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Abstract

Generalized anxiety disorder (GAD) is associated with both autonomic dysfunction, notably decreased vagally-mediated heart rate variability (vmHRV), and neurostructural abnormalities. Regional differences in brain morphometry correlate with vmHRV in healthy individuals. Here, we tested the hypothesis that specific focal abnormalities in cortical structure in GAD underpin decreased vmHRV. Adult female patients with GAD (n = 17) and matched controls (n = 18) underwent structural magnetic resonance imaging after characterization of symptoms and quantification of resting vmHRV derived from continuous pulse oximetry. Cortical reconstruction was performed using the *FreeSurfer* image analysis suite. A priori analysis was conducted only within brain regions involved in vagal control of heart rate. Compared to controls, patients with GAD showed cortical thinning of the (i) left rostral anterior cingulate cortex, (ii) left medial orbitofrontal cortex, and (iii) right isthmus cingulate gyrus. Significant negative relationships were identified between the severity of anxiety symptoms and cortical thickness of the left medial orbitofrontal cortex and right isthmus cingulate gyrus. Compared to controls, patients with GAD showed decreased vmHRV at rest. In controls only, cortical thickness of the left caudal anterior cingulate cortex correlated positively with resting vmHRV. These results extend evidence in GAD for structural abnormalities within cortical areas implicated in emotion regulation and cognition. In addition, these findings may implicate abnormal integrity of anterior cingulate cortex in the psychophysiological expression of GAD and suggest that interventional targeting of this region may normalize autonomic function in GAD.

Keywords: autonomic function; cortical thickness; generalized anxiety disorder; heart rate variability

Abbreviations: ACC = anterior cingulate cortex; GAD = generalized anxiety disorder; GM = grey matter; HC = healthy controls; fMRI= functional magnetic resonance imaging; LH = left hemisphere; OFC = orbitofrontal cortex; PCC = posterior cingulate cortex; RH = right hemisphere; vmHRV = vagally-mediated heart rate variability

1. Introduction

Generalized anxiety disorder (GAD) is conceptualized as an affective disorder, characterized by deficits in emotional expressivity and self-regulation (Mennin et al., 2008). Core symptoms of GAD include excessive, uncontrollable worry and chronic anxiety (American Psychiatric Association, 2013). These experiences are typically accompanied by a number of cognitive (e.g., poor concentration, memory deficits) and physical (e.g., cardiovascular arousal, muscle tension, fatigue) symptoms that together cause substantial functional impairment in daily life (Olfson et al., 1997; Wittchen et al., 1994). GAD has a very high life time prevalence (approximately 5%) and is more than twice as common in women than men (Wittchen, 2002). Nevertheless, GAD's underlying pathophysiology remains poorly understood.

The notion that the autonomic nervous control of physiological arousal is a critical process in emotion regulation is supported by psychophysiological and anatomical evidence (Porges 2007; Thayer and Lane, 2009). The Polyvagal Theory (Porges, 2001; 2007) proposes functional and anatomical relationships between autonomic functioning, adaptive behaviour and potentially psychopathological states. When vagal control becomes dysfunctional, this is typically accompanied by (and may even cause) problems in inhibition (e.g., poor cognitive control, emotion dysregulation). In severe form, emergent problems with cognitive, affective and physiological self-regulation may manifest as GAD or related disorders (Thayer and Lane, 2000; 2009). Vagal modulation of cardiac activity, indexed by vagally-mediated heart rate variability (vmHRV), can therefore be conceived as a transdiagnostic biomarker of psychopathology (Appelhans and Luecken, 2006; Beauchaine and Thayer, 2015; Sgoifo et al., 2015). The theoretical association of GAD with reduced vagal modulation is supported by studies reporting a negative relationship between GAD and vmHRV at rest (Thayer et al., 1996; Pittig et al., 2013; Chalmers et al., 2014). Notably, low resting cardiac vagal modulation is recognized as a potential pathoaetiological mechanism underlying observed increases in cardiac risk in patients with GAD (Cohen and Benjamin, 2006; Thayer et al., 2010). Nevertheless, the presence of a sustained decrease in resting vagal modulation in GAD has been questioned; some studies suggest that patients

with GAD show phasic reductions in vmHRV only during worry and (experimentally) during negative imagery inductions (Fisher and Newman, 2013; Levine et al,. 2016).

