Archival Report

Biological Psychiatry

# Alterations in Amygdala-Prefrontal Functional Connectivity Account for Excessive Worry and Autonomic Dysregulation in Generalized Anxiety Disorder

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**ABSTRACT**

**BACKGROUND:** Generalized anxiety disorder (GAD) is characterized by the core symptom of uncontrollable worry. Functional magnetic resonance imaging studies link this symptom to aberrant functional connectivity between the amygdala and prefrontal cortex. Patients with GAD also display a characteristic pattern of autonomic dysregulation. Although frontolimbic circuitry is implicated in the regulation of autonomic arousal, no previous study to our knowledge combined functional magnetic resonance imaging with peripheral physiologic monitoring in these patients to test the hypothesis that core symptoms of worry and autonomic dysregulation in GAD arise from a shared underlying neural mechanism.

**METHODS:** We used resting-state functional magnetic resonance imaging and the measurement of parasympathetic autonomic function (heart rate variability) in 19 patients with GAD and 21 control subjects to define neural correlates of autonomic and cognitive responses before and after induction of perseverative cognition. Seed-based analyses were conducted to quantify brain changes in functional connectivity with the right and left amygdala.

**RESULTS:** Before induction, patients showed relatively lower connectivity between the right amygdala and right superior frontal gyrus, right paracingulate/anterior cingulate cortex, and right supramarginal gyrus than control subjects. After induction, such connectivity patterns increased in patients with GAD and decreased in control subjects, and these changes tracked increases in state perseverative cognition. Moreover, decreases in functional connectivity between the left amygdala and subgenual cingulate cortex and between the right amygdala and caudate nucleus predicted the magnitude of reduction in heart rate variability after induction.

**CONCLUSIONS:** Our results link functional brain mechanisms underlying worry and rumination to autonomic dyscontrol, highlighting overlapping neural substrates associated with cognitive and autonomic responses to the induction of perseverative cognitions in patients with GAD.

*Keywords:* Amygdala, Functional connectivity, Functional magnetic resonance imaging, Generalized anxiety disorder, Heart rate variability, Perseverative cognition

http://dx.doi.org/10.1016/j.biopsych.2015.10.013

Excessive and uncontrollable worry is an established central feature in the definition of generalized anxiety disorder (GAD). Importantly, worry has to be accompanied by symptoms of negative affect and tension and perceived by the individual as "difficult to control" according to DSM-5. The high prevalence of GAD creates a massive economic burden (1,2), yet its core symptom remains poorly characterized from a neurobiological perspective. The "spontaneous" nature of intrusive thoughts suggests that the neurobiological processes underpinning worry may be better examined over periods of free thinking rather than during behavioral engagement with an external task. Therefore, resting-state neuroimaging would be a useful tool for examining dysfunctional neural circuitry in GAD.

The few published functional connectivity studies of GAD focus largely on the amygdala and associated networks, following evidence for the central contribution of the amygdala to fear and threat processing (3). Resting-state neuroimaging studies support the view that perturbed amygdala-prefrontal connectivity underlies the core features of GAD (4). Decreased connectivity between the amygdala and lateral prefrontal cortex (PFC) was reported in adults (5) and adolescents with GAD (6,7). More recently, aberrant amygdala connectivity with ventromedial PFC and insula was noted in youths with anxiety disorders (8). Amygdala-based connectivity is found to be negatively correlated with anxiety rating scores (9,10).

Taken together, these findings point to a neural basis for emotion regulation deficits in GAD, centered on reduced

SEE COMMENTARY ON PAGE 733

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 ISSN: 0006-3223

functional connectivity within this major frontolimbic pathway. Conversely, effective emotion regulation and anxiety control are predicted by efficient communication between the amygdala and PFC. For example, the positive reappraisal of negative emotional material strengthens connectivity between the amygdala and medial prefrontal regions, with self-reported effectiveness of emotion regulation correlating positively with the degree of functional coupling (11). Moreover, effective emotion regulation evokes a selective increase in connectivity of the amygdala with ventromedial PFC and dorsolateral PFC (12).

Emotion dysregulation in GAD is expressed through poor prefrontal control of worrisome thoughts and chronic failure to downregulate autonomic arousal (13). Medial prefrontal cortices and amygdala are implicated in states of autonomic arousal during mental and emotional stress. These states are characterized by shifts in parasympathetic to sympathetic balance in which baroreflex suppression manifests as increased heart rate (HR) and blood pressure and decreased heart rate variability (HRV) (14,15).

Decreased HRV is a notable autonomic signature of worry states (16,17). However, to our knowledge, no detailed characterization of functional brain processes linking worry to measured changes in autonomic arousal has been conducted in patients with GAD. We combined resting-state functional magnetic resonance imaging (fMRI) with concurrent autonomic measurement, focusing on HRV as a measure of vagally mediated parasympathetic change. The simultaneous assessment of cognitive and physiologic correlates of GAD is particularly relevant in light of a previous study associating self-reported experience of worry and autonomic arousal with distinct patterns of neural connectivity (18).

We used a seed-based approach to analyze our resting-state fMRI data, first to validate earlier findings of decreased amygdala-prefrontal connectivity in patients with GAD compared with healthy control (HC) subjects and second to test the hypothesis that a behavioral induction of perseverative cognition (i.e., worry or rumination) will alter (uncouple) amygdala-prefrontal connectivity. To our knowledge, only one study (focusing on elderly patients) compared the consequences of a worry induction on neural connectivity patterns in GAD (19). The induction may place participants in a task-based state; therefore, our use of the term "resting state," motivated by the absence of direct instructions, should be considered with this caveat in mind.

In line with a dimensional view of psychopathology, we hypothesized that the induction will change the pattern of connectivity in HC subjects to the pattern more typically associated with patients with GAD and that such changes will reflect the dispositional tendencies (trait measures) of individuals to engage in perseverative cognition. We anticipated that resting-state amygdala connectivity reflects ongoing state measures of core GAD symptoms. Drawing on the theoretical model that the PFC downregulates amygdala responses to (real or perceived) threat, we hypothesized that aberrant resting amygdala-PFC would predict increases in self-reported state worry.

Similarly, given the involvement of PFC regions and amygdala in autonomic control (14,15,20–24) and notably in HRV (25), we tested the relationship between amygdala connectivity and changes in HRV in response to the induction. The HRV is a positive marker for emotion regulation (26) and is diminished during maladaptive emotion regulation processes, including worry (27,28). We hypothesized that changes in amygdala-PFC caused by the induction of perseverative cognition would correlate with reductions in HRV evoked by the same induction. We expected these relationships to be amplified in patients with GAD compared with HC subjects (29).

## METHODS AND MATERIALS

### Participants

All participants provided written informed consent. The study was approved by the National Research Ethics Service with local approval of the Brighton and Sussex Medical School Research Governance and Ethics Committee. After excluding one participant who did not complete the full experiment, the sample comprised 19 patients (17 women, 2 men; mean age, 29.58 ± 6.93 years) who met diagnostic criteria for GAD and 21 HC subjects (18 women, 3 men; mean age, 28.67 ± 9.45 years) (Supplement).

