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Structural connectomics of anxious arousal in early adolescence: Translating clinical and ethological findings

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ABSTRACT

Etiological explanations of clinical anxiety can be advanced through understanding the neural mechanisms associated with anxiety in youth prior to the emergence of psychopathology. In this vein, the present study sought to investigate how trait anxiety is related to features of the structural connectome in early adolescence. 40 adolescents (21 female, mean age = 13.49 years) underwent a diffusion-weighted imaging scan. We hypothesized that the strength of several a priori defined structural connections would vary with anxious arousal based on previous work in human clinical neuroscience and adult rodent optogenetics. First, connection strength of caudate to rostral middle frontal gyrus was predicted to be anticorrelated with anxious arousal, predicated on extant work in clinically-diagnosed adolescents. Second, connection strength of amygdala to rostral anterior cingulate and to medial orbital frontal cortex would be positively and negatively correlated with anxious arousal, respectively, predicated on rodent optogenetics showing the former pathway is anxiogenic and the latter is anxiolytic. We also predicted that levels of anxiety would not vary with measures of global network topology, based on reported null findings. Results support that anxiety in early adolescence is associated with (1) the clinical biomarker connecting caudate to frontal cortex, and (2) the anxiogenic pathway connecting amygdala to rostral anterior cingulate, both in left but not right hemisphere. Findings support that in early adolescence, anxious arousal may be related to mechanisms that increase anxiogenesis, and not in a deficit in regulatory mechanisms that support anxiolysis.

1. Introduction

Although it is evident for several biological, psychological and environmental reasons that adolescence is a period of susceptibility for developing psychopathology (Dahl and Hariri, 2005; Telzer et al., 2014), little is known regarding how endogenous and exogenous factors affect relevant neural mechanisms prior to such diseases emerging. To advance knowledge in service of this scientific aim, it is imperative to translate findings and theory from basic neuroscience into hypotheses concerning human development and dysfunction. One such recent, productive endeavor from basic neuroscience, called connectomics, seeks to explicate the structural and functional neural connections across multiple scales of granularity (Sporns, 2012). Indeed, this enterprise has marked a theoretical breakthrough in neuroscience to begin the daunting task of explaining *how* neural ensembles realize psychological functions; that is, to elaborate mechanistic explanations (Thomas and Sharp, under review). Methods used to study the connectome have just begun to be leveraged to investigate psychopathological conditions (Buckholtz and Meyer-Lindenberg, 2012; Van Essen and Barch, 2015). Within this area, little attention has been paid to nonclinical youth, in which such methods could shed light on the developing biology of predisposing emotional traits (Sharp et al., 2015). The present study sought to test if trait anxiety in a nonclinical, early adolescent population was related to features of the structural connectome that have been implicated in basic and applied neuroscience studies on anxiety.

One area that is well suited to advance theory on the mechanisms of human anxiety is ethological work in optogenetics. In this vein, particularly fine-grained insights have recently begun to emerge on the specific connections between amygdala and regions in prefrontal cortex that play causally different roles in anxiety-related behavior. For instance, in rodents, the downstream connection from ventral medial prefrontal cortex (vmPFC) to basomedial amygdala is anxiolytic, whereas basolateral amygdala, primarily connected to dorsal medial prefrontal cortex (dmPFC), promotes freezing behavior under certain conditions (Adhikari et al., 2015). These findings from rodent optogenetics comport with a rich body of literature on the relationship between these two amygdalar pathways and elevated state and trait

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anxiety in both rodents and human adults. Previous molecular neuroscience work in rodents has identified anterior cingulate-amygdala structural connections being necessary for instantiating anxiety (Bissière et al., 2008; Malin et al., 2007). In healthy adult humans, the structural connectivity between amygdala and dorsal anterior cingulate cortex (part of dmPFC) has been shown to positively covary with trait anxiety (Greening and Mitchell, 2015), whereas the structural connectivity between amygdala and medial OFC (part of vmPFC) negatively covaries with trait anxiety (Greening and Mitchell, 2015; Kim and Whalen, 2009). Moreover, functional MRI studies of human adults have found that amygdala-dmPFC functional connectivity increases as state anxiety is induced (Robinson et al., 2012, 2013, 2014). Taken together, there is support that in both rodents and human adults, the amygdala-dmPFC pathway is involved in anxiogenesis whereas the amygdala-vmPFC pathway is involved in anxiolysis.

