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Research paper

When worry may be good for you: Worry severity and limbic-prefrontal functional connectivity in late-life generalized anxiety disorder

M Wu[a], DS Mennin[b], M Ly[a], HT Karim[a], L Banihashemi[a], DL Tudorascu[c,d], HJ Aizenstein[a,e], C Andreescu[a,*]

[a] Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[b] Teachers College, Columbia University, New York City, NY, USA
[c] Department of Internal Medicine, Graduate School of Public health, Pittsburgh, PA, USA
[d] Department of Biostatistics, Graduate School of Public Health, Pittsburgh, PA, USA
[e] Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

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ABSTRACT

Background: Late-life generalized anxiety disorder (GAD) is one of the most common anxiety disorders in older adults. However, its neural markers have received relatively little attention. In this study, we explored the association between worry severity and limbic-prefrontal connectivity during emotional reactivity in late-life GAD.

Methods: We recruited 16 anxious (GAD) and 20 non-anxious (HC) older adults to perform the faces/shapes emotional reactivity task during functional magnetic resonance imaging (fMRI). We investigated the functional connectivity of both the amygdala and the bed nucleus of stria terminalis (BNST) with the prefrontal cortex (PFC) using generalized psychophysiological interaction (gPPI) analysis. We tested for (1) group differences in connectivity, (2) association between worry severity and connectivity, and (3) interaction between group and worry severity and its association with connectivity.

Results: Amygdala-PFC and BNST-PFC functional connectivity were associated with worry severity in an inverse U-shape, and was independent of depression severity, global anxiety, neuroticism, and general cognitive function.

Limitations: Our limitations include slightly skewed PSWQ distributions, lack of non-anxious individuals with high worry, small sample size, and low depression comorbidity in a sample of late-life GAD that may not generalize to GAD in younger populations.

Conclusions: This suggests that moderate worry is associated with maximum engagement of the limbic-PFC connectivity, while severe worry is associated with failure of the limbic-PFC emotional regulation circuit. This may explain the aberrant and exaggerated responses to negative stimuli observed in participants with pathological worry.

1. Introduction

Generalized anxiety disorder (GAD) is the most common anxiety disorder in older adults (Le Roux et al., 2005), with a prevalence matching or exceeding the prevalence of late-life depression (Wittchen and Hoyer, 2001). Despite its contribution to mortality and morbidity (Tully et al., 2013; Lambiase et al., 2014), the underlying neuropathology of anxiety in late-life has received relatively little attention (Ly and Andreescu, 2018).

Emotional dysregulation is the focus of one of the most influential models of GAD. This model posits that GAD individuals have deficits in both emotional reactivity (e.g. strong emotional responses to perceived threats), and in emotional regulation including rigidity to attentional responses to exteroceptive and interoceptive stimuli, excessive use of poor compensatory strategies (e.g., worry), and inability to implement adaptive strategies (e.g., reappraisal) (Mennin et al., 2015). Several studies have explored the neural basis of emotional regulation deficits in midlife GAD (Blair et al., 2012). Most but not all of them focused on the particularities of the limbic-prefrontal connectivity in GAD (Kim et al., 2011; Fitzgerald et al., 2017).

Most studies that have used emotional reactivity tasks such as fearful faces have examined limbic activation in mid-life GAD, pointing

* Correspondence to: University of Pittsburgh, Department of Psychiatry, Western Psychiatric Institute and Clinic, 3811 O'Hara Street, Pittsburgh, PA, USA
E-mail address: Andrcx@upmc.edu (C. Andreescu).

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toward a hyperactive amygdala (Fitzgerald et al., 2017; Hilbert et al., 2014; Fonzo and Etkin, 2017). However, there has been some mixed results with some studies reporting an increased amygdala response to negative faces (Monk et al., 2008; Fonzo et al., 2015; Nitschke et al., 2009) or to an emotion Stroop task (Price et al., 2011), while others failed to describe group differences (Whalen et al., 2008; Andreescu et al., 2015a; Karim et al., 2016a), though two of these were in late-life GAD (Andreescu et al., 2015a; Karim et al., 2016b).

With regard to limbic-PFC connectivity in midlife GAD, most studies indicated an abnormal amygdala-prefrontal connectivity [for a review see Fonzo and Etkin, 2017]. Overall, midlife GAD subjects showed a diminished amygdala/vmPFC connectivity during emotion regulation and an increased amygdala/dmPFC connectivity during emotion reactivity tasks (Fonzo and Etkin, 2017). To our knowledge, there are no published reports specifically analyzing limbic-PFC connectivity in late-life GAD. Our group previously reported on other measures of connectivity in late-life GAD, such as greater functional connectivity (FC) between the amygdala and paraventricular nucleus and lower FC between prefrontal nodes during worry reappraisal in late-life GAD participants (Andreescu et al., 2015b).