### Procedure

The Structured Clinical Interview for DSM-IV was administered by a trained postdoctoral fellow (FM) to patients and HC subjects to confirm or exclude the diagnosis of GAD. To assess comorbid disorders, participants were asked if they currently or previously had a diagnosis of any other psychiatric disorder or had ever been treated by their general practitioner for symptoms other than anxiety. None of the participants had a formal diagnosis of comorbid major depressive disorder. Participants completed a series of online questionnaires on sociodemographic and dispositional traits. Participants were subsequently familiarized with the neuroimaging environment, were connected to the physiologic recording equipment, and underwent the MRI protocol.

### Questionnaires

All participants completed a set of questions assessing sociodemographic and lifestyle information (nicotine, alcohol, and caffeine consumption; physical activity). To assess physical activity, participants were asked to report the type and amount (hours/week) of exercise they regularly did and how active they considered themselves compared with others of the same age and sex. Based on their responses, their perceived physical fitness was classified as low, medium, or high. Dispositional measures of 1) stress-reactive rumination (Stress-Reactive Rumination Scale [SRRS]) (30), 2) depressive rumination (Ruminative Response Scale) (31), and 3) worry (Penn State Worry Questionnaire [PSWQ]) (32) were also obtained.

### Experimental Design

In the MRI scanner, participants underwent a series of four 5-minute resting-state periods, each followed by a 6-minute easy visuomotor tracking task (described elsewhere) (C. Ottaviani, Ph.D., unpublished data, 2015). During resting-state periods, participants were instructed to rest with their eyes open without thinking of anything and not falling asleep.

After the second or third resting block, participants randomly underwent a recorded verbal induction procedure designed to engender perseverative cognition (Supplement). The induction occurred after the second resting-state block in 9 patients with GAD and 11 HC subjects ($n = 20$) and after the third resting-state block in 10 patients with GAD and 10 subjects HC ($n = 20$). The induction has been proved to be particularly effective in evoking worrisome and ruminative thoughts that are prolonged over time (perseverative), and findings have been replicated in different experimental settings in healthy and clinical samples (16). At the end of each resting-state period, participants rated their thoughts over the preceding period using visual analog scales (VASs).

## Visual Analog Scales

To assess levels of perseverative cognition occurring before and after the induction, participants were asked to rate on three separate 100-point VASs: "How much, for the duration of the previous resting period, were you distracted by 1) external stimuli, 2) ruminating/worrying, and 3) internal thoughts?"

## Physiologic Data Processing

The HR was monitored using MRI-compatible finger pulse oximetry (8600FO; Nonin Medical, Inc., Plymouth, Minnesota) recorded digitally as physiologic waveforms at a sample rate of 1000 Hz (via a CED power 1401, using Spike2 version 7 software; Cambridge Electronic Design, Ltd; Cambridge, United Kingdom). Interbeat interval values were visually inspected, and potential artifacts were manually removed. To this pulse data, we applied the root mean square successive difference (RMSSD), which is a reliable parameter for assessing vagally mediated HRV (33). The RMSSD has been shown to be sensitive to changes in the parasympathetic arm of the autonomic nervous system and particularly suited to capture autonomic perturbation in anxiety disorders (34). The RMSSD is known to be stable over short recording intervals (35) and is relatively free of the influences of respiration (36,37). The RMSSD was derived using R Heart Rate Variability (RHRV) 4.0 analysis software from the R Project (http://rhrv.r-forge.r-project.org/) for the duration of each resting-state scanning period. Attention was given to measures before (Pre) and after (Post) the worry induction.

## MRI Acquisition and Preprocessing

MRI was performed on a 1.5-Tesla MAGNETOM Avanto scanner (Siemens AG, Munich, Germany). Structural volumes were obtained using the high-resolution three-dimensional magnetization prepared rapid acquisition gradient echo sequence. Functional data sets used T2*-weighted echo planar imaging sensitive to blood oxygenation–level dependent signal (repetition time = 2.52 seconds, echo time = 43 ms, flip angle = 90°, 34 slices, 3-mm slice thickness, field of view = 192 mm, voxel size = 3 × 3 × 3 mm). Data were preprocessed using Statistical Parametric Mapping (SPM8; Wellcome Department of Imaging Neuroscience, http://www.fil.ion.ucl.ac.uk/spm/) and in-house software implemented in MATLAB (The MathWorks, Inc., Natick, Massachusetts) (preprocessing details are provided in the Supplement). Because global signal removal can potentially change functional connectivity distributions and result in increased negative correlations (38), it was avoided in our preprocessing.

## Statistical Analyses

**Questionnaire, Behavioral, and HRV Analyses.** All data are expressed as means (± SD). Differences at $p \leq .05$ are regarded as significant. Data analysis was performed with IBM SPSS Statistics version 22.0 for Windows (IBM Corp., Armonk, New York). To test for preexisting group differences, a series of $t$ and $\chi^2$ tests was conducted on self-report sociodemographic, physiologic, and personality measures. To test for the effects of the induction on cognitive and autonomic variables, a series of group (GAD vs. HC) × condition (pre vs. post) general linear models was performed on each VAS, HR, and RMSSD. Preinduction values were derived from the average of two or three VASs, HR, and RMSSD values (depending on when the induction took place —after the second or third resting-state period). Similarly, postinduction values consisted of one or two averaged VASs, HR, and RMSSD values.

## Seed-Based fMRI Analysis

Anatomic regions of interest were constructed using an anatomic toolbox in SPM (39) for bilateral amygdala. The average resting-state fMRI time series over the regions of interest were extracted for each participant and for each scan. For each participant, only data obtained from the scan occurring immediately before and immediately after the induction were analyzed.

The time series were then used as a regressor in a first-level SPM analysis, extracting the voxels in the brain showing a significant correlation with it. To test for group differences, second-level analyses were performed in which the first-level contrast images were submitted to a two-sample (GAD vs. HC) $t$ test model. A flexible-factorial design was used to evaluate the induction × group interaction. Gray matter volume was used as a covariate of no interest. To test for the associations between amygdala connectivity and dispositional and autonomic measures, a $t$ test was run to adjust for potential confounds, with group (GAD vs. HC) as factor; VAS, questionnaires, and HRV as covariates of interest; and order (i.e., induction after the second of third resting-state period) as covariate of no interest. Whole-brain gray matter volume was also introduced as a covariate of no interest to correct for possible structural differences between the two groups, which might influence the functional connectivity (40). To investigate whether the neural impact of induction could be predicted by dispositional tendencies to ruminate and worry, we calculated the shift in connectivity after the induction (subtracting connectivity preinduction from connectivity postinduction) in the GAD group and HC group and correlated this connectivity shift with SRRS, Ruminative Response Scale, and PSWQ scores. A statistical threshold was set at $p < .05$, familywise error corrected at cluster level (cluster size defined using uncorrected voxel-level threshold $p < .005$; a more liberal voxel-level threshold $p < .01$ was used occasionally to capture meaningful trends in our data).

## RESULTS

There were no significant differences between the GAD and HC groups in sex, age, years of education, body mass index, physical activity, nicotine use, alcohol use, or caffeine intake (Table 1).