Pharmacological, lesion, and functional neuroimaging approaches both in humans and animals implicate a distributed set of cortical, limbic and brainstem structures in the neural control of HRV (Ter Horst and Postema, 1997; Ahern et al., 2001; Gianaros et al., 2004; Lane et al., 2009; Buchanan et al., 2010; Thayer et al., 2012). These structures form a major part of what is collectively termed the "central autonomic network" (Benarroch, 1993; Thayer and Lane, 2009). Specific regions, for example the anterior cingulate cortex (ACC) are recognized as pivotal in the regulation of HRV (Critchley et al. 2003). Notably, individual differences in vmHRV appear to relate to the structural integrity of the ACC. For example, vmHRV was observed to be positively associated with the grey matter volume of right ACC in 77 predominantly male middle-aged Vietnam war veterans (Woodward et al., 2008). Similarly, several independent studies have found positive associations between resting state vmHRV and cortical thickness of the ACC in healthy adults (Winkelman et al., 2017; Carnevali et al., 2018; Yoo et al., 2018). Subtle structural differences in brain are also observed in patients with GAD, including, with some reliability, higher gray matter (GM) volumes of the amygdala (Etkin et al., 2009; Schienle et al., 2011; Makovac et al., 2016a) and lower GM volumes of the hippocampus (Abdallah et al., 2013; Moon et al., 2014). Increased GM volume of the dorsomedial prefrontal cortex (Schienle et al., 2011) and reduced GM volume of the ACC (Shang et al., 2014) are also documented.

Within the present study, we sought to identify brain structural differences in GAD that potentially underlie concomitant autonomic dysfunction, indexed by reduced vmHRV. To address this aim, we investigated structural differences between patients with GAD and HC in brain regions associated with vagal control of heart rate, i.e. various substructures of the cingulate gyrus (rostral ACC, caudal ACC, posterior cingulate cortex (PCC), isthmus cingulate gyrus), prefrontal cortex (superior frontal gyrus, lateral and medial orbitofrontal cortices (OFC)), and the insula (Gianaros et al., 2004; Critchley et al., 2011; Thayer et al., 2012; Beissner et al., 2013). We employed the measurement of cortical thickness, which is suggested to be a more sensitive parameter with a higher signal-to-noise ratio (Dickerson et

al., 2008; Hutton et al., 2009) and more easily interpretable than the probabilistic GM volumes in voxel-based morphometry (Lehmann et al., 2011).

2. Materials and methods

2.1. Participants

The present study is based on a secondary analysis of baseline data from a subsample of volunteers enrolled in a larger longitudinal functional magnetic resonance imaging (fMRI) by public advertisement (Makovac et al., 2016a; 2016b; 2016c; Meeten et al., 2016; Ottaviani et al., 2016). In order to decrease inter-individual variation, we considered only female participants (n = 37) as they represented the large majority of the initial sample. Two of them were excluded because of severe artifacts in neuroimaging and peripheral physiology data. The final sample comprised 17 women who met diagnostic criteria for GAD, and 18 HC. All participants were right-handed, native English speakers, and had normal or corrected-to-normal vision. Exclusion criteria were: age younger than 18 years, past head injury or neurological disorders, prior history of major medical or psychiatric disorder (other than GAD for the patient group), cognitive impairment, history of substance or alcohol abuse or dependence, diagnosis of heart disease, obesity (body-mass index > 30 kg/m^2), pregnancy, claustrophobia or other general MRI exclusions. Patients and controls were medication free. The average self-reported disease duration was 17.6 (± 7.6) years and none of our patients had a formal diagnosis of any other psychiatric disorder. The study was approved by the National Research Ethics Service (NRES) for the National Health Service (NHS) with local approval of the Brighton and Sussex School Research Governance and Ethics. All procedures were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study, who were compensated (£55) for their participation.

2.2. Procedure

After a pre-screening phone interview to rule out exclusion criteria, participants underwent the *Structured Clinical Interview for DSM-IV* (SCID; First et al., 2002), administered by a trained postdoctoral fellow (FM) to both patients and controls to confirm/exclude the diagnosis of GAD. Participants then completed a series of questionnaires, were subsequently familiarized with the neuroimaging environment, connected to the physiological recording equipment, and underwent the structural MRI brain scan. Subsequently, after a 5-min resting period, participants performed a low demand behavioral task, which was repeated before and after the induction of perseverative cognition and was each time followed by a 5-min resting period. The current study focus on vmHRV data obtained from the first 5-min resting period following the structural brain MRI scan only; therefore, there is no reason to assume that these vmHRV data were influenced by the experimental tasks.

