# Resting-State Functional Connectivity in Trait Anxiety: A Conceptual Replication Study

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**Abstract**: Trait anxiety, a stable personality attribute, has previously been associated with alternations in brain functional connectivity. We use resting-state functional MRI data from a large sample of high anxious individuals to conceptually replicate selected studies with seed-based methods and network-based methods. Seed regions included comprised the lateral amygdala subnuclei (basolateral, centromedial and superficial), bed nucleus of the stria terminalis (BNST) and anterior insula. We investigated anxiety-related alternations in within-network connectivity in the default mode network, salience network and executive control network. The basolateral and centromedial amygdala showed anxiety-related alternations in baseline connectivity. Trait anxiety was further negatively correlated with the functional connectivity of the basolateral amygdala and regions in the ventromedial prefrontal cortex, ventral anterior cingulate cortex and dorsal anterior cingulate cortex. The centromedial amygdala exhibited a stronger functional coupling with regions in the primary somatosensory cortex and premotor cortex with higher levels of trait anxiety. In this regard, we could replicate frequently reported findings to some extend as well as less common results in a large non-clinical sample with elevated trait anxiety. Finally, trait anxiety was not correlated with the functional coupling of the anterior insula and BNST at rest. There was further no effect of trait anxiety on within-network connectivity for all resting-state networks.

**Keywords**: trait anxiety, functional connectivity, amygdala, insula, prefrontal, anterior cingulate cortex, resting-state networks

Anxiety is an affective state which is characterized by temporary changes in emotional, physiological and cognitive responding caused by the anticipation of threat under perceived uncertainty (Grupe & Nitschke, 2013). This manifests in symptoms of physiological arousal, environmental hypervigilance, increased worry as well as attentional bias toward threat-related stimuli (Watson et al., 1988; Craske et al., 2009; Lang et al., 2000; Bishop, 2007; Eysenck et al., 1991; Hazlett-Stevens & Borkovec, 2004). Although anxiety is an evolutionary adaptive response (Brosschot et al., 2016), the general propensity to experience anxiety can become maladaptive in individuals. Trait anxiety measures this stable predisposition in both, quantity and quality of the anxiety response (Eysenck, 1992). Individuals high in trait anxiety are at risk of developing psychiatric conditions later in life, including anxiety disorders (Chambers et al., 2003) and mood disorders (Sandi & Richter-Levin, 2009). Therefore, understanding how trait anxiety is manifested in the brain is a relevant field of research.

Alternations in the functional connectivity (FC) of distributed neural networks including cortical and subcortical regions underlie the manifestation of anxiety disorders and, presumably, trait anxiety (Sylvester et al., 2012). Resting-state fMRI has been used in the investigation of intrinsic FC between remote brain regions involved in various cognitive processes at wakeful rest (Smith, 2013; van den Heuvel & Hulshoff Pol, 2010). Resting-state functional connectivity (RSFC) is defined as the similarity of spontaneous low-frequency fluctuations in the blood oxygen level-dependent (BOLD) signal across time and space (Biswal et al., 1995; Bijsterbosch et al., 2017; Beckmann et al., 2005). RSFC identified different resting-state networks (RSNs) describing functionally organized large-scale networks that are stable across individuals (Damoiseaux et al., 2006; Luca et al., 2006).

Trait anxiety is postulated to underlie anxiety disorders as an early risk factor (Sylvester

et al., 2012). In this regard, variations in RSFC mediated by individual differences in trait anxiety have the potential to serve as a neurological marker of vulnerability to anxiety disorders. Identifying stable and reliable markers could help in the early detection of anxiety disorders. In this paper, we present a conceptual replication to investigate the neural representation of individual differences in trait anxiety at the level of RSFC. This replication study is based on a preceding literature review according to predefined selection criteria. We aim to conceptually replicate previous published studies in a large sample of high anxious individuals. We call our approach a conceptual replication as we selected from relevant literature more prominent target regions, and established analysis methods. This gives the opportunity to validate previous findings derived from small or selective samples. Another advantage is that any effect related to individual differences in anxiety should manifest more prominently in a sample with elevated trait anxiety scores.

In the following, we provide an overview of the target seed regions and networks to rationalize our methodological decisions for this replication study.

**Seed selection**

Several studies investigated the specific functional architecture of the amygdala to indentify its role in different neural circuits with respect to individual differences in trait anxiety (Kim et al., 2011; Loewenstern et al., 2019; Weis et al., 2019). Therefore, we included lateral seed regions of multiple amygdala subnuclei as well as the extended amygdala, to utilize regions that were implicated in previous studies. Selected seeds comprised the left and right basolateral amygdala, centromedial amygdala, the superficial amygdala and the bed nucleus of the stria terminalis (BNST).