### Questionnaires, VAS, HR, and HRV Data

The GAD group reported higher levels of dispositional rumination and worry (SRRS, Ruminative Response Scale, and PSWQ) and had higher HR and lower HRV at baseline (i.e., preinduction) compared with the HC group (Table 1). A main effect of group was evident for ruminating/worrying ($F_{1,38}$ = 6.19, $p$ = .02), with the GAD group engaging in perseverative cognition more than the HC group (GAD = 45.26 ± 19.41, HC = 27.05 ± 26). Patients with GAD were also more distracted by internal stimuli compared with HC subjects, regardless of the induction (GAD = 78.34 ± 15.1; HC = 68.43 ± 17.46), but the difference only approached statistical significance ($F_{1,38}$ = 3.63, $p$ = .06). Lastly, a main effect of group ($F_{1,38}$ = 6.29, $p$ = .02) and induction ($F_{1,38}$ = 15.68, $p$ < .0001) emerged for the VAS "Distracted by external stimuli," with GAD patients being overall more distracted than HC subjects (GAD = 58.34 ± 16.45, HC = 42.17 ± 23.34); both groups were more distracted by external stimuli before the induction (58 ± 23.45) than after the induction (41.7 ± 27.14).

With regard to HR, the general linear model revealed main effects of group, with the GAD group having higher HR compared with the HC group (67.35 ± 8.83 vs. 61.65 ± 7.63 [$F_{1,38}$ = 5.72, $p$ < .001]), and induction, with baseline HR being lower compared with HR after the induction (63.84 ± 9.3 vs. 65.37 ± 8.63 [$F_{1,38}$ = 5.11, $p$ < .05]). No group × induction interaction effect emerged. With regard to HRV, a significant main effect of group emerged ($F_{1,38}$ = 7.92, $p$ < .001), with the GAD group having lower HRV (GAD = 43.53 ± 17.99 vs. HC = 76.43 ± 46.14). No effects of induction or group × induction interaction emerged. When we calculated the shift (post − pre) in HRV after the induction, 14 of 19 patients with GAD reported a negative shift (one-tailed sign test $p$ < .04), whereas only 7 of 21 HC subjects reported a negative shift (not significant).

### Effects of Group and Induction on Amygdala Connectivity

Compared with HC subjects, patients with GAD reported lower connectivity between the right amygdala and right superior frontal gyrus, right paracingulate/anterior cingulate cortex, and right supramarginal gyrus (Figure 1). A group × induction interaction was evident within these same areas, where the induction increased connectivity with the right amygdala in patients with GAD, yet decreased the connectivity between the same areas in HC subjects (Table 2 and Figure 1). These results did not survive familywise error correction.

**Table 1. Sociodemographic, Lifestyle, and Baseline Differences Between Patients With Generalized Anxiety Disorder and Healthy Control Subjects**

| | GAD ($n$ = 19) | HC ($n$ = 21) | $p$ |
|---|---|---|---|
| Age (Years) | 29.58 (± 6.93) | 28.67 (± 9.45) | .72 |
| Gender (M/F) | 2/17 | 3/18 | .72 |
| BMI (kg/m$^2$) | 22.93 (3.21) | 23.14 (3.12) | .51 |
| Education (Years) | 13.10 (± 1.82) | 12.14 (± 2.57) | .18 |
| Disease Duration (Years)[a] | 16.78 (± 8.01) | | |
| Smoking Status | 6 yes, 13 no | 5 yes, 16 no | .58 |
| Cigarettes per Day (Smokers Only) | 1.39 (± 2.80) | .95 (± 2.40) | .59 |
| Alcohol (Units/Week) | 4.12 (± 2.99) | 4.44 (± 3.59) | .75 |
| Coffee/Other Caffeinated Drinks (Cups/Day) | 2.26 (± 1.52) | 2.14 (± 1.85) | .82 |
| Perceived Physical Fitness | 12 H, 5 M, 2 L | 11 H, 9 M, 1 L | .49 |
| RRS | 52.84 (± 11.81) | 37.48 (± 11.93) | < .0005 |
| PSWQ | 54.73 (± 5.69) | 41.81 (± 7.34) | < .0005 |
| SRRS | | | |
| Negative inferential style | 551.05 (± 130.46) | 310.00 (± 143.32) | < .0005 |
| Hopelessness | 199.47 (± 114.48) | 94.76 (± 97.76) | .004 |
| Problem solving | 314.74 (± 88.78) | 355.72 (± 109.39) | .20 |
| Total score | 1378.95 (± 253.99) | 982.86 (± 238.18) | < .0005 |
| Baseline HR (bpm) | 67.35 (± 8.41) | 60.67 (± 9.10) | .01 |
| Baseline RMSSD (ms$^2$) | 47.29 (± 17.47) | 77.93 (± 42.80) | < .0005 |
| VAS Rating (Preinduction) | | | |
| Rumination/worry | 40.84 (± 24.71) | 26.38 (± 26.09) | .08 |
| Distraction | 68.42 (± 17.60) | 48.57 (± 24.42) | .01 |
| Distraction by internal thoughts | 73.94 (± 19.75) | 69.05 (± 18.89) | .43 |

BMI, body mass index; bpm, beats per minute; GAD, generalized anxiety disorder; H, high; HC, healthy control; HR, heart rate; L, low; M, medium; M/F, male/female; PSWQ, Penn State Worry Questionnaire; RMSSD, root mean square successive difference; RRS, Ruminative Response Scale; SRRS, Stress-Reactive Rumination Scale; VAS, visual analog scale.

[a]Assessed by the question, "At what age did anxiety symptoms first appear?"

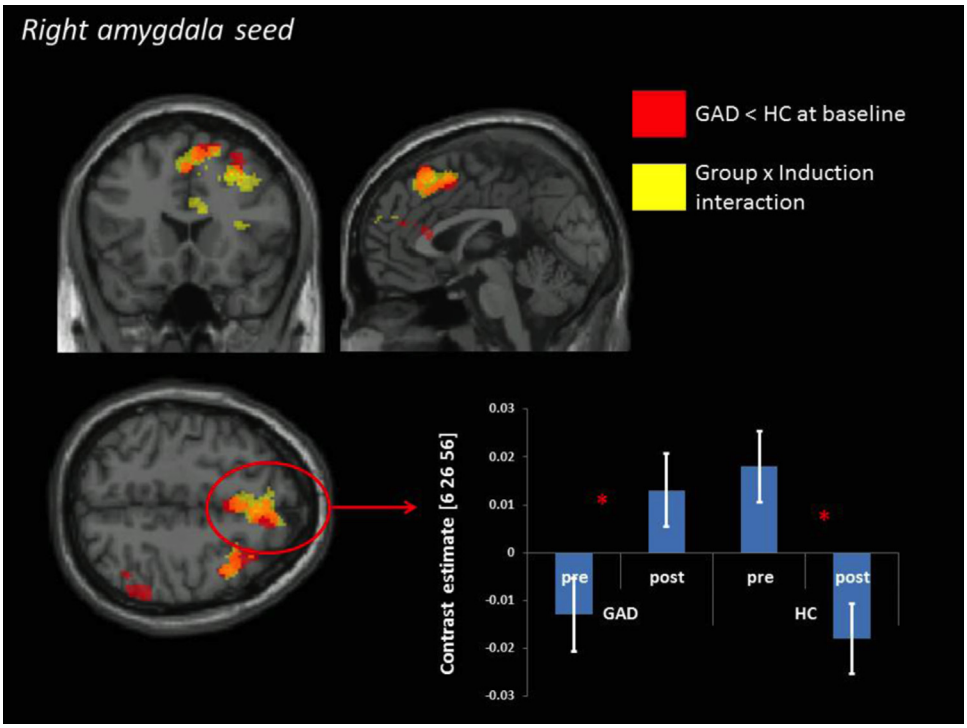


**Figure 1.** Regions showing lower connectivity between the right amygdala and superior frontal gyrus, right paracingulate/anterior cingulate cortex, and right supramarginal gyrus (red areas) in patients with generalized anxiety disorder (GAD) compared with healthy control (HC) subjects. A group × induction interaction was evident in the same areas as a result of an increase of the connectivity in patients with GAD and a decrease of connectivity in HC subjects (yellow areas) after the induction.