Although traditionally severe worry has been confined to categories such as GAD and Major Depressive Disorder (MDD)(Mennin and Fresco, 2013), multiple lines of research support the presence of severe worry in other several other anxiety and mood disorders (Kertz et al., 2012; Tull et al., 2011; Chelminski and Zimmerman, 2003; Mohlman et al., 2004). Of note, while GAD is built around the concept of severe, uncontrollable worry, only 20% of severe older worriers qualify for a GAD diagnosis(Kertz et al., 2012; Ruscio, 2002). This evidence supports a major recent shift in the conceptualization of worry as a transdiagnostic entity most suitable for dimensional investigations (Kertz et al., 2012; Olatunji et al., 2010), in line with the RDoC initiative. Several recent studies used a dimensional approach focused on worry severity. In midlife, two recent studies showed that worry severity was negatively associated with limbic-PFC resting-state functional connectivity (Makovac et al., 2016; Meeten et al., 2016).

In older adults, we found that - independent of diagnosis - high levels of worry were associated with decreased activation in the precuneus and prefrontal cortex, while higher global anxiety (as measured by Hamilton Anxiety Rating Scale) was associated with increased activation in the precuneus and limbic areas during the emotional faces task (Karim et al., 2016a).

Several studies have pointed out a differential involvement of distinct limbic components in mid-life GAD. During anticipatory anxiety, the phasic threat response is modulated by the amygdala, while the sustained response to threat and its anticipation (Davis et al., 2010; Buff et al., 2017) are mediated by the bed nucleus of stria terminalis (BNST). The BNST has been particularly implicated in sustained anticipatory anxiety and apprehension due to threat and loss of control to prevent the threat – all salient features of GAD (Lebow and Chen, 2016). However, few studies have focused their research on the BNST's role in midlife GAD. Under conditions of high uncertainty, individuals with GAD demonstrated *lower* activity in the amygdala and greater activity in the BNST when compared with non-anxious controls (Yassa et al., 2012). During threat anticipation, GAD participants had greater and sustained BNST activity, which was delayed relative to the onset of the threat anticipation (Buff et al., 2017) compared to non-anxious controls. This suggests that both amygdala and BNST activity may be neurobiological markers of anticipatory anxiety but engage at different timepoints (Buff et al., 2017). We have shown that late-life GAD participants had greater connectivity between the BNST and the subgenual cingulate during in-scanner induction of worry (Andreescu et al., 2015b).

In this study, we planned to investigate the limbic-prefrontal connectivity alterations in late-life GAD related to emotional reactivity. Given the differential effects in the amygdala and BNST reviewed above, we investigated the functional connectivity of both regions during exposure to emotional faces.

Additionally, given our previous results indicating specific neural correlations of worry but not of GAD, we also chose to explore the effect of worry from a dimensional perspective. Since worry is a universal phenomenon with possible evolutionary advantages (Price, 2003), we posit that worry encountered in the non-anxious participants may strengthen the limbic-PFC connectivity (facilitating a healthy reactivity and implicit regulation), while pathological severe worry, which accompanies late-life GAD, would compromise this traditional circuit of emotion regulation.

2. Materials and methods

2.1. Participants

We recruited 16 anxious and 20 age-matched, non-anxious elderly adults (aged 60–90 years) at the University of Pittsburgh. Inclusion criteria for anxious participants were a primary diagnosis of GAD for at least six months according to the Structured Clinical Interview for DSM-IV and a score of 17 or greater on the Hamilton Anxiety Rating Scale (HARS). Participants with other secondary anxiety disorders were also included and 3 (18.8%) of 16 were diagnosed with other anxiety disorders (1 social phobia, 1 panic disorder and 1 post-traumatic stress disorder). We excluded participants with a current diagnosis of major depressive disorder, with ongoing psychotherapy or current antidepressant or anxiolytic use, life time psychosis or bipolar disorder, dementia or a score of 24 or lower on the Mini-Mental State Examination (MMSE). Non-anxious elderly participants had no current or past diagnosis of psychiatric disorders. Participants were required to be off antidepressant/antianxiety medications for at least two weeks prior to scanning (six weeks for fluoxetine). All participants provided written informed consent. The institutional review board of the University of Pittsburgh approved this study.

2.2. Clinical measures

We assessed: worry severity with the Penn State Worry Questionnaire (PSWQ); anxiety symptoms with the Hamilton Anxiety Rating Scale (HARS); depressive symptoms with the Hamilton Depression Rating Scale (HAMD); cognitive function with the Mini-Mental State Examination (MMSE); neuroticism with the Five Factor Inventory (FFI)-Neuroticism subscale (FFI-N); and medical comorbidities with the Cumulative Illness Rating Scale for Geriatrics (CIRS-G).

The PSWQ is a 16-item questionnaire based on a factor analysis of 161 items. Each of the 16 items is designed to gather information regarding the frequency and intensity of worry (Meyer et al., 1990). Several studies have shown that this scale has high internal consistency and temporal stability, as well as favorable discriminant validity (correlation with Hamilton Anxiety Rating Scale and Hamilton Depression Rating Scale are $r = -0.02$ and 0.04 respectively) (Brown et al., 1992; Meyer et al., 1990). Principal component analysis showed that PSWQ items are loaded on a single, independent factor (worry) (Brown et al., 1992), making it the ideal questionnaire for measuring worry severity.