2.3. Questionnaires

Participants completed a series of sociodemographic (age, years of education), lifestyle (nicotine, alcohol and caffeine consumption, physical activity) and psychometric questionnaires, including the *State-Trait Anxiety Inventory* (STAI; Spielberg et al., 1983), and the *Penn State Worry Questionnaire* (PSWQ; Meyer et al., 1990). The severity of trait anxiety was measured using the trait version of the STAI (STAI-T), which is a quadruplet Likert type scale consisting of 20 items assessing how the patient feels, independent from the current status and circumstances. The lowest score that can be obtained is 20 and the highest is 80. Greater scores indicate greater anxiety levels and lower scores indicate lower levels of anxiety. The validity of the scale has been repeatedly confirmed, with reliability coefficients ranging from 0.71 to 0.86 and good internal consistency and homogeneity coefficients between 0.83 and 0.87 (Aydemir and Köroğlu, 2000). Of note, STAI-T scores highly correlated with scores of the state version of the STAI ($r = 0.87$, $p < 0.01$), indicating that self-reported "trait" estimates were not influenced by state anxiety differences. The severity of worry was measured using the PSWQ (Meyer et al., 1990), which consists of 16 items assessing trait worry closely associated

with GAD. The items are rated on a 5-point Likert scale and are summarized in a total score ranging from 16 to 80. The PSWQ shows test-retest correlations of at least .85, and excellent convergent validity with other measures of worry (Molina and Borkovec, 1994).

2.4. MRI acquisition and processing

Brain structural MRI scans (0.9 mm isometric voxels, 192 sagittal slices, repetition time 11.4 ms, echo time 4.4. ms, inversion time 300 ms) were acquired using a Siemens Avanto 1.5 T scanner (32-channel head coil, Siemens, Erlagen, Germany). The T1 weighted (MPRAGE) volumes from all participants were visually reviewed to exclude the presence of macroscopic artifacts. The acquired data were processed using *FreeSurfer* for preliminary pre-processing, tissue classification, reconstruction of the grey matter and the grey/white matter boundary surfaces, and cortical parcellation by means of the Desikan-Killany atlas (Desikan et al., 2006). Those steps are comprehensively described in the related literature (Dale et al., 1999; Fischl et al., 1999). As a result, we quantified global and regional mean cortical thickness by computing the distance between the grey matter and the grey/white matter boundary surfaces. This technique guarantees good reliability (Han et al., 2006; Jovicich et al., 2006).

2.5. Physiological data recording and processing

We adhered to the *Guidelines for Reporting Articles on Psychiatry and Heart rate variability* (GRAPH) in our reporting of vmHRV processing and analyses (Quintana et al. 2016). Pulse-to-pulse intervals – an approximation of beat-to-beat intervals– were measured using a photoplethysmograph placed on the right index finger (50 Hz) during a 5-min resting period in which participants were asked to keep their eyes open, without falling asleep and without thinking about anything. While pulse interval is not a direct interbeat interval measure, as it is derived from both the time between pulse wave initiation (i.e. sinoatrial node firings) and the changes in pulse transit time, it offers an accurate approximation of interbeat intervals during immobility (Lu et al., 2009; Gil et al., 2010). As per recommendations (Task

Force, 1996), raw data were up-sampled at 1000 Hz to refine the R-wave fiducial point for vmHRV calculation using RHeart Rate Variability (RHRV) 4.0 analysis software from the R Project (http://rhrv.r-forge. r-project.org/). Potential artifacts were visually identified and manually removed and the root mean square successive difference (RMSSD), which is a reliable index of vmHRV (Task Force 1996), was derived. RMSSD is stable over short recording intervals (Nussinovitch et al,. 2012), is relatively free of respiratory influences (Penttila et al., 2001), and is particularly suited to capture autonomic perturbation in the context of anxiety disorders (Alvares et al., 2013). From now on, we will use the term vmHRV to refer to RMSSD.

2.6. Statistical analyses

Data analysis was performed with SPSS 24.0 for Windows (SPSS Inc, USA). Statistical significance was set at $p \leq 0.05$. Assumptions for normality were tested for all continuous variables using the Shapiro-Wilk test. We accounted for non-normal distribution of vmHRV values ($p < 0.001$) by calculating its natural logarithm (ln(vmHRV)). STAI-T scores were normally distributed across the whole sample ($p = 0.329$), which was not the case for the PSWQ ($p = 0.024$; see Figure 1 for the distributions of these questionnaire scores within each group).

First, socio-demographic and lifestyle variables were compared between the two groups using *t*- and Chi-square tests, as appropriate. Second, to test for group differences in the main variables of the study, STAI-T scores and ln(vmHRV) values were compared by means of t-tests, while PSWQ scores were compared by means of the Mann-Whitney test.