Nevertheless, given the anatomic overlap with the areas of lower connectivity in the GAD group compared with the HC group, we deemed it appropriate to describe this trend, as it meaningfully contributes to the interpretation of our data. No differences between the GAD and HC groups emerged for the postinduction connectivity of the right amygdala and the rest of the brain. No significant connectivity results emerged for the analysis using the left amygdala seed.

### Amygdala Connectivity and Correlations With Trait Measures

As depicted in Figure 2, a negative correlation was observed between the connectivity of the left amygdala with posterior paracingulate gyrus and anterior paracingulate gyrus/frontal medial cortex and dispositional worry (PSWQ). Similarly, a negative correlation was observed for connectivity of the right amygdala with thalamus and right middle frontal gyrus and the tendency to ruminate after the occurrence of a stressor (SRRS).

### Amygdala Connectivity and Correlations With State Measures

**Worry.** The baseline connectivity between the right amygdala and the paracingulate cortex was positively associated with Δ state worry (post − pre induction), indicating that in both groups, higher connectivity between the right amygdala and the paracingulate cortex predicted higher self-reported levels of worry after the induction (Figure 3).

**HRV.** The baseline connectivity between the right amygdala and subcallosal cortex and between the left amygdala and left caudate nucleus was negatively correlated with Δ HRV (post − pre) across groups (Figure 4A), where more negative Δ HRV values indicated a stronger decrease in HRV after the induction. This result indicates that a higher degree of connectivity between the amygdala and subcallosal cortex/caudate nucleus predicted a stronger decrease in HRV after the induction. At the same time, a Δ HRV (post − pre) × group interaction was evident for the connectivity between bilateral amygdala and PFC/cingulum, driven by a positive correlation in the GAD group and no correlation in the HC group. The positive correlation showed that a higher baseline connectivity between bilateral amygdala and PFC/cingulum predicted less decrease in HRV, acting as a protective factor (Figure 4B).

## DISCUSSION

We combined resting-state fMRI techniques with peripheral physiologic monitoring to disentangle the interplay between core psychological and physiologic expressions of GAD (i.e., excessive worry and autonomic dysfunction). We drew on preexisting evidence for the central role of the amygdala in GAD pathology (41) to quantify the relationship between amygdala connectivity and subjective and physiologic correlates of worry, particularly after an induction of perseverative cognition.

As in previous studies, at baseline, patients with GAD showed lower connectivity between the right amygdala and right superior frontal gyrus, right paracingulate/anterior cingulate cortex, and right supramarginal gyrus compared with HC subjects, supporting the hypothesis that disruption within the amygdala-PFC and amygdala-paracingulate networks (42–44) underlies the core features of GAD. Lower baseline connectivity in GAD may reflect failure to recruit PFC in the regulation

**Table 2. Brain Areas Showing Significant Connectivity Alteration in Patients With Generalized Anxiety Disorder Versus Healthy Control Subjects or Correlation With Behavioral and Autonomic Measures**

| | | Cluster | | Voxel | | | |
|---|---|---|---|---|---|---|---|
| | | | | | MNI | | |
| Brain Region | Side | *k* | *p* FWE | *Z* | x | y | z |
| GAD < HC | | | | | | | |
| Right amygdala seed | | | | | | | |
| Frontal pole | R | 374 | .004 | 4.84 | 40 | 48 | 16 |
| Superior frontal gyrus | R | 1330 | .001 | 4.83 | 6 | 30 | 52 |
| Paracingulate/ACC | R | 323 | .001 | 4.26 | 10 | 40 | 22 |
| Group × Induction Interaction | | | | | | | |
| Right amygdala seed | | | | | | | |
| Paracingulate/superior frontal gyrus | R | 332 | .07 | 3.72 | 6 | 26 | 56 |
| Middle frontal gyrus | R | 347 | .06 | 3.47 | 42 | 12 | 46 |
| Negative Correlation With SRRS and PSWQ Scores | | | | | | | |
| Right amygdala seed—SRRS | | | | | | | |
| Middle frontal gyrus | R | 270 | .04 | 3.81 | 30 | 34 | 48 |
| Thalamus | | 250 | .055 | 3.46 | −4 | −16 | 8 |
| Left amygdala seed—PSWQ | | | | | | | |
| Posterior cingulate cortex | | 276 | .03 | 4.58 | −6 | −20 | 40 |
| Paracingulate cortex/medial frontal gyrus | R | 482 | .001 | 4.26 | 12 | 52 | 2 |
| Positive Correlation With Δ Worry [Post − Pre] Across Both Groups | | | | | | | |
| Right amygdala seed | | | | | | | |
| Paracingulate gyrus | R | 262 | .02 | 4.06 | 12 | 26 | 40 |
| Negative Correlation With Δ HRV [Post − Pre] Across Both Groups | | | | | | | |
| Right amygdala seed | | | | | | | |
| Subcallosal cortex/ACC | L | 619 | .001 | 3.98 | −4 | 32 | −8 |
| Paracingulate gyrus/frontal medial cortex | L | | | 3.58 | −8 | 54 | −2 |
| Left amygdala seed | | | | | | | |
| Caudate/accumbens | L | 604 | .001 | 4.44 | −14 | 22 | −6 |
| Frontal orbital cortex | L | | | 3.88 | −34 | 28 | 2 |
| Δ HRV [Post − Pre] × Group Interaction | | | | | | | |
| Right amygdala seed | | | | | | | |
| Frontal orbital cortex | R | 339 | .006 | 4.86 | 20 | −28 | 22 |
| Superior frontal gyrus | R | 773 | .001 | 4.59 | 20 | 36 | 50 |
| Frontal pole | L | | | 3.85 | −10 | 50 | 48 |
| Cerebellum: vermis | L | 400 | .004 | 4.33 | −2 | −68 | −8 |
| ACC | R | 937 | .001 | 4.32 | 14 | 44 | 10 |
| Left amygdala seed | | | | | | | |
| Middle frontal gyrus | R | 499 | .001 | 4.66 | 50 | 24 | 44 |
| Frontal pole | R | | | 4.12 | 16 | 46 | 52 |
| Middle frontal gyrus | L | 614 | .001 | 4.6 | −42 | 30 | 42 |
| Frontal pole | L | | | 3.48 | −6 | 46 | 52 |
| Lateral occipital cortex | R | 431 | .001 | 4.24 | 38 | −64 | 28 |
| Supramarginal gyrus | R | 5.07 | .001 | 4.21 | 66 | −36 | 34 |

ACC, anterior cingulate cortex; FWE, familywise error; GAD, generalized anxiety disorder; HC, healthy control; HRV, heart rate variability; L, left; MNI, Montreal Neurological Institute; PSWQ, Penn State Worry Questionnaire; R, right; SRRS, Stress-Reactive Rumination Scale.

of an anxiety state, leading to increased amygdala activity and difficulties in emotion regulation (4), which is also supported by the chronically lower baseline HRV found in our pathologic group and reported by others (17).