2.3. Faces and shapes emotional reactivity task

Adapted from the work of Hariri et al. (2002), the faces and shapes task is a well-validated paradigm to probe emotional faces processing and reliably engage the limbic and prefrontal regions (Hariri et al., 2002). The paradigm consisted of 9 blocks: 5 control blocks of matching shapes, interspersed with 4 experimental blocks of matching emotional faces. Each block lasted 24 s and contained six 4-second sequential matching trials with a total scan time of ~3.9 min.

2.4. MRI acquisition

All MR scanning was performed on a 3T Siemens Trio scanner with a 32-channel head coil at the University of Pittsburgh Magnetic Resonance Research Center. Whole-brain fMRI data were acquired axially using gradient-echo-planar imaging (EPI) with the following parameters: repetition time (TR) = 2 s, echo time (TE) = 34 ms, flip angle (FA) = 90°, field of view (FOV) = 256 × 256 mm^2, voxel size = 2 × 2 × 3 mm^3 (no gap), and 28 axial slices. T1-weighted structural images were acquired using a magnetization-prepared rapid gradient echo sequence (T1w MPRAGE) with the following parameters: TR = 2 ms, TE = 3.4 ms, FA = 9°, FOV = 256 × 254 mm^2, voxel size = 1 × 1 × 1 mm^3 (no gap), 160 slices, and GRAPPA = 2. T1w MPRAGE images were used to facilitate and improve the normalization of fMRI data into the Montreal Neurological Institute (MNI) template space. To assess white matter hyperitensities burden (WMH), we acquired whole-brain T2-weighted fluid-attenuated inversion recovery (T2-weighted FLAIR) images with TR = 9002 ms, T3 = 56 ms, FOV = 256 × 212 mm^2, and voxel size = 1 × 1 × 3 mm^3(no gap).

2.5. MRI data preprocessing

Functional images were preprocessed in SPM12 (Wellcome Trust Centre for Neuroimaging, http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab (Mathworks, Natick, MA). The T1w MPRAGE was segmented and warped into the Montreal Neurological Institute (MNI) common template space using SPM's unified segmentation/normalization procedure. The MPRAGE was skull stripped using a mask generated from gray, white, and cerebrospinal fluid segmentations. The functional data were first realigned to the mean functional image using the two-pass realignment procedure for motion correction, then resliced and warped into the MNI common space using the fMRI-structural co-registration matrix and the structural-MNI deformation field.

As in our previous studies (Wu et al., 2006) an automated WMH segmentation and localization pipeline was performed on the T2w FLAIR images to compute the WMH volume. Normalized WMH (nWMH) was calculated as the percentage of WMH over the total brain volume (gray matter + white matter).

2.6. Functional connectivity

Generalized psychophysiological interaction (gPPI) analysis (McLaren et al., 2012) was performed to estimate functional connectivity from three seed regions, i.e., left and right amygdala and BNST, during the faces and shapes task. Left and right amygdala were created with the anatomically defined automated anatomical labeling (AAL) atlas (Tzourio-Mazoyer et al., 2002) using the WFU Pick-Atlas tool. The BNST (bilateral) was hand drawn using MRIcron (version 6/2013) on a built-in MNI template (ch2better) as reported in Banihashemi et al. (2012) and Andreescu et al. (2015b). Principal time series were generated for each seed region using singular value decomposition (SVD).

Principal time series of the seed region, task conditions (face and shape matching) convolved with the hemodynamic response function, interaction variables (seed times series by task condition), as well as motion parameters were included in the design matrix. An autoregressive filter was used to account for serial correlations due to aliased biorhythms and unmodeled activation and a high-pass filter (1/128 Hz) was used to model low-frequency noise. PPI connectivity maps (amygdala or BNST) for face matching versus shape matching were computed for every participant.

2.7. Statistical analysis

We compared clinical and demographic characteristics between the healthy controls (HC) and late-life GAD groups using independent-sample t tests for continuous variables and $\chi 2$ tests (or Fisher's exact as appropriate) for categorical variables. All statistical analyses were carried out with SPSS version 24 software (IBM Corporation, Chicago, IL).

We conducted all voxel-wise statistical tests using statistical non-parametric mapping method (SnPM, http://warwick.ac.uk/snpm). We used a prior prefrontal mask created as a combination of prefrontal regions in the AAL atlas (Tzourio-Mazoyer et al., 2002) using the WFU Pick-Atlas tool and with a total volume of 386,016 mm^3 (48,252 voxels, 2 × 2 × 2 mm^3). We used the non-parametric method SnPM's permutation-based method (5000 permutations) to calculate non-parametric *p*-values and a cluster-wise correction method with a cluster-forming threshold of $p < 0.001$ to control for multiple comparisons (at family wise error [FWE] $p < 0.05$).

We conducted independent t-tests to identify differences between groups (GAD vs. HC). We conducted a regression to identify the association between connectivity and worry severity (PSWQ) across both groups. We then conducted two more similar regressions except within the GAD and HC groups independently.

As a post-hoc analysis, in clusters with significant group x worry interactions, we extracted the functional connectivity between amygdala or BNST and the corresponding cluster for each participant. We plotted the association (as measured by Pearson correlation) between connectivity and PSWQ scores in both groups. We then examined the partial correlations between functional connectivity and PSWQ worry scores in each group, controlling for FFI-N, HARS, HDRS, MMSE and WMH burden.