The role of the amygdala in anxiety is attributed to its involvement in the evaluation and

processing of threat-related stimuli (LeDoux, 2003; Fitzgerald et al., 2017; Straube et al., 2011; Etkin et al., 2004; Dickie & Armony, 2008). Alternations in amygdala activity have been reported for anxiety disorders and trait anxiety during emotion regulation tasks (Fitzgerald et al., 2017) and the processing of stimuli signaling threat (Straube et al., 2011; Etkin et al., 2004; Dickie & Armony, 2008), suggesting aberrant network dynamics with frontal regions that are involved in emotional regulatory functions over the amygdala through top-down or bottom-up processes (Dixon et al., 2017; Kohn et al., 2014; Berboth & Morawetz, 2021; Dolcos et al., 2011). In line with this notion, anxiety disorders (for a review see, Roberts, 2020) and anxiety proneness (Bishop, 2007) have been associated with hyperactivity of the amygdala accompanied by hypoactivity of frontal regions including the medial prefrontal cortex (mPFC), the lateral PFC (lPFC) and the anterior cingulate cortex (ACC). Consistently, previous studies that investigated trait anxiety have implicated aberrant RSFC between the amygdala and the mPFC (Delli Pizzi et al., 2017; Kim et al., 2011) and dorsolateral PFC (Loewenstern et al., 2019; Weis et al., 2019), as well as the ventral ACC (Berry et al., 2019). In particular, alternations in the FC between the amygdala and a network comprising the mPFC and ACC have frequently been associated with trait anxiety. It has been shown that subdevisions of the mPFC and ACC have different roles in emotional processing. The ventral portion has been associated with emotional conflict regulation and the dorsal portion with emotional conflict detection and appraisal (for a review see Etkin et al., 2011). Based on previous studies, trait anxiety might influence the dissociation of amygdala RSFC with the ventral and dorsal mPFC and ACC. However, disagreement remains regarding the directionality of the association between FC and trait anxiety. Aiming to replicate these findings in a highly anxious population, we hypothesize to find anxiety-induced alternations in amygdala RSFC that differ for the dorsal and ventral subsection of the mPFC and ACC.

In addition to the amygdala, we included the anterior insula as a seed region to investigate its association with trait anxiety. Studies that looked at the bilateral anterior insula RSFC either investigated variations in trait anxiety of whole-brain RSFC (Huggins et al., 2018) or the direct coactivation of the amygdala and insula (Baur et al., 2013; Huggins et al., 2018). The anterior insula has a role in interoception through the integration of information about stimulus salience and current internal body states (Paulus & Stein, 2006). In this regard, it informs about homeostatic significance of stimuli and contributes to the instatiation of the subjective awareness of feelings (Craig, 2011; Critchley et al., 2004). The anterior insula is involved in the anticipation of uncontrollable aversive events (Alvarez et al., 2011; Grupe et al., 2012; Somerville et al., 2012; Simmons et al., 2006; Carlson et al., 2011) and hypervigilant threat-monitoring in anxious individuals (Somerville et al., 2010).

**Resting-state network (RSN) selection**

Alternations in RSNs that underlie individual differences in anxiety are associated with relative hypo- or hyper-connectivity, either within or between networks. We chose to primarily include the three core networks that are thought to be affected in anxiety and anxiety disorders (Sylvester et al., 2012; Xu et al., 2019; Peterson et al., 2014) in our analysis to investigate trait anxiety changes in within-network connectivity. These include the salience network (SN), the default mode network (DMN) and the executive control network (ECN). The SN encompasses cortical hubs in the dorsal anterior cingulate cortex (dACC) and frontoinsula and additional subsections comprising the amygdala, hypothalamus, ventral striatum, thalamus, and specific brainstem nuclei (Seeley, 2019) and is involved in stimulus-driven salience processing (Seeley et al., 2007). Changes in within-network FC have previously been reported for anxiety disorders (Etkin et al., 2009; Xu et al., 2019) and anxiety-related personality dimensions (Markett et al.,

2016; Geng et al., 2016; Xu et al., 2019). The majority of these studies reported a stronger functional coupling within the SN. The DMN comprises core regions in the medial prefrontal cortex, the posterior cingulate cortex and precuneus and the inferior parietal lobule (Shulman et al., 1997; Buckner et al., 2008). Further, the hippocampus is frequently coupled with the DMN and it has subcortical connections to the amygdala (Buckner et al., 2008). The DMN is a task-negative network. While being suspended during goal-oriented or attention-demanding tasks (Raichle & Snyder, 2007), DMN activiation is evoked by (affective) self-referential thoughts, repetitive negative thinking, episodic memory retrieval, and interospection about one's mental states (Andrews-Hanna et al., 2010; Feurer et al., 2021). The DMN has shown to be affected in anxiety disorders (Zhao et al., 2007; Northoff, 2020). These alternations are assumed to arise from a hypo-connectivity within the network (Peterson et al., 2014; Xu et al., 2019). The ECN is defined by primary regions in the dorsolateral prefrontal cortex and lateral posterior parietal cortex (Seeley et al., 2007). Like the SN, the ECN is often referred to as a task-positive network (Fox et al., 2005) which is involved in top-down cognitive control, working memory and executive functioning (Daniels et al., 2010; Fox et al., 2006; Sylvester et al., 2012; Xu et al., 2019). The ECN has been associated with decreased functioning in anxiety disorders (Sylvester et al., 2012; Picó-Pérez et al., 2017) and in individuals with high trait anxiety (Bishop, 2009). In addition, decreased within-network connectivity of the ECN has been associated with anxiety disorders at rest (Geiger et al., 2017).