The current findings implicate more superior regions of the right frontal lobe compared with the decreased connectivity between ventrolateral PFC and amygdala associated with greater anxiety found in previous studies and consistent with the inhibitory function of ventrolateral PFC. However, this is not an unexpected result if we consider that the superior frontal gyrus is involved in cognitive processes and effortful regulation of affect, and its activity has been found to be negatively correlated with that of the amygdala (45).

Following the induction, although only at a trend level, the connectivity between the same areas and the right amygdala increased in patients with GAD but decreased in HC subjects.

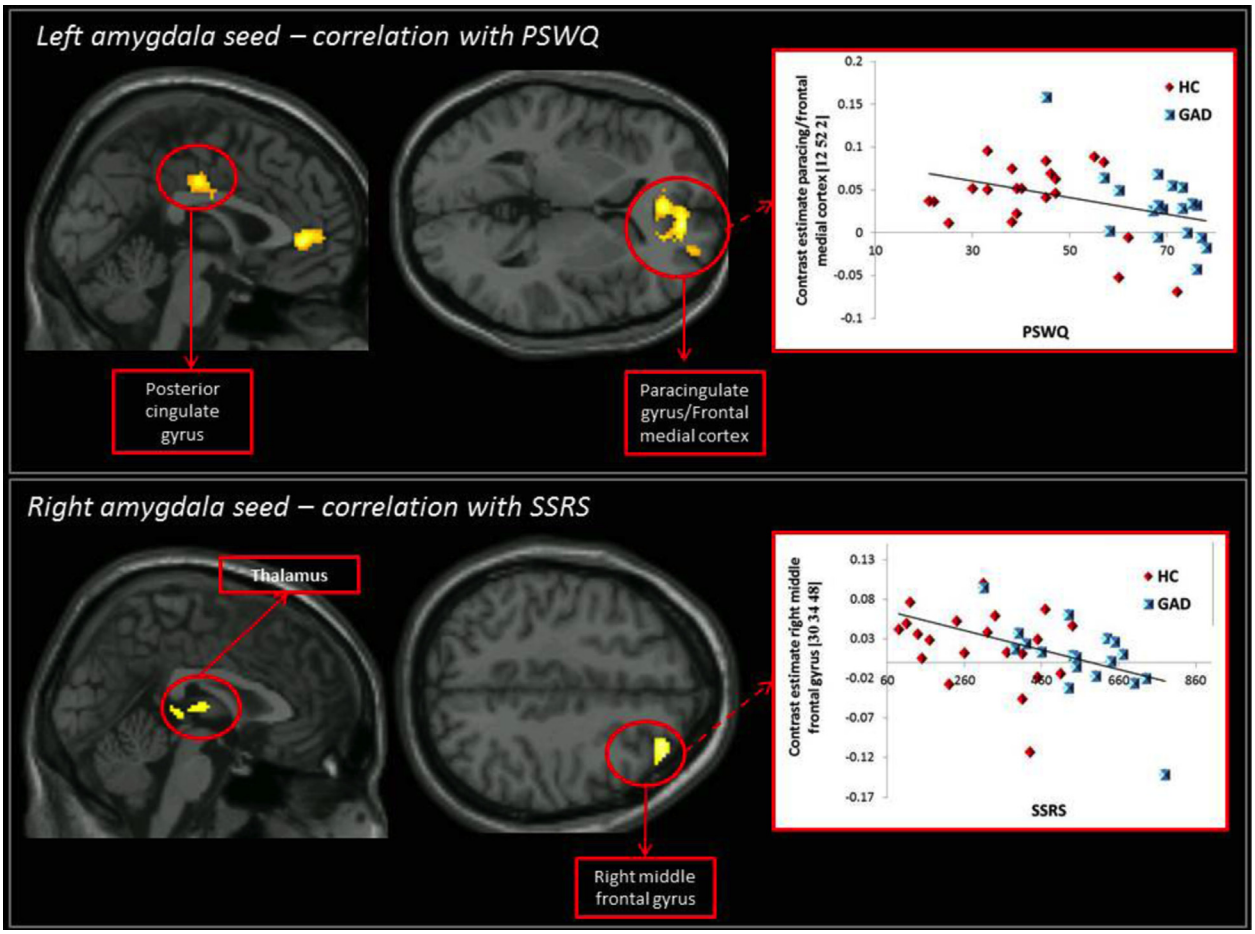


**Figure 2.** Correlations between dispositional measures of stress reactive rumination (Stress-Reactive Rumination Scale [SRRS]) and worry (Penn State Worry Questionnaire [PSWQ]) and functional connectivity of the left and right amygdala with the cortex. GAD, generalized anxiety disorder; HC, healthy control.

This finding is in line with a dimensional perspective of anxiety disorders, which led us to predict that the perseverative cognition induction would bring connectivity within the HC group closer to that observed in the GAD group. The HC subjects showed a tendency to respond to the perseverative cognition induction, becoming "neurally" more similar to the usual state of the patients with GAD. At rest, HC subjects habitually perceive the environment as safe, and their PFC exerts tonic inhibitory control on the amygdala and sympathoexcitatory neural circuits (24,25), as reflected in their functional integration between these neural structures. In these individuals, the perseverative cognition caused by the induction acts as a threat response that temporarily takes the regulatory role of PFC cortex "off-line," disinhibiting these

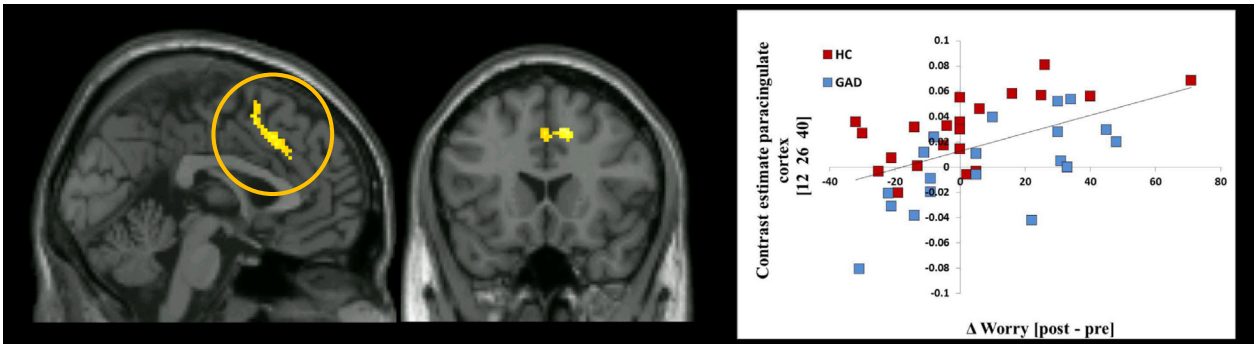


**Figure 3.** Positive correlation between state worry change score [Δ = post − pre] and connectivity of the right amygdala and paracingulate cortex across groups. GAD, generalized anxiety disorder; HC, healthy control.