Finally, we tested the association between the amygdala/BNST functional connectivity and PSWQ, HARS, HDRS and FFI-N. To test the linear and quadratic relationships (across groups) between connectivity and worry we performed two regression analyses (Model 1 predictor: PSWQ versus Model 2 predictors: PSWQ and $PSWQ^2$).

3. Results

3.1. Participants characteristics

Table 1 summarizes the demographic and clinical characteristics of the sample.

Older GAD participants and older non-anxious participants did not differ on age, sex, race, education, or medical burden (as measured by CIRS-G). GAD participants compared to non-anxious participants had greater levels of worry (PSWQ $p < 0.001$), anxiety (HARS $p < 0.001$) and depression (HDRS $p < 0.001$). The histogram of PSWQ scores in Fig. 1 shows the distribution of worry severity scores in the GAD group and the non-anxious control group. While both groups were cognitively normal based on their MMSE scores, GAD participants had marginally lower MMSE scores than elderly non-anxious participants (MMSE $p = 0.052$).

3.2. Functional connectivity

Table 2 and Fig. 2 present effects of group and worry severity on the amygdala- and BNST-prefrontal functional connectivity. When looking across both groups, there were no differences in connectivity between groups and worry was not associated with connectivity.

Significant group x worry interactions were observed for left amygdala-prefrontal and BNST-prefrontal functional connectivity. Results for the right amygdala were non-significant for both main effects and interaction. Henceforth, when referring to the amygdala we refer to the left amygdala. Scatterplots of the interactions (Fig. 2) show that left amygdala-prefrontal (OFC, ACC/mPFC) and BNST-prefrontal (OFC) functional connectivity were associated with PSWQ differently between older GAD and HC.

Table 3 presents associations between these measures before and after adjusting for specific factors. Specifically, amygdala-prefrontal

Table 1
Summary of participant demographic variables and clinical characteristics.

Characteristic	Group; mean (SD) Late-life GAD, $n = 16$	HC, $n = 20$	Group Comparison (χ, t, p value)
Age, year[†]	67.38 (5.78)	67.80 (7.88)	$t(34) = 0.18$, $p = 0.86$
Sex (F)	9	10	$\chi^1 = 0.14$, $p = 0.71$
Race (W/AA)[‡]	15/1	18/2	$p = 0.59$
Education, year	15.19 (2.99)	16.90 (4.14)	$t(34) = 1.39$, $p = 0.17$
PSWQ	59.19 (12.00)	34.00 (9.39)	$\mathbf{t(34) = -7.07, p < 0.001}$
FFI -N	8.70 (5.52)	26.00 (9.62)	$\mathbf{t(34) = -6.78, p < 0.001}$
HARS	20.38 (3.44)	3.20 (2.02)	$\mathbf{t(34) = -18.70, p < 0.001}$
HDRS	12.69 (2.44)	1.35 (1.31)	$\mathbf{t(34) = -17.85, p < 0.001}$
MMSE	28.88 (0.96)	29.45 (0.76)	$\mathbf{t(34) = 2.01, p = 0.052}$
Duration of illness, years	25.38 (23.33)	N/A	N/A
CIRS-G[§]	5.81 (2.23)	4.68 (2.16)	$t(34) = -1.52$, $p = 0.14$

[†] Age range 60–90 years.
[‡] Fisher's exact test.
[§] CIRS-G score was available on 35 out 36 participants.
PSWQ – Penn State Worry Questionnaire; FFI-N – Five Factor Inventory Neuroticism subscale; HARS – Hamilton Anxiety Rating Scale; HAMD – Hamilton Depression Rating Scale; MMSE – Mini-Mental State Examination and CIRS-G – Cumulative Illness Rating Scale for Geriatrics.

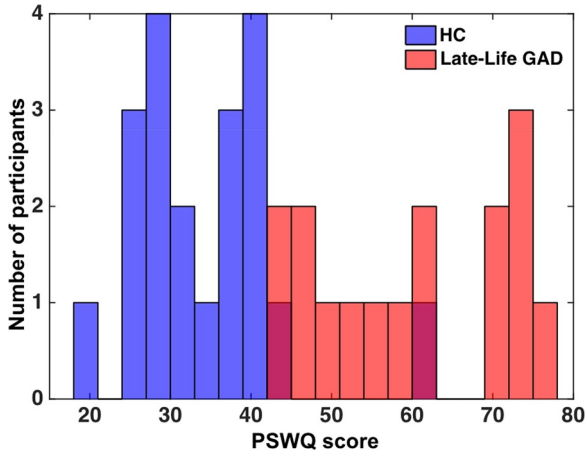

Fig. 1. Distribution of the Penn State Worry Questionnaire (PSWQ) in non-anxious healthy control (HC) and late-life generalized anxiety disorder (GAD) participants.

functional connectivity was *negatively* associated with PSWQ scores in GAD participants and was *positively* associated with PSWQ scores in non-anxious participants. Similarly, BNST- prefrontal functional connectivity was *negatively* correlated with PSWQ scores in GAD participants and *positively* correlated with PSWQ scores in non-anxious participants. These associations remained when adjusting for nWMH, except for the association between PSWQ and BNST-PFC connectivity in non-anxious participants. Correlations between functional connectivity and PSWQ scores remained significant after controlling for FFI-N, HARS, HDRS, and MMSE scores except for BNST-PFC connectivity in non-anxious participants.