Existing literature on the structural biomarkers of trait anxiety in human adolescence specifically, in both clinical and subclinical samples, has generated mixed findings (Gee et al., 2013; Jalbrzikowski et al., 2017; Swartz et al., 2014), with some studies finding connection strength between the amygdala and prefrontal cortex correlating negatively with anxiety (Swartz et al., 2014), whereas others finding positive correlations with anxiety (Jalbrzikowski et al., 2017). Part of the reason for these conflicting findings may be the overreliance on fitting the diffusion tensor model to estimate characteristics of white matter morphology, as is done in analyses focusing on fractional anisotropy. Alternatively, in many connectomics analyses that leverage probabilistic tractography, more complex models (e.g., ball and stick) that do not assume a predominant single fiber direction within a given voxel may yield quite different information. In one of the only studies on adolescent anxiety using the structural connectome approach, adolescents diagnosed with depression and comorbid anxiety, compared to healthy controls, showed reduced connectivity in right caudate to right middle frontal gyrus, but showed no differences in global graph-theoretical measures of network topology (Tymofiyeva et al., 2017). These findings are consistent with work demonstrating that diminished frontostriatal functional connectivity is associated with depression, anxiety, and general deficits in emotion regulation in human adults (Furman et al., 2011; Vaghi et al., 2017) and in human adolescents (Forbes and Dahl, 2012).

It is vital to test whether these neurobiological correlates of trait anxiety manifest in adolescence prior to the emergence of clinically-relevant psychopathology, as the results of such studies can help identify biomarkers of psychological dysfunction that precede disease onset and can inform theories regarding the pathophysiology of the disorder. In the present study, we leveraged diffusion-weighted MRI data to examine how a type of trait anxiety, anxious arousal, is related to changes in the structural connectome in a sample of nonclinical adolescents. Given that anxiety is a broad concept of which there are putative subtypes, we chose to focus on anxious arousal (also called somatic anxiety), as its cluster of symptomatology (lower fear threshold, hypervigilance, sympathetic hyperarousal) is proximal to the phenomena probed in ethological work on which some of our hypotheses were based (e.g., Adhikari et al., 2015; Bissière et al., 2008; Watson et al., 1995). Anxious arousal is conceptualized as a trait measure, given that it has high reliability across time and can predict temporally distal behavior and neural activity (Sharp et al., 2015). By contrast, anxious apprehension is characterized by verbal rumination and worry, two phenomena that qualitatively differ from the more rudimentary anxious phenomenology rodents engage in. Moreover, anxious apprehension includes states marked by rich verbal content, can be about temporally or conceptually distal threats, and engages higher-order cognitive functions (Sharp et al., 2015).

We tested three hypotheses regarding how levels of anxious arousal covary with features of the structural connectome. First, in line with animal work in rodents (e.g., Adhikari et al., 2015), we predicted that anxious arousal would be positively related to connection strength between rostral anterior cingulate cortex (rACC) and amygdala and would be negatively correlated with the connection between medial orbitofrontal cortex (OFC) and amygdala. We focused on rostral anterior cingulate as a homolog of rodent dmPFC (which is defined as the rodent cingulate cortex; Adhikari et al., 2015) due to a convergence across histological, ethological and human neuroscience work in regards to its association with (1) anxiety behavior and phenomenology and (2) connectivity with amygdala (Greening & Mitchell, 2015; Vogt and Paxinos, 2014). Indeed, rACC is positioned between limbic and cortical connections and is critical for amygdala-dependent learning (Bissière et al., 2008). Because of their more precise anatomical designation and because of their nomenclature in the atlas from which we extracted such regions, we will refer to rACC and medial OFC instead of dmPFC and vmPFC, respectively.

Second, we predicted that the same marker of structural connectome dysfunction found in clinically anxious youth (e.g., Tymofiyeva et al., 2017) would bear out in our younger, non-clinical adolescent sample. In particular, we tested whether anxious arousal would be negatively associated with connectivity strength between the caudate to middle frontal gyrus (MFG). Based on previous literature, albeit using diffusion-tensor imaging, we predicted a medium effect size for the relationship between anxiety and the strength of structural neural connections (Baur et al., 2013; Phan et al., 2009). To achieve 80% power, a medium effect between a correlation of 0.3 and 0.4 requires a minimum sample size of 34 participants (Faul et al., 2007).