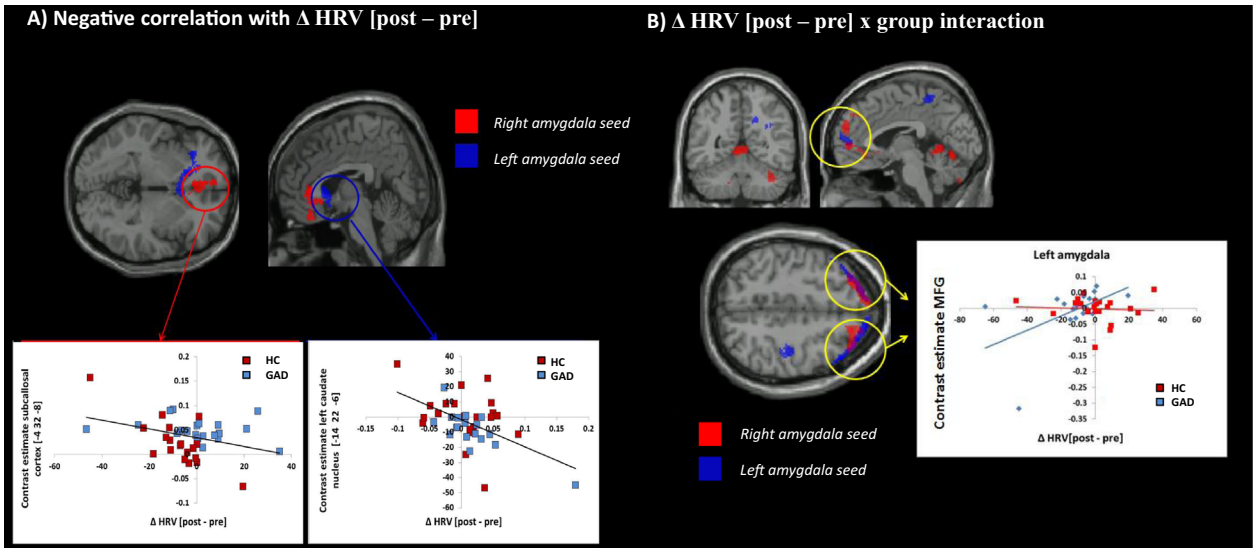


**Figure 4.** **(A)** Negative correlation between Δ heart rate variability (HRV) [post − pre] and connectivity of the right amygdala and subcallosal cortex (red areas) and of the left amygdala and left caudate nucleus (blue areas). **(B)** Δ HRV [post − pre] × group interaction driven by a positive correlation between Δ HRV [post − pre] and connectivity of bilateral amygdala and bilateral middle frontal gyrus (MFG) and frontal orbital cortex. GAD, generalized anxiety disorder; HC, healthy control.

circuits and being characterized by a reduction in amydgala-PFC connectivity.

Alternatively, the postinduction amygdala-PFC coupling in the patients with GAD may reflect the habitual engagement in cognitive strategies with the aim to regulate excessive arousal. According to the most prominent psychological model of worry, Borkovec's avoidance theory (13), patients with GAD use worry as a maladaptive cognitive avoidance strategy in an attempt to "keep under control" physiologic arousal associated with anxiety. Our results fit well with Borkovec's model because only in the patients with GAD was increased connectivity between bilateral amygdala and PFC/cingulum, presumably reflecting a stronger engagement of the PFC as consistently suggested by brain activation studies on this topic (46), associated with the attenuation of dysregulated autonomic arousal, confirming that worry may be an effective coping strategy to suppress physiologic arousal in this clinical population. This finding provides additional insight into how this maladaptive strategy is maintained in this psychopathologic disorder. Nevertheless, with time, worry becomes dysfunctional in GAD, ultimately recalibrating the effective PFC control over structures including amygdala (diminished functional connectivity) and downgrading autonomic regulation (decreasing tonic HRV). The present data suggest the potential utility of therapeutic interventions aimed at enhancing connectivity in high arousal states and reducing connectivity in low arousal states. However, future studies are needed to consolidate the role of amygdala-PFC connectivity as a predictive or modifiable biomarker in GAD.

Our results showing reduced connectivity at baseline but enhanced connectivity during worry may help explain inconsistent results on amygdala-PFC coupling found in anxiety (47). Moreover, the present findings partially support a recently proposed view of anxiety symptoms as subserved by different neural mechanisms, such as reduced connectivity within a dorsolateral PFC–thalamo-striatal network associated with trait anxiety and increased dorsolateral PFC functional connectivity with default mode regions associated with worry (48). Our results also suggest a functional lateralization of amygdala, with the functional connectivity between the right amygdala and PFC being preferentially involved in anxiety and state worry lending support to the idea of different roles for the left and right amygdala in emotional processing (49).

The only previous work that examined the effects of a worry induction on GAD studied elderly participants, using insula as a seed region. Keeping in mind these differences, the previous study also observed stronger connectivity between insula and orbitofrontal cortex in participants with GAD during a worry induction compared with reappraisal (19). In agreement with our group difference and in line with a dimensional view of psychopathology, trait measures of stress-reactive rumination and worry were negatively associated with baseline connectivity between the amygdala and areas of the frontal and cingulate cortex—again presumably reflecting efforts to suppress arousal.

Consistent with the effects of induction in increasing functional amygdala-PFC connectivity in GAD, baseline connectivity between the right amygdala and paracingulate cortex predicted the postinduction shift in state worry, with higher connectivity being associated with stronger increases in worry after induction. Such an association fits well also with data on patients with generalized social phobia displaying stronger connectivity between the amygdala and dorsomedial PFC than control subjects during self-referential criticism (50). Similarly, a more neurotic individual shows greater connectivity between the right amygdala and right dorsomedial PFC when processing angry and fearful faces compared with neutral faces (51). An alteration in the

connectivity of the amygdala with the paracingulate cortex resulted as a significant group effect, changed from preinduction to postinduction; was associated with trait and state measures of perseverative cognition; and was implicated in the Δ HRV × group interaction, suggesting overlapping neural mechanisms for the expression of (dispositional and state) worry and the accompanying autonomic dysregulation in GAD.

When the examined network did not involve the PFC but encompassed connectivity of the amygdala with limbic and subcortical structures, a stronger coupling between these areas predicted a stronger decrease in HRV after the induction, indicating a threat mode response. The caudate nucleus is implicated in HRV regulation in patients with social anxiety disorder (52) and healthy subjects (53,54). Moreover, interactions between the striatum and amygdala are of particular interest in the context of reward processing (55), and the caudate nucleus is implicated in the processing of threatening face stimuli (56). A positive correlation has been reported between the activation of the caudate nucleus and HRV in patients with social anxiety disorder, whereas a negative correlation is observed in healthy individuals (57). Our results provide further novel insight into striatolimbic interaction relevant to perseverative cognition. Such interactions are reminiscent of observations in patients with obsessive-compulsive disorder, in whom caudate nucleus is implicated in the expression of repetitive obsessions evoked by contamination fears (58,59).