There were no significant associations between any functional connectivity measure and HARS or HDRS. The neuroticism score from the FFI was negatively correlated with the extracted functional connectivity indices in elderly GAD participants only with BNST-OFC connectivity ($r(14) = -0.53$, $p = 0.04$). For non-anxious controls results were nonsignificant (p's > 0.23).

The association between PSWQ and connectivity was not significant across both groups, however after adding the quadratic term ($PSWQ^2$) there was a significant change in R^2 and F-values compared to the model with just the linear term. Specifically, the quadratic model had significantly greater R^2 and F values (compared to the linear model) for: amygdala-dACC connectivity [R^2change = 0.20, F(1,33) change = 8.23, $p = 0.007$], amygdala-OFC connectivity [R^2change = 0.16, F(1,33) change = 6.73, $p = 0.014$], and BNST-prefrontal connectivity [R^2change = 0.20, F(1,33) change = 8.93, $p = 0.005$], suggesting the quadratic model was a better fit for the combined group (GAD and HC).

The quadratic terms between worry severity and amygdala-PFC connectivity were significant for: amygdala-dACC connectivity [F(2, 35) = 4.45, $p = 0.02$, PSWQ vertex/peak = 46.5]; amygdala-OFC connectivity [F(2, 35) = 4.26, $p = 0.02$, PSWQ vertex/peak = 44.8], and BNST-PFC connectivity [F(2, 35) = 5.47, $p = 0.009$, PSWQ vertex/peak = 45.1] (Fig. 3). One non-anxious participant had a PSWQ of 62, and we tested whether that participant's exclusion altered these analyses and found that it did not.

Table 2
Group-by-worry ANOVAs of functional connectivity during the faces-shapes task (corrected $p < 0.05$).

Group-by-worry ANOVA	Brain region	Brodmann area (BA)	Peak MNI coordinates (x,y,z)	t-score ($df = 32$)	Size (mm^3)
Left amygdala functional connectivity					
Main effect of group	None				
Main effect of worry	None				
Group by worry interaction	L OFC	BA 47	−28, 32, −4	5.37	1088
	mPFC/L ACC	BA 32, 10	−10, 48, −6	4.27	560
	mPFC/R ACC	BA 32, 10, 11	6, 44, −8	4.07	872
BNST functional connectivity					
Main effect of group	None				
Main effect of worry	None				
Group by worry interaction	L OFC	BA 47, 11	−34, 34, −8	4.75	1080

Abbreviations: MNI-Montreal Neurologic Institute; L-left; OFC– orbitofrontal, mPFC-medial prefrontal cortex, ACC-anterior cingulate cortex.
Groups: late-life generalized anxiety disorder (GAD) vs. healthy control (HC).
Worry: Penn State Worry Questionnaire (PSWQ). Results for the right amygdala were non-significant for both main effects and interaction.