Previous RSFC studies have reported changes in within-network connectivity of the DMN in relation to trait anxiety (Modi et al., 2015; Saviola et al., 2020). Based on these observations and findings from patients with anxiety disorder we hypothesize to replicate these findings, i.e a reduction in the within-network functional connectivity of the DMN at rest.

## Method

### Participants

All analyses were conducted on the data provided by the high anxiety sample taken from Brehl and colleagues (see Brehl et al., 2020). In their study, 2206 individuals were pre-screened via the online survey platform soscisurvey.com. For all participating candidates, anxiety levels on the HADS (Hospital Anxiety and Depression Scale; Zigmond & Snaith, 1983), demographic data and MRI compatibility were assessed. The HADS is a screening tool for anxiety and depression symptoms that comprises two 7-item subscales generating a separate score for anxiety and depression. Participants with a score on the HADS anxiety-subscale (HADS-A) ≥ 8 were invited to participate in the study. Since a score on the HADS-A score above 7 indicates moderate anxiety symptoms, this ensures oversampling for elevated anxiety levels in the sample distribution in order to facilitate the detection of anxiety-driven effects. Participants with normal anxiety scores ≤ 7 were selected for participation in the study based on a 25%-probability. Individuals with HADS-A scores > 15, a score reflecting severe levels of anxiety, were subsequently assessed by the study psychologist to exclude clinical cases. Eventually, 248 subjects were recruited to participate in the study. Subsequently, 14 subject were excluded, one subject due to suspected psychiatric symptoms, 13 subjects due to reduced data quality, incomplete data, incidental findings, or falling asleep during the scanning session. Finally, 234 healthy participants (Mean age: 22.8 ± 4.85; m = 63, f = 171) completed the whole test session. Of those, 184 participants had elevated HADS-A scores (≥ 8) and 50 participants had HADS-A scores in the normal range (<8). Participants were paid 10 Euro per hour for their participation in the study. The study has been approved by the Commissie Mensgebonden Onderzoek (CMO) of the Radboudumc in Nijmegen/ The Netherlands (CMO 2017/3588).

Resting-state data was available for 221 of the 247 subjects participating in the study by Brehl and colleagues (2021). One participant was further excluded due to an aberrant FOV, resulting in a final sample of 220 participants.

**Measurements**

All participants completed multiple self-assessment questionnaires after the fMRI scanning session. To ensure comparability with existing literature on resting-state functional connectivity, trait anxiety was assessed with the trait version of the Spielberger's State and Trait Anxiety Inventory (STAI-T; Spielberger, 1983). The STAI is developed to assess short-lived state and stable trait anxiety in two separate scales (STAI-S and STAI-T, respectively). Scores on each scale ranges from 20 to 80, with higher scores reflecting higher anxiety. The STAI-T is regarded as a stable measurement with good test-retest reliability and internal consistency (Barnes et al., 2002). However, STAI-T items have been postulated to measure anxious and depressive dimensions of negative affect, a general variable of negative emotions (Knowles & Olatunji, 2020; Mathews et al., 1996; Balsamo et al., 2013; Bado et al., 2010; Bijsterbosch et al., 2014). We therefore included the IDS-SR (The Inventory of Depressive Symptomatology, Self-Report; Rush et al., 1996) as a covariate, to account for depressive symptoms in the analysis.

**Literature search and selection**

A literature search was conducted to obtain a pre-selection of potential articles to be included in this study. For this purpose, the following databases were used: PubMed (searched on October 21 2020) and Web of Science (searched on October 25 2020). Search results were derived using the keywords (anxiety) AND (resting state). We included resting-state fMRI studies that used seed-based whole-brain RSFC or independent component analysis to correlate with individual anxiety scores measured with the STAI-T. A manual search of the titles and

abstracts of the remaining articles was conducted based on the following inclusion criteria: 1) English article, 2) human studies, 3) RSFC studies, 4) trait anxiety assessed with the STAI-T, 5) no history of or current neurological or psychiatric disorders, 6) adult sample. The inclusion criteria were derived to match the sample that had been used in this study. In order to avoid potential confounds like neurodevelopmental and neurodegenerative effects, only studies conducted in adult samples have been included.