In conclusion, our data provide important new insight into neural mechanisms through which emotional regulation and autonomic dysfunction interact in GAD. This study also has a broader relevance, shedding light into potentially opposing processes that contribute to the relationship between anxiety disorders and cardiovascular risk (a still unresolved debate) (60). The present findings suggest that the aberrant engagement of amygdala-PFC circuitry might be one of the key factors underlying the pathophysiology of GAD. If the robustness of this prediction is confirmed in future research, resting-state connectivity could potentially be used as a biomarker of treatment response.

## ACKNOWLEDGMENTS AND DISCLOSURES

This work was supported by the Italian Ministry of Health Young Researcher Grant No. GR-2010-2312442 (CO).

The authors report no biomedical financial interests or potential conflicts of interest.

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Received Jun 27, 2015; revised Sep 23, 2015; accepted Oct 13, 2015.

Supplementary material cited in this article is available online at http://dx.doi.org/10.1016/j.biopsych.2015.10.013.

## REFERENCES

1. Hoffman DL, Dukes EM, Wittchen HU (2008): Human and economic burden of generalized anxiety disorder. Depress Anxiety 25:72–90.
2. Olesen J, Gustavsson A, Svensson M, Wittchen HU, Jönsson B (2012): CDBE2010 study group; European Brain Council. The economic cost of brain disorders in Europe. Eur J Neurol 19: 155–162.
3. LeDoux J (2003): The emotional brain, fear, and the amygdala. Cell Mol Neurobiol 23:727–738.
4. Hilbert K, Lueken U, Beesdo-Baum K (2014): Neural structures, functioning and connectivity in generalized anxiety disorder and interaction with neuroendocrine systems: A systematic review. J Affect Disord 158:114–126.
5. Etkin A, Prater KE, Schatzberg AF, Menon V, Greicius MD (2009): Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. Arch Gen Psychiatry 66:1361–1372.
6. Monk CS, Telzer EH, Mogg K, Bradley BP, Mai X, Louro HM, *et al*. (2008): Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety disorder. Arch Gen Psychiatry 65:568–576.
7. Roy AK, Fudge JL, Kelly C, Perry JS, Daniele T, Carlisi C, *et al*. (2013): Intrinsic functional connectivity of amygdala-based networks in adolescent generalized anxiety disorder. J Am Acad Child Adolesc Psychiatry 52:290–299.
8. Hamm L, Jacobs RH, Johnson MW, Fitzgerald DA, Fitzgerald KD, Langenecker SA, *et al*. (2014): Aberrant amygdala functional connectivity at rest in pediatric anxiety disorders. Biol Mood Anxiety Disord 4:15.
9. Ball TM, Ramsawh HJ, Campbell-Sills L, Paulus MP, Stein MB (2013): Prefrontal dysfunction during emotion regulation in generalized anxiety and panic disorders. Psychol Med 43:1475–1486.
10. McClure EB, Adler A, Monk CS, Cameron J, Smith S, Nelson EE, *et al*. (2007): fMRI predictors of treatment outcome in pediatric anxiety disorders. Psychopharmacology 191:97–105.
11. Banks SJ, Eddy KT, Angstadt M, Nathan PJ, Phan KL (2007): Amygdala-frontal connectivity during emotion regulation. Soc Cogn Affect Neurosci 2:303–312.
12. Erk S, Mikschl A, Stier S, Ciaramidaro A, Gapp V, Weber B, *et al*. (2010): Acute and sustained effects of cognitive emotion regulation in major depression. J Neurosci 30:15726–15734.
13. Borkovec TD, Alcaine O, Behar ES (2004): Avoidance theory of worry and generalized anxiety disorder. In: Heimberg R, Mennin D, Turk C, editors. Generalized Anxiety Disorder: Advances in Research and Practice. New York: Guilford, 77–108.
14. Critchley HD (2005): Neural mechanisms of autonomic, affective, and cognitive integration. J Comp Neurol 493:154–166.
15. Gianaros PJ, Onyewuenyi IC, Sheu LK, Christie IC, Critchley HD (2012): Brain systems for baroreflex suppression during stress in humans. Hum Brain Mapp 33:1700–1716.
16. Ottaviani C, Thayer JF, Verkuil B, Lonigro A, Medea B, Couyoumdjian A, *et al*. (2016): Physiological concomitants of perseverative cognition: A systematic review and meta-analysis. Psychol Bull 142:231–259.
17. Thayer JF, Friedman BH, Borkovec TD (1996): Autonomic characteristics of generalized anxiety disorder and worry. Biol Psychiatry 39: 255–266.
18. Bijsterbosch J, Smith S, Forster S, John OP, Bishop SJ (2014): Resting state correlates of subdimensions of anxious affect. J Cogn Neurosci 26:914–926.
19. Andreescu C, Sheu LK, Tudorascu D, Gross JJ, Walker S, Banihashemi L, *et al*. (2015): Emotion reactivity and regulation in late-life generalized anxiety disorder: Functional connectivity at baseline and post-treatment. Am J Geriatr Psychiatry 23:200–214.
20. Critchley HD, Mathias CJ, Josephs O, O'Doherty J, Zanini S, Dewar BK, *et al*. (2003): Human cingulate cortex and autonomic control: Converging neuroimaging and clinical evidence. Brain 126:2139–2152.
21. Chang C, Metzger CD, Glover GH, Duyn JH, Heinze H, Walter M (2013): Association between heart rate variability and fluctuations in resting-state functional connectivity. Neuroimage 68:93–104.