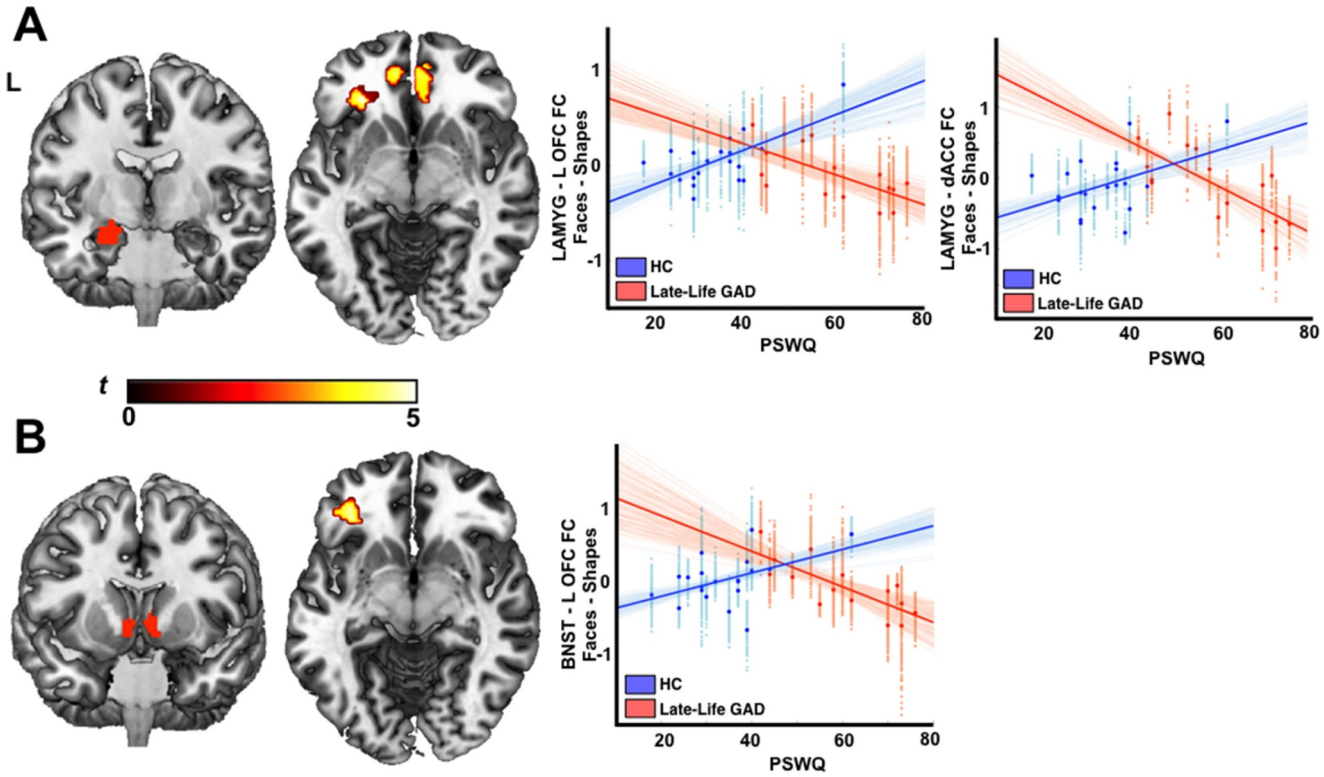

Fig. 2. T There are significant group differences (HC vs GAD) in the associations between worry severity (PSWQ) and Amygdala/BNST-PFC functional connectivity during emotional faces processing (corrected $p < 0.05$). Amygdala/BNST-prefrontal (PFC) functional connectivity is positively correlated with the PSWQ in HC and is negatively correlated with the PSWQ in GAD. A) interaction of Group x PSWQ on left amygdala functional connectivity. B) interaction of Group x PSWQ on the bed nucleus of stria terminalis (BNST) functional connectivity. Left panel – seed regions (upper left amygdala, lower BNST), middle panel – interaction *t* maps, and right panel - scatterplots of PSWQ and functional connectivity. Note: L-left; FC-functional connectivity; PSWQ-Penn State Worry Questionnaire; AMYG- left amygdala; dACC-dorsal anterior cingulate; OFC-orbitofrontal cortex; BNST- bed nucleus of stria terminalis.

4. Discussion

Our primary findings are 1) worry severity and amygdala/BNST-PFC functional connectivity were negatively correlated in GAD and positively correlated in HC, and 2) in the entire sample, the limbic-PFC functional connectivity displayed a reverse U-shape correlation with worry severity such that very low and very high levels of worry were associated with a lower limbic-PFC connectivity. These results remained significant after controlling for neuroticism, global anxiety, depression severity, and cognitive status (MMSE).

The limbic-PFC connectivity during emotional reactivity/implicit emotion regulation has been extensively studied, especially in adolescent and midlife anxiety (Hare et al., 2008; Kim et al., 2011). Most studies reported that the disruption of the limbic-PFC connectivity is one of the main neurobiological features of GAD (Prater et al., 2013). This neural GAD "trait" has been linked to exaggerated negative reactivity associated with increased attention toward negative stimuli in mid-life GAD (Roy et al., 2008; Mennin et al., 2007) but also to deficits in implicit down-regulation of negative affect (Fitzgerald et al., 2017; Fonzo and Etkin, 2017). Of note, the disruption in limbic-PFC connectivity has been described as both increased (Robinson et al., 2014) and decreased (Etkin et al., 2010) amygdala-PFC connectivity during

Table 3
Associations between limbic-PFC connectivity and PSWQ, FFI-N, HARS, and HDRS.

Association	Left Amygdala - OFC	Left Amygdala - ACC/mPFC	BNST - OFC
	GAD		
Connectivity and PSWQ	**r(14) = −0.75, p = 0.001**		**r(14) = −0.80, p < 0.001**
Adjusting for nWMH	**r(13) = −0.74, p = 0.001**		**r(13) = −0.80, p < 0.001**
Adjusting for FFI-N, HARS, HDRS, and MMSE	**r(10) = −0.61, p = 0.03**		**r(10) = −0.65, p = 0.02**
Connectivity and FFI-N	r(14) = −0.46, p = 0.07	r(14) = −0.44, p = 0.09	**r(14) = −0.53, p = 0.04**
Connectivity and HARS	r(14) = 0.05, p = 0.86	r(14) = 0.24, p = 0.37	r(14) = 0.20, p = 0.45
Connectivity and HDRS	r(14) = −0.31, p = 0.25	r(14) = −0.42, p = 0.10	r(14) = −0.47, p = 0.07
	HC		
Connectivity and PSWQ	**r(18) = 0.61, p = 0.004**		**r(18) = 0.46, p = 0.04**
Adjusting for nWMH	**r(17) = 0.59, p = 0.008**		r(17) = 0.43, p = 0.06
Adjusting for FFI-N, HARS, HDRS, and MMSE	**r(14) = 0.54, p = 0.03**		r(14) = 0.48, p = 0.06
Connectivity and FFI-N	r(18) = 0.23, p = 0.23	r(18) = 0.20, p = 0.40	r(14) = 0.08, p = 0.75
Connectivity and HARS	r(18) = 0.31, p = 0.18	r(18) = 0.31, p = 0.18	r(18) = 0.03, p = 0.90
Connectivity and HDRS	r(18) = 0.14, p = 0.55	r(18) = 0.12, p = 0.61	r(18) = 0.22, p = 0.34