A multivariate General Lineal Model (GLM) was implemented to compare mean cortical thickness of specific regions of interest (ROIs) between patients with GAD and HC, controlling for the effects of age and global mean cortical thickness. As already mentioned, we focused on those brain regions implicated in vagal control of HR, a process that is compromised in anxious individuals. We systematically ran post hoc pair-wise comparisons, which were corrected for multiple comparisons using the Bonferroni test.

Then, given that dimensional models of psychopathology have steered emphasis beyond categorical group comparisons (American Psychiatric Association, 2013; Cuthbert, 2014), partial correlations of severity of anxiety (STAI-T score) and worry (PSWQ score) symptoms alongside cortical thickness of all selected ROIs were computed, adjusting for age and global mean cortical thickness.

To answer the question whether brain structural differences in the selected ROIs significantly predict vmHRV as a function of Group (GAD vs HC) and/or dimensional symptoms of anxiety (scores on the STAI and PSWQ), we performed a series of GLMs, using ln(vmHRV) as the outcome variable, and incorporating as factors 1) age, 2) global mean cortical thickness, 3) Group (GAD vs HC), 4) STAI-T, 5) PSWQ, 6) cortical thickness of each determined region (one for each model), and the interaction terms of cortical thickness with 7) Group, 8) PSWQ, and 9) STAI as predictors. GLMs for each brain region were performed separately for right and left hemispheres.

3. Results

3.1. Socio-demographic, lifestyle and clinical data

Patients with GAD and HC were well matched regarding all socio-demographic and lifestyle characteristics, which are listed in Table 1.

As expected, patients with GAD showed significantly higher scores on STAI-T and PSWQ than HC. In addition, individuals with GAD had significant lower resting ln(vmHRV) compared to HC with a medium size effect. Table 2 reports clinical and peripheral physiology characteristics of the two groups.

3.2. Structural brain MRI results

There was no overall difference between GAD and HC participants in global mean cortical thickness (GAD = 2.44 ± 0.2 mm vs HC = 2.46 ± 0.3 mm). However, individuals with GAD showed reduced mean cortical thickness across specific regions including (i) the left rostral ACC (explaining 12% of the variance), (ii) the left medial orbitofrontal cortex (OFC) (explaining 17% of the variance), and (iii) the right isthmus cingulate gyrus (explaining 23% of the variance), when compared to HC. These

differences were apparent after adjusting for the effects of age and global cortical thickness (Table 3; Figure 2). No other significant group differences in mean cortical thickness were found for the other ROIs (Table 3). In dimensional analyses, we observed a negative relationship between cortical thickness in the left medial OFC and ratings of worry ($r = -0.368$, $p = 0.035$) and trait anxiety severity ($r = -0.448$, $p = 0.009$), independently from group. Similarly, cortical thickness in the right isthmus cingulate gyrus was negatively associated with ratings of worry ($r = -0.501$, $p = 0.003$) and trait anxiety severity ($r = -0.473$, $p = 0.005$), independently from group.

3.3. Cortical structural characteristics predicting vmHRV as a function of GAD diagnosis

Controlling for age and global mean cortical thickness, we observed that ln(vmHRV) was predicted by left caudal ACC cortical thickness (explaining 14% of the variance). ln(vmHRV) was also predicted by a significant interaction between group and left caudal ACC cortical thickness (explaining 18% of the variance) (Table 4). *Post hoc* exploration of this interaction showed that left caudal ACC cortical thickness strongly predicted ln(vmHRV) in HC ($r^2 = 0.409$), but not in patients with GAD ($r^2 = 0.006$) (Figure 2).

4. Discussion

This study tested the hypothesis that autonomic dysfunction (indexed by reduced vmHRV) is associated with brain structural changes in patients with GAD. Overall, the present results extend earlier observations on the brain structural correlates of GAD by providing evidence for cortical thinning in areas involved in emotion regulation and cognition, notably the rostral ACC, the medial OFC, and the isthmus cingulate gyrus, and confirm the presence of reduced vmHRV in this clinical group. Intriguingly, cortical thickness of the left caudal ACC appears to diverge in its association with vmHRV as a function of GAD, providing preliminary evidence of disrupted central-autonomic integration in GAD.