We also sought to examine whether adolescence is marked by similar focal dysfunction (i.e., specific subnetworks) in the structural connectome as are adults. Connectomes comprise two basic components: nodes and edges. Nodes, in this study, are gray-matter regions defined by the volume, and the edges are the weighted strength of each pathway between nodes. Indeed, extant connectomics literature in adult humans has *not* found significant differences across clinically anxious groups and healthy participants in measures of global network topology (Korgaonkar et al., 2014; Tymofiyeva et al., 2017). These metrics tend to accompany broader dysfunction in cognition, such as the positive symptoms common in schizophrenia (e.g., van den Heuvel et al., 2013). For this reason, we predicted that correlations between global graph-theoretic neural measures (global efficiency, characteristic path length, and node strength) and self-reported anxiety would not be significantly different from the null-hypothesis.

To maximize the sensitivity of our structural connectivity analyses, we employed a model to estimate multiple fibers within each voxel (Behrens et al., 2007). This method is superior to traditional diffusion tensor imaging (DTI) analyses given that DTI studies that derive fractional anisotropy assume each voxel contains one single major fiber direction, which is not the case, as over 90% of voxels contain more than one fiber orientation (Jeurissen et al., 2010). Thus, it is essential to use models that do not assume a predominant single fiber direction within a given voxel.

2. Methods

2.1. Participants

54 adolescents participated in the present study. 14 adolescents were excluded from present analyses due to corrupted diffusion weighted data (see Quality Control section below). Our final sample included 40 adolescents (21 females; mean age = 13.49 years, range = 12.16–14.78 years). All participants completed a phone screen, during which parents confirmed their child had no history of a clinical diagnosis of mental health disorders, were not taking any psychotropic medications, did not have a learning or developmental disability, and were free of all MR contraindications. All participants provided written informed assent and parents provided informed consent which were approved, along with the entire study protocol, by the Institutional Review Board.

2.2. Mood and anxiety symptom questionnaire

Participants filled out the mini version of the Mood and Anxiety Questionnaire (Casillas and Clark, 2000), which asks participants to report about their mood and anxiety symptoms experienced over the past two weeks on a 5 point Likert scale. The anxious arousal scale consists of 10 questions measuring sympathetic hyperarousal symptomatology, such as, “my hands were shaky” or “I felt dizzy and lightheaded”. The scale had good internal consistency (Cronbach's $\alpha = 0.82$). To ensure findings were specific to anxiety, we controlled for depression as measured by the 8 question depression subscale (Cronbach's $\alpha = 0.70$) on this same measure.

2.3. Imaging acquisition

Diffusion-weighted imaging (DWI) data were collected using a Siemens 3T Trio MRI scanner and a 32-channel head coil. The acquisitions consisted of 30-direction DTI data with a b-value of 1000 s/mm^2 and 2 b = 0 s/mm^2 images acquired at the beginning of the run. The imaging consisted of 72-slices, 2 mm thick acquired with 1.9 mm × 1.9 mm in-plane resolution. A single-shot, spin-echo EPI acquisition was used with TE of 100 ms, TR of 5 s, an SMS multiband factor of 2 (https://www.cmrr.umn.edu/multiband/) (Auerbach et al., 2013; Setsompop et al., 2012a; Setsompop et al., 2012b; Xu et al., 2013) using the CMRR sequence, and a GRAPPA factor of 2 for parallel imaging (Griswold et al., 2002). In addition to the DTI scan, a structural T1-weighted magnetization-prepared rapid acquisition of gradient echo (MPRAGE) acquisition was acquired with 0.9 mm isotropic resolution, TE of 2.32 ms, TR of 1.9 s, and a magnetization preparation pulse with an inversion time, TI, of 900 ms.

2.4. Quality control

Prior to transforming data and conducting any analyses, each raw DWI file was manually checked to determine if there were artifacts that corrupted > 3 volumes in order to yield high-fidelity tractography results (Oguz et al., 2014). 11 participants met this threshold for corrupted data, yielding the 40 participants out of 51 that comprise present analyses. We chose a stringent standard for eliminating the data given that only 30 directions were encoded in the diffusion-weighted acquisition protocol, and thus irreparable volumes would significantly impact the estimate of the orientation distribution function on which the connectome reconstruction is based (Behrens et al., 2007). Moreover the overwhelming majority of artifacts were unrecoverable venetian-blind artifacts due to excessive movement.