Abbreviations: PSWQ – Penn State Worry Questionnaire; OFC – Orbitofrontal Cortex; ACC – Anterior Cingulate; mPFC – medial Prefrontal Cortex; BNST – Bed Nucleus Stria Terminalis; nWMH – normalized white matter hyperintensities; GAD – Generalized Anxiety Disorder; HC – Non-anxious controls; FFI-N – five factor inventory neuroticism subscore; HARS – Hamilton Anxiety Rating Scale; HDRS – Hamilton Depression Rating Scale; MMSE – mini mental state examination.

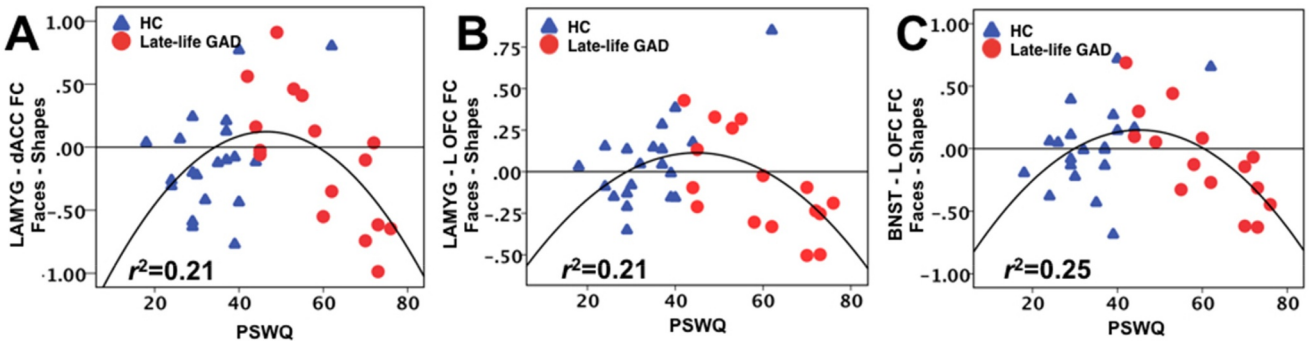

Fig. 3. Significant quadratic relationships between worry severity (PSWQ) and amygdala/BNST functional connectivity during emotional faces processing across entire sample. The y-axis represents differences in limbic-PFC connectivity during emotional face processing and shape matching, so a value of zero represents similar limbic-PFC connectivity during face processing and shape matching and a positive value indicates greater limbic-PFC connectivity during face processing than during shape matching. Scatter plots show quadratic associations of PSWQ and A) left amygdala– dorsal anterior cingulate (dACC) functional connectivity ($F(2, 35) = 4.45$, $p = 0.02$, PSWQ vertex/peak = 46.5), B) left amygdala – left orbitofrontal cortex (L OFC) connectivity ($F(2, 35) = 4.26$, $p = 0.02$, PSWQ vertex/peak = 44.8), and C) BNST– L OFC connectivity ($F(2, 35) = 5.47$, $p = 0.009$, PSWQ vertex/peak = 45.1). Note: L-left; FC-functional connectivity; PSWQ-Penn State Worry Questionnaire; AMYG-amygdala; dACC-dorsal anterior cingulate; OFC-orbitofrontal cortex; BNST- bed nucleus of stria terminalis.

exposure to emotional stimuli (Fonzo and Etkin, 2017), suggesting a heterogeneity in both the tasks and GAD symptomatology.

Most of these studies focused however on group differences in younger adults and only a few recent studies parsed apart the effect of worry severity. Makovac et al. showed that PSWQ was negatively associated with left amygdala-ACC/mPFC resting-state functional connectivity (Makovac et al., 2016) and that the amygdala-paracingulate connectivity was associated with higher self-reported levels of worry after an in-scanner worry induction task (Makovac et al., 2016). Meeten et al. measured resting state connectivity before and after worry induction on the same sample and found that higher PSWQ scores were associated with diminished connectivity changes (post – pre-induction) between left amygdala and the inferior frontal gyrus (Meeten et al., 2016).

Despite the emerging evidence of the role of BNST in the pathophysiology of anxiety, there are relatively few neuroimaging studies that explored the activation or connectivity of BNST in healthy adults or GAD (Herrmann et al., 2016), mostly due to resolution difficulties related to the neuroanatomical particularities of the ventral basal forebrain (Pedersen et al., 2017). These studies indicate an increased BNST activation during threat monitoring in healthy participants with greater anxiety (Somerville et al., 2010). Buff et al. showed a temporally dissociable involvement of BNST and the amygdala during threat anticipation in mid-life GAD, with an increased but delayed BNST activation relative to the onset of threat anticipation (Buff et al., 2017).