**MRI acquisition and preprocessing**

Scans were acquired on a 3-T Magnetom P rismaF it MRI Scanner (Siemens AG, Healthcare Sector, Erlangen, Germany) using a 32-channel head coil at the Donders Centre for Cognitive Neuroimaging. For each subject a high resolution T1-weighted anatomical scan (slice number = 192, TR = 2300 ms, TE = 3.03 ms, flip angle = 8°, voxel size = 1x1x1 mm3 , FOV = 256x256x192 mm3 , base resolution = 256, sagittal acquisition) was obtained. Images of resting-state functional MRI were acquired with a T2*-weighted EPI BOLD-fMRI multiband 4 sequence (slice number = 68, TR = 1500 ms, TE = 39.6 ms, flip angle = 75°, voxel size = 2x2x2 mm3, slice gap = 0 mm, FOV = 210 mm). During the resting-state scan, participants were instructed to keep their eyes open and stay awake while focusing on a fixation cross and think of nothing in particular. The resting-state scan was acquired after the completion of three functional MRI tasks over a time window of seven minutes. This acquisition period has been proven to be sufficient to detect RSNs (Van Dijk et al., 2010).

Functional MRI data was preprocessed according to the preprocessing pipeline of fMRIPrep version 1.4.1 (Esteban et al., 2019; RRID:SCR_016216). Prior to the preprocessing the first five volumes were discarded to allow the early transient magnetization to settle at a steady-state. To account for inhomogeneities in B0, distortion correction was performed using

fieldmaps. A Boundary-Based Registration approach (9 degrees of freedom) was used to co-register the unwarped EPI functional data to the corresponding T1-weighted reference image. Head-motion correction was applied by registering each volume to native space. The data was spatially smoothed using a 6 mm full-width-half-maximum Gaussian kernel and high-pass temporal filtered (0.01 Hz cut-off period). Subsequently, the data was normalized to MNI-standard space (MNI152NLin6Asym) and resampled to 2mm3 resolution with FMRIB's Nonlinear Image Registration Tool (FNIRT). Brain extraction was performed using the binarized whole-brain mask from fMRIPrep. ICA-based data denoising was performed using ICA-AROMA (ICA-based Automatic Removal Of Motion Artifacts) (Pruim et al., 2015) in FSL version 6.0.1 (FMRIB Software Library, University of Oxford, UK; www.fmrib.ox.ac.uk/fs) to identify and remove subject motion components from the data. Lastly, average time series of eroded white matter and CSF masks were extracted and regressed out of the preprocessed data.

**Resting-state fMRI analysis**

***Region of interest (ROI) creation***

ROI masks for the amygdala subregions, BNST and insula seed regions were created. For the lateral amygdala ROI masks, templates of the basolateral, centromedial and superficial subregions were obtained from the MNI Juelich probability atlas as implemented in FSLeyes (FSL version 6.0.3). We included voxels that had a probability of 60% and above of being labeled as the respective subregion in the corresponding masks. The chosen threshold ensured minimal overlap between voxels of adjacent ROIs. Voxels that subsequently belonged to more than one ROI were assigned to the ROI mask with the higher probability. Voxels with equal probabilities were excluded from mask creation. This resulted in six non-overlapping ROI masks. All calculations on the thresholded masks were performed with Matlab (Version

9.10.0.1602886 (R2021a) MATLAB, 2021) and further visually verified with FSLeyes.

Next, left and right insula ROI masks were defined with the Harvard Oxford probability atlas and thresholded at 50% in FSLeyes. The resulting masks were further separated into anterior and posterior portions based on visual guidance of known parcellation of the insula (Naidich et al., 2004). Final ROIs included the left and right anterior insula. Additionally, ROI masks of the left and right BNST were obtained from the probabilistic mask defined by (Theiss et al., 2017). This ROI defining approach of the BNST was adopted from (Weis et al., 2019). The mask was resampled from MN152I-standard space 1mm3 resolution to 2mm3 resolution to match the data resolution of this study. Finally, all ROI masks were binarized. All masks are available at NeuroVault (https://neurovault.org/collections/WYHXBEQF/).

***Seed-based connectivity analysis and correlation with anxiety scores***

Resting-state seed-based analyses were conducted with dual regression (Beckmann et al., 2009) to generate subject-specific spatial maps and corresponding timeseries, one for each ROI. The dual regression technique consists of two stages involving one regression analysis each. In the first stage, the seed-based ROI maps are regressed into the preprocessed BOLD datasets from each subject. The result of stage 1 is a timecourse for each subject and each ROI map, representing the subject-level timecourse associated with each ROI. Subject-level timeseries were variance-normalized before entering the second stage, to test for differences in strength and amplitude. In the second stage, the obtained timecourses are regressed against the same BOLD dataset, generating subject-specific spatial maps, again, one for each ROI map. These spatial maps containing the parameter estimates were used for further second-level analyses. To test for differences in functional connectivity due to STAI-T scores FSL's randomise permutation testing (with 5000 permutations) was used. IDS-SR scores, sex and age were included as covariates.