2.5. Preprocessing

Prior to connectome reconstruction, diffusion weighted data were preprocessed by converting DICOM files to NIFTI format, followed by eddy current correction using an affine registration to the b = 0 image (i.e. without gradients). Finally, in preparation for probabilistic tractography, FSL's bedpostx (Behrens et al., 2007) was run, which estimates a probability distribution of primary fiber orientations at each voxel using Markov chain Monte Carlo sampling.

2.6. Cortical parcellation

Freesurfer's recon-all (Fischl et al., 1999) was run on each subject's high-resolution T1-weighted structural image. This outputted a cortical parcellation from which regions were defined for subsequent probabilistic tractography. The present analysis used the 68 cortical regions defined by Freesurfer that cover the entire cortex, and 14 from subcortical regions, which comprise an 82-region connectome (Desikan et al., 2006). To prepare these regions for tractography, each region was registered to diffusion-weighted space, first using Freesurfer's bbregister tool (using FSL's FLIRT initialization) to compute the transformation matrix from diffusion-weighted space to T1 space. This was followed by Freesurfer's mri_vol2vol to bring the Freesurfer parcellations into diffusion-weighted space using the inverse of the previously computed transformation matrix. Bbregister has been shown to improve registration beyond more traditional methods, in which the cost function examines gradient directions and magnitudes across tissue boundaries (Greve and Fischl, 2009).

2.7. Probabilistic tractography

FSL's probtrackx2 (Behrens et al., 2007) was used to carry out probabilistic tractography. 5000 “seed” streamlines were generated from each voxel within each of the 82 regions, and targets were defined as any voxel within the 81 additional regions. Using protrackx2 in network mode, the output included a matrix, fdt_network_matrix, which contained the number of streamlines from each seed volume that reached all other 81 target regions. All other options for probtrackx2 were set to default inputs.

2.8. Connectome reconstruction

Each entry in the connectome was normalized by the average volume of each ROI comprising the pathway (see Fig. 1 for full pipeline). This method of weighting is a modified version of the connectome density function (Cammoun et al., 2012), in which the penalization for long tracks was not included. We note that the penalty for track lengths is only appropriate for tractography methods that seed white matter voxels, whereas our approach seeds from ROI volumes in gray matter. Connectomes were then symmetrized, by averaging identical entries that were initiated from opposite ends (e.g., amygdala to insula and insula to amygdala). This was done for two reasons: (1) the graph-theoretic algorithms require symmetric matrices and (2) one cannot determine whether axonal bundles comprise efferent or afferent pathways (Jones et al., 2013) reconstructed from diffusion-weighted data.

2.9. Data analysis

2.9.1. Graph-theoretical analyses

To carry out subsequent graph-theoretical analyses, a Python implementation, bctpy (LaPlante et al., 2014) of the Brain Connectivity Toolbox (Rubinov and Sporns, 2010) was used. Three graph-theoretical metrics were computed. These metrics have been shown to be reliable across time using the same pipeline we followed to reconstruct connectomes (Owen et al., 2013). Measures included mean strength (K), global efficiency (E), and mean clustering coefficient (C). We did not expect any of these to covary with measures of anxious arousal.

2.9.2. Connection of interest analyses

Analyses compared three a priori connections of interest, including amygdala to rostral ACC, amygdala to medial OFC, and caudate to MFG. Each of these regions was defined by the Freesurfer parcellation according to the Desikan et al. (2006) atlas. Such connections were extracted from the overall connectome matrix, in which each of these connections comprised the connection density between the aforementioned pair of cortical or subcortical regions.

3. Results

3.1. Global network topology

To compute graph-theoretical measures of *topological* distance, we transformed the original connectome into a connection-length matrix. Connection-length matrices are computed as the element-wise inverse of connection strengths. In graph theory, stronger connections are considered to be more proximal, regardless of the underlying physical