4.1. Reduced resting state vmHRV in GAD

As expected, female patients with GAD show decreased resting state vmHRV, reinforcing similar results from previous studies (Thayer et al., 1996; Pittig et al., 2013; Chalmers et al., 2014). Current psychophysiological theories provide a framework to interpret the link between GAD and decreased resting vagal activity. Among these, the Polyvagal Theory (Porges, 2001; 2007) highlights the importance of vagal activity in attention, expression of emotions, social bonding, and flexible adjustment to environmental demands, which are all compromised in patients with GAD (Bradley et al., 1999). Vagal activity serves to dampen stress-induced cardiovascular sympathetic activation and promote states of physiological and behavioral calm, and self-regulation (Porges, 2001; 2007). Without this protective function of vagal activity, individuals are more vulnerable to anxious apprehension and worry, which are core symptoms of GAD (Bradley et al., 1999). The Neurovisceral Integration Model also elaborates the conceptualization of decreased resting vagal activity as a core feature of GAD, emphasizing the inability to disengage from states of threat detection. This sustained threat response consequently serves to perpetuate hyperarousal and worry, even when no real threat exists (Thayer and Lane, 2000; 2009).

4.2. Brain structural characteristics underlying reduced vmHRV in GAD

Neuroimaging research in GAD provides increasing evidence for structural alterations in medial temporal lobe regions including the hippocampus and the amygdala (Etkin et al., 2009; Schienle et al., 2011; Abdallah et al., 2013; Moon et al., 2014; Makovac et al., 2016a), consistent with a large body of evidence implicating the latter in fear and threat processing (LeDoux, 2003), as well as in autonomic regulation (LeDoux, 2000; Makovac et al., 2016a). The literature on structural alterations in other regions in GAD is inconsistent. For example, findings of reduced GM volume in the ACC, especially in its rostral-ventral subdivision (Shang, et al. 2014) or increased GM volume in the dorsomedial prefrontal cortex (Schienle et al., 2011) are reported, yet not replicated in other studies. Additionally, it

is unclear if structural alterations explain disrupted neural control of vmHRV, leading to autonomic dysfunction.

We found that the GAD group is characterized by cortical thinning of the left rostral subdivision of the ACC. This result extends earlier work documenting structural changes (i.e. reduced GM volume) of the rostral ACC in patients with GAD (Shang et al., 2014). Human neuroimaging studies show that the rostral ACC plays a key role in the regulation of emotional processing via top-down regulation of the amygdala, and that ACC-amygdala circuitry appears to be disrupted in anxiety disorders, particularly when processing threatening information (Goodkind et al., 2013; Kim et al., 2011). The rostral ACC is more active when participants are asked to regulate conflicting emotional information (Etkin et al., 2006), avoid attending to irrelevant emotional information (Bishop et al., 2004), or exercise top-down control upon processing of emotional stimuli (Ochsner et al., 2004), highlighting its joint roles in emotion representation and regulation (Smith et al., 2014, Smith and Lane, 2015). In line with this view, inflexible activation in the rostral ACC has been associated with the inability to shift attention away from negative self-related stimuli (Wagner et al., 2015). Interestingly, in a previous fMRI study reduced activation of the ACC was reported during feelings of guilt in patients with obsessive-compulsive disorder, who, similarly to the present sample with GAD, had significantly higher STAI trait scores than controls (Basile et al., 2014). It is critical to note that the cortical thinning of the rostral ACC was not significantly associated with the severity of anxiety symptoms in the present study. Taken together, data suggest that this particular region may be associated with emotion regulation difficulties to a greater extent than anxiety per se, a possibility that is supported by the ability of the rostral ACC to predict psychotherapeutic outcome in anxiety and mood disorders (e.g., Siegle et al., 2012).

Recent computational models revealed that the ACC is a central hub for the interactions between cognitive control, emotion regulation, and vagal modulation (Smith et al., 2017). In this study, despite the absence of significant differences in terms of cortical thickness within the caudal ACC between patients and controls, structural characteristics of this specific sub-region appear to diverge in their

association with vmHRV as a function of group. More specifically, cortical thickness in this area is positively associated with resting vmHRV in HC, replicating previous results (Woodward et al., 2008; Winkelman et al., 2017; Carnevali et al., 2018; Yoo et al., 2018), but not in patients with GAD. One speculative interpretation for this finding is that cortical thinning of the rostral subdivision of the ACC disrupts neural control of vmHRV by the caudal ACC, leading to autonomic dysfunction in patients with GAD. However, given that HRV is a marker of poor peripheral cardiovascular health and cardiovascular disease risk (Thayer et al., 2010), we cannot rule out the possibility that poor peripheral cardiovascular health might lead to poor neurovascular health and loss of cortical tissue. Longitudinal studies are necessary to clarify the relationship between cortical thinning of the ACC and reduced vmHRV in patients with GAD to address potential (1) top–down (i.e., decline in cortical thickness favoring reduced vmHRV), (2) or bottom–up (i.e., decline in vmHRV favoring reduced cortical thickness) causality. However, the demonstration that cortical thickness of the left caudal ACC correlates with a proxy (i.e., vmHRV) of neurovisceral regulation in controls but not in patients with GAD opens an interesting avenue to understanding the hidden neural code of visceral regulation (see Lucini et al., 2018), in line with historical models emphasizing the unitary organization of bodily functions (Hess, 2014).