Correction for multiple comparisons was done with family-wise error correction (FWE) ($p < 0.05$) using threshold-free cluster enhancement (TFCE).

***Independent component analysis and correlation with anxiety scores***

Pre-processed resting-state fMRI data of all 220 participants was entered into a Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC) independent component analysis (ICA) from the FSL software package to obtain group-ICA spatial maps. The number of independent components (ICs) was manually set to 30. A decomposition at this low model order has shown to be sufficient to detect large-scale brain networks (see Abou Elseoud et al., 2011).

A-priori selected networks of interest included the salience network (SN), default mode network (DMN), and executive control network (ECN). To identify the corresponding group ICs, reference network templates (Kohn et al., 2021) of the three networks were matched to each IC. Therefore, a spatial cross-correlation coefficient was calculated between any given reference network template and each group IC volume. The IC-maps yielding the highest correlation value with the templates were subsequently used in the analysis. To further verify our selection, a second correlation with established network templates was done. Therefore, individual networks identified as the anterior and posterior SN, dorsal and ventral DMN as well as the left and right ECN were taken from (Shirer et al., 2012). These template networks were acquired from 90 functionally defined clusters across 14 ICNs resulting from a MELODIC ICA. In addition, visual inspection of the ICs with template networks further ensured component reliability.

First- and second-level analyses were conducted similar to the approach taken for the seed-based connectivity analysis using dual regression and non-parametric permutation testing. For this analysis the group ICA-map showing the highest correlation with the template maps

from Kohn et al., 2021 for each relevant network was used as the input of the first stage of dual regression. Correction for multiple comparisons was done with family-wise error correction (FWE) ($p < 0.05$) using threshold-free cluster enhancement (TFCE).

## Results

### Demographic and clinical measurements

The demographic characteristics and self-report questionnaire scores of the subjects are shown in Table 1. No significant difference was found for STAI-T scores between males ($M = 41.71$, $SD = 12.14$) and females ($M = 43.92$, $SD = 10.66$; $t(218) = -1.31$, $p = 0.192$). A strong correlation was found between HADS-A and STAI-T scores ( $r= .717$, $p < .001$). The sample distribution of STAI-T scores is given in Figure 4 in the supplementary materials.

### Seed-based correlation analysis

#### *Amygdala*

We found a main effect for correlations of STAI-T scores with resting-state functional connectivity (RSFC) of the left basolateral (BLA) and left centromedial (CM) amygdala ROIs. The results are reported in Table 2. In Figure 1 and 2 we present plots depicting significant clusters reported for the BLA and CM amygdala, repectively. In addition, we provide the mean amygdala RSFC for each subject and each significant cluster for illustrative purposes. Analysis of the BLA amygdala RSFC yielded a significant small cluster within the left pregenual anterior cingulate cortex (pgACC; peak voxel: $x = -6$, $y = 42$, $z = 12$) for the correlation with anxiety at the $p<0.05$ corrected threshold. The correlation was negative, implying a stronger functional connectivity between the BLA amygdala and the significant cluster with lower STAI-T scores. Individual FC strength indicates that lower STAI-T scores associated with slightly positive RSFC becomes negative with higher scores. Further, a region in the left medial frontal gyrus

(peak voxel: x = -10, y = 60, z = 8) exhibited anxiety-related changes with regard to its FC with the BLA at rest. The association between coupling strength and trait anxiety scores was negative. A third cluster was detected for the left BLA ROI with RSFC negatively covaring with STAI-T scores, located in the left dorsal ACC (peak voxel: x = -14, y = 24, z = 30). For the correlation of the left CM RSFC with STAI-T scores significant clusters located in the left primary somatosensory cortex and premotor cortex were obtained at the $p<0.05$ corrected threshold. Two cluster were located in the left primary somatosensory cortex (peak voxel: BA1: x = -44, y = -30, z = 64; BA2: x = -36, y = 38, z = 54) and one cluster in the premotor cortex (peak voxel: x = -24, y = -16, z= 60). The RSFC of the CM with all clusters was positively correlated with anxiety scores, implying that higher scores on the STAI-T were associated with higher RSFC between the basolateral amygdala and the reported cluster. Considering the individual strength in RSFC, as shown in Figure 2, lower anxiety scores are likely correlated with negative FC between the left CM amygdala and clusters in the sensorimotor cortex. This association is likely reversed with higher anxiety scores. No effect of IDS-SR scores, sex or age for any of the ROIs was found. Subsequent combination of bilateral ROIs into either three lateral ROI masks or a single amygdala mask showed no effect for all covariates.

***BNST and insula***

For the left and right BNST and insula no significant association between STAI scores and RSFC was found. Further, no effect was found for any additional covariates.