22. Beissner F, Meissner K, Bär KJ, Napadow V (2013): The autonomic brain: An activation likelihood estimation meta-analysis for central processing of autonomic function. J Neurosci 33:10503–10511.
23. Makovac E, Garfinkel SN, Bassi A, Basile B, Macaluso E, Cercignani M (2015): Effect of parasympathetic stimulation on brain activity during appraisal of fearful expressions. Neuropsychopharmacology 40:1649–1658.
24. Thayer JF, Lane RD (2009): Claude Bernard and the heart-brain connection: Further elaboration of a model of neurovisceral integration. Neurosci Biobehav Rev 33:81–88.
25. Thayer JF, Ahs F, Fredrikson M, Sollers JJ 3rd, Wager TD (2009): A meta-analysis of heart rate variability and neuroimaging studies: Implications for heart rate variability as a marker of stress and health. Neurosci Biobehav Rev 33:81–88.
26. Williams DP, Cash C, Rankin C, Bernardi A, Koenig J, Thayer JF (2015): Resting heart rate variability predicts self-reported difficulties in emotion regulation: A focus on different facets of emotion regulation. Front Psychol 10:261.
27. Ottaviani C, Shapiro D, Couyoumdjian A (2013): Flexibility as the key for somatic health: From mind wandering to perseverative cognition. Biol Psychol 94:38–43.
28. Ottaviani C, Medea B, Lonigro A, Tarvainen M, Couyoumdjian A (2015): Cognitive rigidity is mirrored by autonomic inflexibility in daily life perseverative cognition. Biol Psychol 107:24–30.
29. Olatunji BO, Broman-Fulks JJ, Bergman SM, Green BA, Zlomke KR (2010): A taxometric investigation of the latent structure of worry: Dimensionality and associations with depression, anxiety, and stress. Behav Ther 41:212–228.
30. Robinson MS, Alloy LB (2003): Negative cognitive styles and stress-reactive rumination interact to predict depression: A prospective study. Cognit Ther 27:275–292.
31. Nolen-Hoeksema S, Morrow J (1991): A prospective study of depression and posttraumatic stress symptoms after a natural disaster: The 1989 Loma Prieta earthquake. J Pers Soc Psychol 61:115–121.
32. Meyer TJ, Miller ML, Metzger RL, Borkovec TD (1990): Development and validation of the Penn State Worry Questionnaire. Behav Res Ther 28:487–495.
33. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996): Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. Circulation 93:1043–1065.
34. Alvares GA, Quintana DS, Kemp AH, Van Zwieten A, Balleine BW, Hickie IB, *et al*. (2013): Reduced heart rate variability in social anxiety disorder: Associations with gender and symptom severity. PLoS One 8:e70468.
35. Nussinovitch U, Cohen O, Kaminer K, Ilani J, Nussinovitch N (2012): Evaluating reliability of ultra-short ECG indices of heart rate variability in diabetes mellitus patients. J Diabetes Complications 26:450–453.
36. Goedhart AD, van der Sluis S, Houtveen JH, Willemsen G, de Geus EJ (2007): Comparison of time and frequency domain measures of RSA in ambulatory recordings. Psychophysiology 44:203–215.
37. Penttilä J, Helminen A, Jartti T, Kuusela T, Huikuri HV, Tulppo MP, *et al*. (2001): Time domain, geometrical and frequency domain analysis of cardiac vagal outflow: Effects of various respiratory patterns. Clin Physiol 21:365–376.
38. Saad ZS, Gotts SJ, Murphy K, Chen G, Jo HJ, Martin A, *et al*. (2012): Trouble at rest: how correlation patterns and group differences become distorted after global signal regression. Brain Connect 2: 25–32.
39. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N, *et al*. (2002): Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. Neuroimage 15:273–289.
40. Dukart J, Bertolino A (2014): When structure affects function—the need for partial volume effect correction in functional and resting state magnetic resonance imaging studies. PLoS One 9:e114227.
41. Kim MJ, Gee DG, Loucks RA, Davis FC, Whalen PJ (2011): Anxiety dissociates dorsal and ventral medial prefrontal cortex functional connectivity with the amygdala at rest. Cereb Cortex 21:1667–1673.
42. Prater KE, Hosanagar A, Klumpp H, Angstadt M, Phan KL (2013): Aberrant amygdala-frontal cortex connectivity during perception of fearful faces and at rest in generalized social anxiety disorder. Depress Anxiety 30:234–241.
43. Liao W, Qiu C, Gentili C, Walter M, Pan Z, Ding J, *et al*. (2010): Altered effective connectivity network of the amygdala in social anxiety disorder: A resting-state FMRI study. PLoS One 5:e15238.
44. Hahn A, Stein P, Windischberger C, Weissenbacher A, Spindelegger C, Moser E, *et al*. (2011): Reduced resting-state functional connectivity between amygdala and orbitofrontal cortex in social anxiety disorder. Neuroimage 56:881–889.
45. Roy AK, Shehzad Z, Margulies DS, Kelly AM, Uddin LQ, Gotimer K, *et al*. (2009): Functional connectivity of the human amygdala using resting state fMRI. Neuroimage 45:614–626.
46. Paulesu E, Sambugaro E, Torti T, Danelli L, Ferri F, Scialfa G, *et al*. (2010): Neural correlates of worry in generalized anxiety disorder and in normal controls: A functional MRI study. Psychol Med 40:117–124.
47. McClure EB, Monk CS, Nelson EE, Parrish JM, Adler A, Blair RJ, *et al*. (2007): Abnormal attention modulation of fear circuit function in pediatric generalized anxiety disorder. Arch Gen Psychiatry 64:97–106.
48. Forster S, Nunez Elizalde AO, Castle E, Bishop SJ (2015): Unraveling the anxious mind: Anxiety, worry, and frontal engagement in sustained attention versus off-task processing. Cereb Cortex 25:609–618.
49. Baas D, Aleman A, Kahn RS (2004): Lateralization of amygdala activation: A systematic review of functional neuroimaging studies. Brain Res Brain Res Rev 45:96–103.
50. Blair K, Geraci M, Devido J, McCaffrey D, Chen G, Vythilingam M, *et al*. (2008): Neural response to self- and other referential praise and criticism in generalized social phobia. Arch Gen Psychiatry 65: 1176–1184.
51. Cremers HR, Demenescu LR, Aleman A, Renken R, van Tol MJ, van der Wee NJ, *et al*. (2010): Neuroticism modulates amygdala-prefrontal connectivity in response to negative emotional facial expressions. Neuroimage 49:963–970.
52. Ahs F, Sollers JJ 3rd, Furmark T, Fredrikson M, Thayer JF (2009): High-frequency heart rate variability and cortico-striatal activity in men and women with social phobia. Neuroimage 47:815–820.
53. Critchley HD, Rotshtein P, Nagai Y, O'Doherty J, Mathias CJ, Dolan RJ (2005): Activity in the human brain predicting differential heart rate responses to emotional facial expressions. Neuroimage 24:751–762.
54. Lane RD, McRae K, Reiman EM, Chen K, Ahern GL, Thayer JF (2009): Neural correlates of heart rate variability during emotion. Neuroimage 44:213–222.
55. Balleine BW, Delgado MR, Hikosaka O (2007): The role of the dorsal striatum in reward and decision-making. J Neurosci 27:8161–8165.
56. Almeida I, van Asselen M, Castelo-Branco M (2013): The role of the amygdala and the basal ganglia in visual processing of central vs. peripheral emotional content. Neuropsychologia 51:2120–2129.
57. Gaebler M, Daniels JK, Lamke JP, Fydrich T, Walter H (2013): Heart rate variability and its neural correlates during emotional face processing in social anxiety disorder. Biol Psychol 94:319–330.
58. Baxter LR (1995): Neuroimaging studies of human anxiety disorders: Cutting paths of knowledge through the field of neurotic phenomena. In: Bloom F, Kupfer D, editors. Neuropsychopharmacology: The Fourth Generation of Progress. New York: Raven Press, 1287–1299.
59. Gillan CM, Apergis-Schoute AM, Morein-Zamir S, Urcelay GP, Sule A, Fineberg NA, *et al*. (2015): Functional neuroimaging of avoidance habits in obsessive-compulsive disorder. Am J Psychiatry 172: 284–293.
60. Player MS, Peterson LE (2011): Anxiety disorders, hypertension, and cardiovascular risk: A review. Int J Psychiatry Med 41; 365–337.