4.3. Brain structural changes in GAD

Besides cortical thinning of the left rostral ACC, the GAD group is characterized by reduced cortical thickness of the left medial OFC and right isthmus cingulate cortex. Importantly, cortical thickness of the left medial OFC and right isthmus cingulate cortex is negatively associated with self-report ratings of worry and trait anxiety. Notably, these brain areas overlap mainly with default mode network areas that play a central role in self-referential processes (Davey et al., 2016). Volume reductions in the medial OFC have previously been reported in individuals diagnosed with post-traumatic stress disorder (Rauch et al., 2003) and obsessive-compulsive disorder (Szeszko et al., 1999). Moreover, trait anxiety is negatively correlated with cortical thickness of the right, but not left, medial OFC in a

healthy sample (Kuhn et al., 2011). Cortical thinning of the medial OFC might therefore constitute a structural neural correlate of different types of anxiety spectrum disorders, including GAD. Human neuroimaging studies demonstrate that cortical thickness (Milad et al., 2005) and brain activity of the medial OFC (Milad et al., 2007) correlate positively with extinction recall, indicating that structural alterations in this region may lead to less flexibility in controlling fear, and may make people more susceptible to emotional trauma. Animals with an orbitofrontal lesion act as if they were using the same reinforcement learning algorithms as non-lesioned animals, but operating on an impoverished representation of the task structure, leading several authors to conclude that the OFC may be the repository for consolidated representations of the causal structure of a task (Gershman et al., 2015; Wilson et al., 2014). Less is known about the function of the isthmus cingulate, yet there is evidence of its involvement in memory and pain processing (Nielsen et al., 2005), and in mood symptoms (e.g. anhedonia and affective flattening (Whitford et al., 2014)).

4.4 Study limitations and conclusion

There are limitations to this study: first, due to difficulties in recruiting GAD patients who were not on medication, the sample size is modest and should be extended in the future. Second, we included only female participants in order to decrease inter-individual variation and therefore these results cannot be generalized to men. Nevertheless, the clinical group can be considered as homogeneous since the patients did not suffer from co-morbid psychiatric disorders, notably major depression, at the time of the testing and they were currently not on medication. Third, the present study used pulse oximetry data to calculate vmHRV, rather than the more common approach of using ECG. This limits direct comparison with studies that use ECG. Moreover, data acquisition using photoplethysmography may be suboptimal because these devices cannot discriminate between sinus and non-sinus beats (Lucini et al., 2017). Therefore, caution must be paid in interpreting our vmHRV results. Furthermore, cortical thickness measures only permit evaluation of the cortical surface, and thus do not allow analysis of deeper structures including amygdala or even hippocampus. Lastly, as discussed above, the present

study cannot answer the question of whether the structural abnormalities in the GAD group are acquired and progressive, reflecting the course of the illness, or the extent to which they represent a developmental predisposition to GAD. It will thus be important to investigate whether cortical thickness in these brain areas undergoes a progressive decrease over time. Further research with larger samples and a longitudinal design is needed to further examine structural brain concomitants of autonomic dysfunction in GAD.

To conclude, this is not the first study that has described disrupted cortico-autonomic integration in psychopathological disorders (e.g., Smith et al., 2015 and Koenig et al., 2018a for major depression; Thome et al., 2017 for PTSD). A recent pilot study, however, found that it is possible to increase cortical thickness in the orbitofrontal cortex of adolescents with depression by means of 8-week treatment with a selective serotonin reuptake inhibitor, and that this is associated with increased HRV (Koenig et al., 2018b). Our study is the first to implicate abnormal structural integrity of the ACC in the expression of reduced vmHRV in GAD, suggesting that interventional targeting of this region may represent a clinically valid approach to normalize autonomic function in this psychopathological condition.

Tables

Table 1. Sociodemographic and lifestyle characteristics.