**ICA**

To identify targeted networks in the group ICA components, all 30 components were correlated with a reference templates of the salience network (SN), default mode network (DMN) and executive control network (ECN). The following components were identified, listed

in order of decreasing correlation strength: IC 2 and 13 (SN), IC 3 and 19 (DMN) and IC 10, 9 and 13 (ECN). The following peak correlations between template ICs and group-ICA maps were found: r= .23 (SN), r= .32 (DMN), and r = .20 (ECN). Correlation values and explained variance of each identified IC are reported in Table 3 in the supplementary materials section. Selected group ICA components corresponding to the three networks are visualized in Figure 3. We did not find an association between trait anxiety and within-network connectivity for any of the networks. Therefore, we performed subsequent correlation analyses with additional networks derived from Shirer et al. (2012). A description can be found in the supplementary materials.

## Discussion

In this study, the relationship between resting-state functional connectivity (RSFC) and trait anxiety scores was investigated by means of seed-based analysis and independent component analysis of various seed regions and networks attenuated in anxiety. We found that different subregions of the amygdala RSFC-patterns reflected trait anxiety assessed on the STAI-T differently. We found a correlation of trait anxiety scores with RSFC of the left centromedial and basolateral subregions of the amygdala. A left-lateralization of amygdala RSFC in relation to trait anxiety has previously been reported by only one study (He et al., 2015). The majority of studies reported a correlation of trait anxiety with the RSFC of the right amygdala (Zidda et al., 2018; Kim et al., 2011; Delli Pizzi et al., 2017) or bilateral amygdala (Berry et al., 2019; Loewenstern et al., 2019). In contrast, we could not replicate anxiety-mediated differences in RSFC when including only the lateral or bilateral amygdala. Functional lateralization of the amygdala in relation to task-based emotional processing has previously been discussed. Here, the right amygdala was associated with fast detection of threat signals and the left with sustained stimulus processing (Markowitsch, 1998; Costafreda et al., 2008). However, these differential

associations to stimuli processing are difficult to interpret in the context of baseline activity, as investigated in the present study. It can be speculated that a baseline shift in left amygdala FC in high levels of trait anxiety reflects ongoing hypervigilance and sustained threat-monitoring at rest.

We found a positive correlation of trait anxiety with the FC of the centromedial subregion of the amygdala and regions in the left primary somatosensory cortex (postcentral gyrus) and left premotor cortex (precentral gyrus). This presumably underlies an attenuation of negative intrinsic coupling between the centromedial amygdala and sensorimotor regions found in individuals with low trait anxiety. Alternations in amygdala-sensorimotor coupling has not frequently been reported in the context of trait anxiety. As to our knowledge, only one study reported a positive correlation between trait anxiety and baseline FC of the left amygdala and the somatomotor network, here defined as spanning the precentral and postcentral gyri (He et al., 2015).

The premotor cortex is involved in the preparation of movement behavior with respect to current sensory information, previous experiences and future goals (Pressman & Rosen, 2015; Gazzaniga et al., 2009). For example, the premotor cortex exhibits increased activation during the processing of facial expression (Sato et al., 2004; Kilts et al., 2003) and body expressions (Pichon et al., 2008) signaling anger, presumably related to the preparation of defensive action. Functional (Rizzo et al., 2018; Toschi et al., 2017) and structural (Grèzes et al., 2014) connectivity between the premotor cortex and the amygdala have previously been reported. The role of this limbic-motor network is assumed to reflect evolutionary mechanisms of salience driven motor inhibition and goal-directed behavior that facilitate adaptive responding related to threat avoidance. The somatosensory cortex is assumed to contribute to the initiation and

regulation of emotions. Like the insula, it is an upstream target of the amygdala (Kropf et al., 2019). Together with these two structures it is implicated in threat anticipation and interoceptive predictive processing that are related to hyperarousal and somatic symptoms of anxiety. Thus, the somatosensory cortex plays a role in somatization and has been linked to cognitive deficits in the evaluation of negative stimuli present in anxiety (Perez et al., 2015). Further, a relationship between the amygdala and somatosensory areas could serve to maintain attentional bias towards threat in anxiety (Greening et al., 2016).

Our finding that trait anxiety mediated changes in amygdala-sensorimotor FC at rest may particularly relate to the experimental setup during data acquisition specific to our sample. Functional parcellation of the motor and somatosensory cortex have derived a topographic representation which relates cortical areas to corresponding body parts. Comparing the particular location of our results in the premotor cortex and primary somatosensory cortex with their respective topography, suggest that those regions are associated with finger movement (Roux et al., 2020, 2018). The resting-state scans were assessed after the participants performed three functional tasks. During the tasks and throughout the resting-state scanning session they held a button box for behavioral responding in their right hand. Theoretically, traces of task activation could have remained longer present in individuals with high trait anxiety, representing a delayed shift back to baseline. Alternatively, a diminished negative baseline FC of the amygdala with regions in the sensorimotor network in individuals with elevated trait anxiety scores could reflect aberrant introspective processing and increased anxiety sensitivity. The amygdala, insula and somatosensory regions are involved in interoception, predictive processing of stimulus salience with respect to somatic homeostasis and allocation of appropriate behavior. This is further reflected by functional connections with motor areas that are involved in the initiation of

approach/avoidant behavior in anxiety.