In our study, limbic-PFC connectivity had opposite correlations with worry severity in healthy controls and GAD. While these results may be difficult to interpret in a categorical framework, they fit much better in a dimensional framework. Thus, most healthy controls have worry scores going from very low to moderate, while GAD participants exhibit worry scores from moderate to severe (see Fig. 1). The dissociation noticed in the group analysis is probably carried by the distribution of the worry severity in the two groups.

In the observed reverse U-shape relationship between worry severity and limbic-PFC connectivity (Fig. 3), very low or high worry was associated with a low limbic-PFC connectivity, and an intermediate level of worry was associated with a greater limbic-PFC connectivity [participants with low or high worry severity had lower limbic-PFC connectivity than participants with moderate levels of worry].

One potential explanation for these results is that PFC-limbic connectivity is driven by amygdala reactivity. According to this hypothesis, low worriers would have lower PFC-limbic connectivity due limited amygdala reactivity during the faces-shapes task (therefore requiring minimal PFC intervention for regulation), while severe worries would have a lower PFC-limbic connectivity due to excessive amygdala reactivity but poor prefrontal downregulation. However, previous analysis from our group (Karim et al., 2016a) and post-hoc analyses of the participants in the current study did not indicate a significant positive correlation between amygdala activation and worry severity. Therefore, the results reported here indicate that the limbic-PFC connectivity appears to be a distinctive marker, potentially independent from amygdala reactivity, a marker that in this context may reflect the efficacy of implicit emotion regulation that peaks at moderate levels of worry.

These results are reflective of the classic Yerkes-Dodson law regarding the effect of arousal on performance. Thus, according to the Yerkes-Dodson law, performance increases with mental arousal/anxiety up to a certain point, after which performance decreases at high levels of arousal/anxiety (Curtin, 1984). This bell-curve correlation may explain the overall lack of effect of worry severity when the GAD and non-GAD groups are collapsed. This reverse U-shaped correlation also suggests that a moderate level of worry, associated with maximum limbic-PFC connectivity, may be the “sweet spot” at which implicit emotion regulation is most effective.

Limbic-PFC connectivity was greatest individuals with a PSWQ score of 44–46. The maximum coupling for BNST- OFC appeared to occur at a slightly higher level of worry than the maximum coupling for Amygdala-OFC, and the maximum coupling of amygdala-dACC occurred at the highest level of worry. Based on these very preliminary results, we may speculate that the modulation of emotional reactivity is more complex than simple prefrontal tonic inhibition of limbic structure. Although our study was not designed to test this, we may speculate that these results suggest a step-wise engagement of both limbic (amygdala/BNST) and PFC (OFC/ACC) structures associated with worry severity and that the intervention of dACC signals a need for implicit regulation at levels of worry associated with the appraisal and expression of negative emotions, supporting the emotion dysregulation model of GAD (Etkin et al., 2010).

Several limitations of this study should be considered. First, in our sample, the distribution of PSWQ scores is typically skewed across both groups. There was a lack of non-anxious individuals with high PSWQ scores or anxious participants with low PSWQ scores. Thus, in this sample, the GAD diagnosis was confounding with the PSWQ worry score. Nevertheless, our results suggested that pathological worry in GAD participants adversely impact amygdala/BNST-PFC functional connectivity. The opposite associations of functional connectivity and the PSWQ between the GAD and control groups or the inverted U-shape relationship when collapsing the two groups may be due to different segments of PSWQ (normal worry versus pathological worry), or GAD diagnosis, or a combination of both. Second, this study had a small-to-

moderate sample size (16 GADs and 20 controls), which may have prevented us from pinpointing more fine-grained differences in connectivity between BNST, the amygdala and the PFC. Third, we used a relatively "pure" GAD sample, with minimal depressive comorbidity. While this allowed us to isolate the effects of GAD/worry, it probably limits the generalizability of the results given the well-known GAD-major depressive comorbidity. This study was performed in late-life GAD and may not generalize to GAD in younger populations. Overall, a larger sample size, a recruitment strategy centered on worry severity, and multimodal MR imaging would benefit future studies in order to better understand the neural correlates of emotional reactivity and regulation in participants with severe worry.

Overall, our study brings two original contributions to the field 1) exploring the PFC-limbic connectivity in late-life GAD and 2) describing the non-linear association between worry severity and PFC-connectivity in late-life. Future studies may involve larger samples and test the effectiveness of limbic-PFC connectivity using both implicit and explicit emotion regulation tasks.

Declaration of interest

The authors declare no conflict of interest.

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Institutional review board

This research study was approved by the University of Pittsburgh Institutional Review Board.

CRediT authorship contribution statement

M Wu: Methodology, Formal analysis, Writing - original draft, Validation. **DS Mennin:** Conceptualization, Methodology, Writing - review & editing, Validation. **M Ly:** Conceptualization, Methodology, Writing - review & editing, Validation. **HT Karim:** Conceptualization, Methodology, Writing - review & editing, Validation. **L Banihashemi:** Conceptualization, Methodology, Writing - review & editing, Validation. **DL Tudorascu:** Formal analysis, Validation. **HJ Aizenstein:** Conceptualization, Methodology, Writing - review & editing, Validation. **C Andreescu:** Conceptualization, Funding acquisition, Methodology, Writing - original draft, Validation.

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