	GAD (*n* = 17)	HC (*n* = 18)	*t/χ2*	*p*
Age (years)	30.7±2.0	27.2±1.7	1.29	0.206
BMI (kg/m^2)	22.8±0.8	23.3±0.7	0.44	0.663
Education (years)	13.1±0.4	12.2±0.6	1.21	0.223
Smoking status	5Y, 12N	3Y, 15N	0.81	0.369
Cigarettes per day (smokers only)	4.1±1.7	4.3±2.8	0.08	0.942
Alcohol (units/week)	4.2±0.8	3.9±0.9	0.32	0.748
Coffee/caffeinated drinks (cups/day)	2.5±0.4	2.1±0.4	0.70	0.488
Perceived physical fitness	11H, 5M, 1L	8H, 9M, 1L	1.59	0.452

Note. Data are reported as means ± standard errors. Abbreviations: GAD = generalized anxiety disorder; HC = healthy controls; BMI = body mass index; Y = yes; N = no; H = high; M = moderate; L = low.

Table 2. Differences between the two groups in the clinical and cardiac variables of the study.

	GAD (*n* = 17)	HC (*n* = 18)	*t/U*	*p*	Effect size (Cohen's *d*)
STAI-T (score)	52.6±2.3	37.1±2.4	4.70	< 0.001	1.592
PSWQ (score)	67.6±2.7	44.3±3.3	28.5	< 0.001	1.857
ln(vmHRV)	3.8±0.1	4.2±0.2	2.05	0.048	0.690

Note. Data are reported as means ± standard errors. Abbreviations: GAD = generalized anxiety disorder; HC = healthy controls; STAI-T = State-Trait-Anxiety-Inventory, Trait version; PSWQ = Penn State Worry Questionnaire; ln(vmHRV) = natural logarithm of vagally-mediated heart rate variability.

Table 3. Differences in cortical thickness of predefined regions of interest (ROIs) between patients with generalized anxiety disorders and healthy controls, controlling for the effects of age and total cortical thickness.

Brain region	Side	GAD (n = 17)	HC (n = 18)	F	p	Effect size (η_p^2)
Lateral OFC	LH	2.54±0.03	2.56±0.03	0.019	0.891	0.001
Lateral OFC	RH	2.49±0.03	2.51±0.04	0.004	0.949	0.000
Medial OFC	LH	2.38±0.04	2.49±0.03	6.578	0.015	0.175
Medial OFC	RH	2.31±0.04	2.36±0.04	0.396	0.534	0.013
Superior frontal gyrus	LH	2.75±0.04	2.74±0.04	1.996	0.168	0.060
Superior frontal gyrus	RH	2.14±0.02	2.14±0.03	0.414	0.524	0.013
Rostral ACC	LH	2.73±0.04	2.83±0.04	4.187	0.049	0.119
Rostral ACC	RH	2.67±0.05	2.69±0.04	0.005	0.943	0.000
Caudal ACC	LH	2.60±0.06	2.67±0.07	0.107	0.745	0.003
Caudal ACC	RH	2.45±0.04	2.50±0.04	0.159	0.693	0.005
PCC	LH	2.43±0.04	2.42±0.04	1.249	0.272	0.039
PCC	RH	2.36±0.02	2.44±0.04	3.046	0.091	0.089
Isthmus cingulate gyrus	LH	2.35±0.05	2.41±0.04	0.658	0.423	0.021
Isthmus cingulate gyrus	RH	2.27±0.03	2.41±0.04	9.435	0.004	0.233
Insula	LH	2.89±0.03	2.95±0.05	1.064	0.310	0.033
Insula	RH	2.89±0.03	2.98±0.05	1.953	0.172	0.059

Note. Data are reported as means ± standard errors and expressed in mm. Abbreviations: GAD = generalized anxiety disorder; HC = healthy controls; ACC = anterior cingulate cortex; OFC = orbitofrontal cortex; PCC= posterior cingulate cortex; LH = left hemisphere; RH = right hemisphere.

Table 4. General lineal model for the prediction of ln(vmHRV) via cortical thickness of the left caudal ACC as a function of categorical and dimensional models of GAD pathology, adjusting for the effects of age and total cortical thickness.