In sum, we found a positive correlation between trait anxiety and amygdala-sensorimotor RSFC, this has not been frequently reported in the literature and may underlie baseline alternations in stimulus-driven motor inhibition and interoception.

We observed a negative correlation between trait anxiety and the RSFC of the basolateral amygdala and regions in the dorsal anterior cingulate cortex (dACC), ventral anterior cingulate cortex (vACC) and ventromedial prefrontal cortex (vmPFC). Correlations of individual FC with anxiety scores suggest that higher trait anxiety was associated with a negative coupling between these regions and the basolateral amygdala that was attenuated in low levels of trait anxiety.

We could replicate the negative correlation between trait anxiety and functional coupling of the amygdala and the ventral ACC and mPFC at rest that has been reported in an earlier study by Kim et al. (2011). However, contradictory to our findings, several sudies reported a positive baseline coupling that increased with trait anxiety scores (Qin et al., 2014; Berry et al., 2019; He et al., 2015). In this regard, future work is needed to clarify which factors contribute to the differences in directionality. The vmPFC is involved in emotional control through inhibitory regulation (Andrewes & Jenkins, 2019; Koenigs & Grafman, 2009; Etkin et al., 2011). It has been postulated that under baseline conditions the vmPFC is presumably positively coupled with the amygdala (Kim et al., 2011; Roy et al., 2009). This positive coupling could reflect a continuous inhibition of the amygdala by the vmPFC that becomes suspended under threat conditions. Consistently, increased RSFC between the vmPFC and amygdala during aversive anticipation has been associated with positive treatment outcomes in generalized anxiety disorder (Nitschke et al., 2009). Our findings show that the amygdala is likely not coupled with the vmPFC at rest for lower trait anxiety scores. Furthermore, task-based FC reported mixed results

regarding the direction of the amygdala-vmPFC coupling. During threat-inducing tasks increased FC has been reported (Gold et al., 2016), whereas a negative coupling has been found in tasks involving stimulus evaluation and emotional conflict processing (Etkin et al., 2011).

We would like to point out that the cluster located in the vmPFC extended into the frontal pole, whereas the cluster located in the ventral ACC extended to the more dorsal part of the mPFC. Further, it should be noted that there is no universal cytoarchitectonic parcellation of the ACC. Therefore, the boundaries are arbitrary and hinder comparisons between results that pertain to particular subregions. However, we classify the cluster located in the ventral ACC as belonging to the pregenual ACC. Within the vmPFC, the pregenual ACC in particular has been associated with the regulation of emotional responses (Etkin et al., 2011; Yarkoni et al., 2011) and emotional conflict (Etkin et al., 2006; Egner et al., 2008) by top-down control over the amygdala (Etkin et al., 2006). Our data also showed a negative correlation between trait anxiety and RSFC of the amygdala and dorsal ACC. A previous study has reported a positive association between state anxiety scores and the RSFC of the amygdala and dorsal ACC/mPFC (Kim et al., 2011). These findings could be translated to trait anxiety to a lesser degree. The dorsal ACC is a diverse region that is involved in both salience evaluation and subsequent cognitive control (Etkin et al., 2011; Geng et al., 2016). Together with the amygdala, the dorsal ACC is grouped with the salience network which is involved in stimulus-driven salience processing (Seeley et al., 2007). In accordance, the dorsal ACC is involved in the processing of stimuli signaling threat and displays stronger structural connections with the amygdala in individuals with high levels of trait anxiety (Greening & Mitchell, 2015). These findings may underlie an amplification mechanism of the dorsal ACC/mPFC over the amygdala (Robinson et al., 2012, 2014).

In summary, the basolateral amygdala has shown a negative baseline coupling with

regions in the dorsal ACC and ventral ACC/mPFC with high levels of trait anxiety. However, the interpretation of these findings remains difficult with regard to regional specificity of dorsal and ventral subdivisions and their differential role in regulatory pathways with the amygdala. Moreover, we could not find a differentiation in the associative direction between trait anxiety scores and the ventral and dorsal portion of the ACC and mPFC. Therefore, further clarification with regard to functional specificity with regard to mPFC and ACC subdevisions and its connectivity with the amygdala is needed. Also, the temporal covariation between two brain regions may not indicate a direct communication between these regions. For example, the present findings may reflect a direct influence between the amygdala and both dorsal and ventral areas. Alternatively, a direct influence of the amygdala with either region and an indirect influence with the other is possible. Finally, we could not replicate anxiety-mediated change in the RSFC between the amygdala and areas in the dorsolateral PFC. This is in contrast to earlier studies investigating amygdala RSFC in trait anxiety (Loewenstern et al., 2019; Weis et al., 2019).