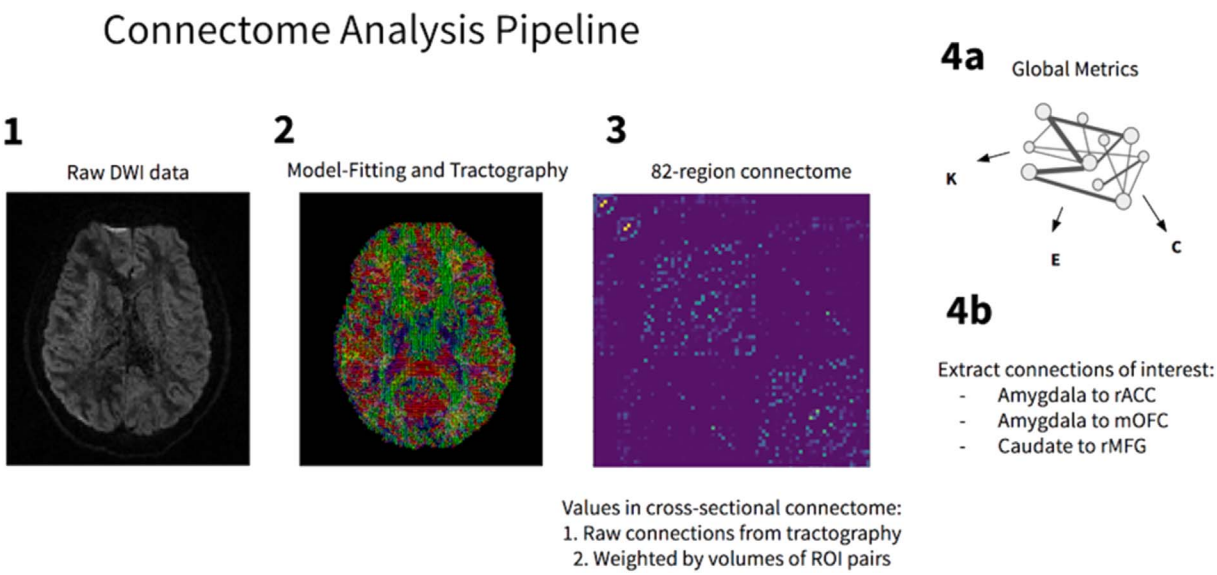

Fig. 1. Full connectome pipeline. (1) Raw diffusion data was preprocessed. (2) FSL's bedpostx estimated the posterior distribution of fiber orientations for each voxel. Tractography was run on these data between each gray matter region defined in Freesurfer's recon-all parcellation (not shown here). (3) A connectome was built which describes how strongly interconnected a given cortical or subcortical region is with all other regions. (4a) Global graph-theoretical summary measures derived from the whole connectome included mean strength (K), global efficiency (E), and mean clustering coefficient (C). (4b) Three connections of interest, including amygdala to rACC, amygdala to mOFC and caudate to rMFG (from each hemisphere) were extracted for subsequent correlational analyses.

distance. E is computed as the average inverse topological distance between nodes, and is a measure of integration. C indicates the average density of clustering for a given node, and is a measure of modularity or network segregation (Owen et al., 2013). Thus, selected graph-theoretical measures comprised indicators of overall network integration (L, K) and segregation/modularity (C) to overall network topology. As predicted, the relationship between anxious arousal and measures of global network topology were insignificantly different from the null hypothesis (largest effect size for global efficiency; $r = -0.127$, $p = 0.436$).

3.2. A priori edge-wise pathways

To carry out the edge-wise hypotheses, we extracted six entries from the overall connectome matrix, and computed Pearson correlations with anxious arousal scores (see Table 1). Two edge-wise pathways were significantly related to anxious arousal in the left hemisphere in the predicted direction. As shown in Fig. 2, for left rACC to left amygdala, there was a positive correlation between connectivity strength and anxious arousal ($r = 0.466$, $p = 0.002$) and for left caudate to left rMFG, there was a negative correlation between connectivity strength and anxious arousal ($r = 0.381$, $p = 0.01$). See Table 1 for full results.

Table 1
Correlations between selected edges in connectome and anxious arousal.

	L Amyg – L mOFC	R Amyg – R mOFC	L Amyg – L rACC	R Amyg – R rACC	L Caudate – L rMFG	R Caudate – R rMFG
Anxious arousal	0.058	− 0.152	− 0.466**	− 0.031	− 0.379*	− 0.194

Note. Each correlation is between a given brain metric and family connectedness scores.
* < 0.05.
** < 0.01.

3.3. Outlier estimation and controlling for concurrent depression

To ensure effects were not due to outliers or concurrent levels of depression, we computed multiple regressions accounting for these potential confounds. We used the criterion that any data point from the MRI metrics over 2.5 standard deviations from the mean should be considered an outlier. This resulted in removing two subjects. Multiple regressions without the 2 outliers and controlling for depression demonstrated that the effects still held in left rACC to amygdala ($\beta = 0.612$, $p = 0.0003$) and in left caudate to left rMFG ($\beta = -0.313$, $p = 0.04$).

