z	cFWE
Worry: linear positive relationship								
Striatum		R	1771	5.19	8	20	1	0.016
Caudate nucleus		L	623	4.55	-5	17	1	n.s.
		R	434	4.73	33	-19	-3	n.s.
Worry: linear negative relationship								
MCC		L	343	4.29	-14	23	34	n.s.
Paracentral lobule		R	262	4.06	11	-30	57	n.s.
Supplementary motor area		R	271	4.63	12	-6	55	0.041

R: right side; L: left side; Voxels: number of voxels per cluster; x, y, z: MNI coordinates of peak voxel; n.s.: non-significant; main analysis: minimum cluster size = 200; $p < 0.001$, corrected for non-stationarity. Additionally, the level of significance using clusterwise FWE (p_{cFWE}) is given.

Table 5
Whole brain analysis: Associations between dimensional worry severity and white matter volume.

Contrast	Region	Side	Voxels	t	x	y	z	cFWE
Worry: linear positive relationship: no significant differences								
Worry: linear negative relationship								
	Cerebellum	R	446	4.63	35	-48	-51	n.s.
	Middle frontal lobe	L	270	5.12	-32	9	46	n.s.
	Precentral lobe	R	236	4.85	20	-25	69	n.s.

R: right side; L: left side; Voxels: number of voxels per cluster; x, y, z: MNI coordinates of peak voxel; n.s.: non-significant; main analysis: minimum cluster size = 200; $p < 0.001$, corrected for non-stationarity. Additionally, the level of significance using clusterwise FWE (p_{cFWE}) is given.

Table 6

Whole brain analysis: associations between dimensional intolerance of uncertainty and gray matter volume.

Contrast	Region	Side	Voxels	t	x	y	z	cFWE
Intolerance of uncertainty: linear positive relationship								
Superior temporal pole	R		425	5.28	36	12	-23	0.038
	R		277	4.82	23	0	-41	n.s.
Fusiform gyrus	R		719	3.84	20	0	-9	n.s.
Intolerance of uncertainty: linear negative relationship								
MCC	L		392	4.76	-14	24	34	n.s.
Paracentral lobule	R		514	4.51	11	-31	58	n.s.

R: right side; L: left side; Voxels: number of voxels per cluster; x, y, z: MNI coordinates of peak voxel; n.s.: non-significant; main analysis: minimum cluster size = 200; $p < 0.001$, corrected for non-stationarity. Additionally, the level of significance using clusterwise FWE ($pcFWE$) is given.

Table 7
Whole brain analysis: Associations between dimensional intolerance of uncertainty and white matter volume.

Contrast	Region	Side	Voxels	t	x	y	z	cFWE
Intolerance of uncertainty: linear positive relationship: no significant results								
Intolerance of uncertainty: linear negative relationship								
Cerebellum		R	635	5.00	9	-33	-12	n.s.

L: left side; Voxels: number of voxels per cluster; x, y, z: MNI coordinates of peak voxel; n.s.: non-significant; main analysis: minimum cluster size = 200; $p < 0.001$, corrected for non-stationarity. Additionally, the level of significance using clusterwise FWE (p_{cFWE}) is given.