**Limitations**

Despite previous assumption that the BNST is involved in anxiety, we did not find trait anxiety-related changes in RSFC. Evidence of BNST involvement in anxiety are based on findings reporting increased BNST activation when exposed to sustained and unpredictable threat. This association has been found in animal studies (Walker et al., 2003; Davis, 2006) and human studies (McMenamin et al., 2014; Somerville et al., 2010). To our knowledge, a single RSFC study investigated the association between the BNST and the amygdala with high field strength imaging in trait anxiety (Weis et al., 2019). In line with our results, they did not find correlations between RSFC and trait anxiety. The small size of the BNST poses a challenge on

imaging of this structure. Compared to our dataset, Weis and colleagues used a higher imaging resolution and applied less smoothing, implying that our chances to find effects was even more restricted due to constrains of the applied imaging methods.

We could not find an association between insula RSFC and trait anxiety. This is in line with previous studies that investigated the resting-state functional connectivity of the insula (Baur et al., 2013; Huggins et al., 2018). Given, the insula's function of signaling interoceptive states, it is likely that this structure is rather associated with anxiety sensitivity. Anxiety sensitivity is defined as the fear and awareness of the physiological symptoms of anxiety (Reiss et al., 1986) and has been postulated to be mediated by a circuitry involving the anterior insula (Paulus & Stein, 2006). In contrast, trait anxiety might be rather associated with the cognitive aspect of anxiety.

The present study has further limitations, that need to be regarded when generalizing the results. First, we could not find a right-shift distribution of STAI-T scores that was present for scores on the HADS in our sample. Nevertheless, the sample showed an elevated mean STAI-T score when contrasted with similar studies.

Furthermore, the mean score still reflects a general elevation in trait anxiety scores in our sample (Bieling et al., 1998). Furthermore, we assessed trait anxiety with a particular psychometric test, the STAI-T. However, the STAI-T scale has been postulated to measure negative affect in general (Knowles & Olatunji, 2020). Therefore, including multiple measurement tools could reduce bias distinct to different anxiety measurements. Lastly, it should be pointed out that the resting-state scans where collected after three task-related fMRI blocks, this could have resulted in task-induced changes in resting-state connectivity.

## Conclusion

Overall, the results suggest that trait anxiety is associated with alternations in the functional connectivity of the basolateral and centromedial amygdala at rest. Trait anxiety levels were related to differences in the functional coupling of the amygdala with regions in the ACC, medial PFC and sensorimotor cortex. No other region or RSNs exhibited anxiety-related changes in baseline connectivity. With regard to the amygdala, we could replicate common findings from three out of six studies to some extend in our large sample. Specifically, amygdala RSFC displayed alternations with comparable regions in the ACC and mPFC, however, differences relate to amygdala lateralization and/or direction of correlation with anxiety scores. We could further replicate findings of one study in relation to the direction of correlation, amygdala lateralization as well as specific regions coupled with the amygdala. Interestingly, this relates to a finding less frequently reported in the literature. It is of further notice that this particular study was also conducted in a large sample. Together, these results show that common findings in RSFC studies on trait anxiety could be replicated in a large sample of high anxious individual. However, further investigated is needed to interpret and incorporate these findings in the functional architecture of amygdala circuitry in anxiety.

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# Supplementary materials

## Methods

### *Demographics*

The sample distribution of STAI-T scores (mean = 43.33, SD = 11.09) can be inspected in Figure 4. For reference, mean STAI-T score in the general population is 36.3 (SD= 11.4) (Crawford et al., 2011). In patients with anxiety disorders such as GAD mean scores above 50 have been reported Lissek et al. (2014). To test whether STAI-T scores reflect the sampled right-shifted distribution of HADS-A scores a Saphiro-Wilk test of normality was conducted. Results indicate that STAI-T scores are normally distributed in the sample (Saphiro-Wilk: $W(220) = 0.99$, $p = .137$; skewness = .243, kurtosis = 2.8).

### *IC selection*

For selection of network components representing the SN, DMN and ECN, all 30 ICs derived from the MELODIC ICA were correlated with template networks from Kohn et al. (2021) and (Shirer et al., 2012). Results are summarized in Table 3.

### *RSNs of minor interest*

We did not find anxiety-related changes in within-network connectivity for any of the preselected RSNs including the default mode network, the salience network and the executive control network. Therefore, we chose to subsequent investigate additional networks to test for correlations with trait anxiety. The following networks were included: Auditory network, basal ganglia network, higher visual network, primary visual network, visual network, visuospatial network, language network, sensorimotor network, precuneus network. Networks templates were taken from (Shirer et al., 2012) and correlated with alle 30 group ICA components. However, no correlation with trait anxiety was found for any of these additional networks.