Parameter	*β*	*SE*	*t*	*p*	95 % *CI*	Effect size (η_p^2)
Intercept	14.11	5.30	2.67	0.013	3.21,25.02	0.221
Age (years)	-0.56	0.02	-3.09	0.005	-0.09,-0.02	0.227
Global cortical thickness (mm)	-0.33	1.06	-0.31	0.761	-2.51,1.86	0.004
Group (GAD, HC)	8.06	3.46	2.33	0.028	0.93,15.20	0.178
STAI-T (score)	-0.10	0.18	-0.06	0.956	-0.38,0.36	0.000
PSWQ (score)	-0.23	0.17	-1.36	0.187	-0.57,0.12	0.068
Caudal ACC LH thickness (mm)	-2.73	1.34	-2.04	0.052	-5.50,-0.03	0.142
Caudal ACC LH thickness * Group	-3.09	1.32	-2.34	0.028	-5.81,-0.36	0.179
Caudal ACC LH thickness * STAI-T	-0.06	0.07	-0.81	0.936	-0.15,0.14	0.000
Caudal ACC LH thickness * PSWQ	-0.09	0.07	1.38	0.180	-0.04,-0.22	0.071

Abbreviations: ln(vmHRV) = natural logarithm of vagally-mediated heart rate variability; GAD = generalized anxiety disorder; STAI-T = State-Trait-Anxiety-Inventory, Trait version; PSWQ = Penn State Worry Questionnaire; ACC = anterior cingulate cortex; LH = left hemisphere; SE = standard error; CI = confidence interval.

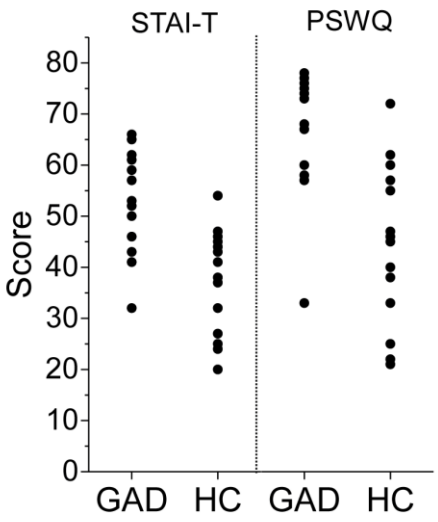

Fig. 1 Distribution of symptom severity by group. Abbreviations: GAD = generalized anxiety disorder; HC = healthy controls; STAI-T = State-Trait Anxiety Inventory, Trait version; PSWQ = Penn State Worry Questionnaire

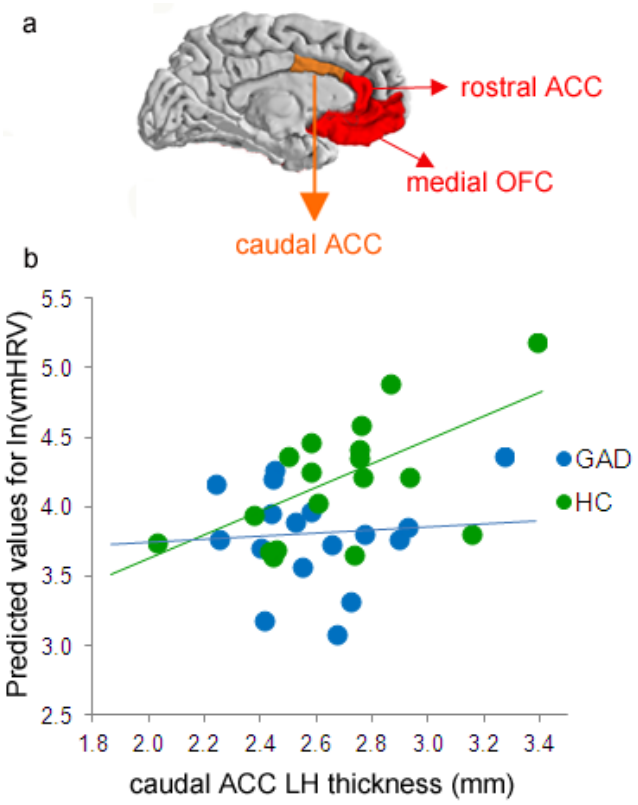

Fig. 2 Cortical surface representation of brain areas of the left hemisphere (in red) where patients with GAD showed reduced cortical thickness compared to HC, namely the medial OFC and rostral ACC. Panel a also highlights (in orange) the left caudal ACC, whose thickness differed in its association with vmHRV as a function of GAD. Panel b depicts the scatter plot of the prediction of ln(vmHRV) by cortical thickness of the left caudal ACC, in GAD (*n* = 17) and HC (*n* = 18). Abbreviations: ln(vmHRV)= natural logarithm of vagally-mediated heart rate variability; ACC = anterior cingulated cortex; OFC = orbitofrontal cortex; LH = left hemisphere; GAD = generalized anxiety disorder; HC = healthy controls

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