4. Discussion

The present study establishes features of the structural connectome that are associated with a type of trait anxiety, anxious arousal, in early adolescence. Specifically, anxiety was positively related to connection strength between left amygdala to left rostral ACC and negatively associated with connection strength between left caudate and left rostral MFG. Moreover, no global topological features of the connectome varied with anxious arousal. Results demonstrate the utility of using structural connectomics to translate basic findings on circuit dynamics to the study of nonclinical adolescents with predisposing emotional traits.

It is interesting that effects were found in the left hemisphere, given that some argue right hemisphere homologs tend to be recruited more than their left counterparts in affective processing (e.g., Heller et al., 1997) and associated with trait anxiety (e.g., Eden et al., 2015). However, findings on lateralization of emotion processing are mixed, with a recent study germane to the present focus on anxiety showing the central importance of left hemisphere function and structure. Specifically, the study leveraged machine learning to predict childhood anxiety, and found that left basolateral amygdala functional connectivity and structural morphology were the strongest predictors of all features in their model (Qin et al., 2014).

The fact that the medial OFC to amygdala pathway was not related to anxiety reflects that anxiety in early adolescence may be in part due to hyperactivity in anxiogenic mechanisms as opposed to a failure of top-down regulation in anxiolytic mechanisms. This may be an

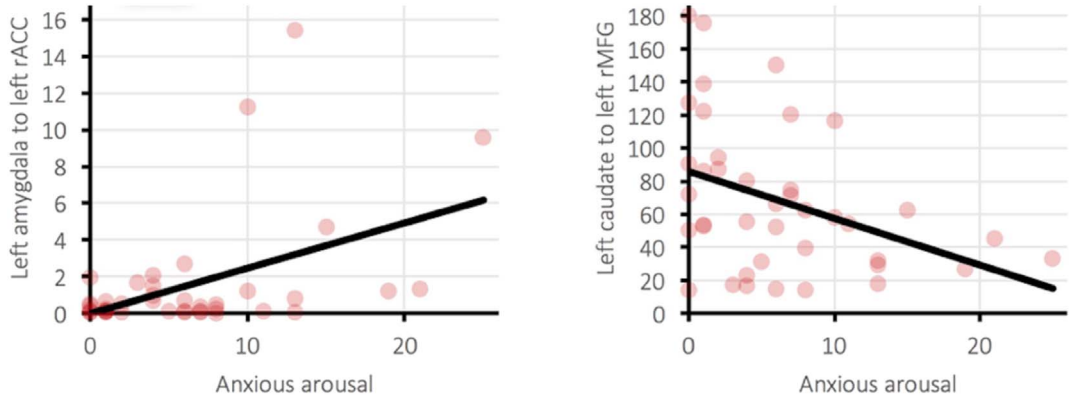

Fig. 2. Edge-wise findings. Two significant edge-wise findings that are associated with anxious arousal. The chart on the left reflects a positive correlation between left amygdala to left rACC, which matches the prediction that this neural pathway implements anxiogenesis. By contrast, the chart on the right reflects a negative correlation between left caudate and left rMFG, supporting the hypothesis that this relationship found in clinically-diagnosed adolescents is a biomarker in nonclinical adolescents with high anxiety.

explanation for why the medial OFC-amygdala pathway, which has been found in adults to be significantly related to trait anxiety, may not present as early in the development of trait anxiety (Clewett et al., 2014; Greening and Mitchell, 2015; Kim and Whalen, 2009). That is, the rostral ACC to amygdala pathway that was positively related to anxiety in the present study has been shown in rodents to be fear-producing. This comports with several fMRI studies in humans that have shown that amygdala and rACC experience heightened coupling when adaptively responding to threat of mild electric shock, and are chronically co-active in individuals with forms of anxiety disorders (Casement et al., 2014; Robinson et al., 2012, 2013, 2014; Vytal et al., 2014).

The second finding reflecting that lower connection strength between left caudate to rostral MFG is related to higher levels of anxiety comports with studies on anxiety and depression, two highly comorbid conditions which some consider to be a single pathology (Korgaonkar et al., 2014; Tymofiyeva et al., 2017). Heightened positive functional connectivity during rest between caudate and MFG has also been observed in those with social anxiety disorder (Arnold Anteraper et al., 2014). Even though the direction of correlation is opposite in the aforementioned functional study compared to present results, findings may not be contradictory, as the relation between functional and structural connectivity is varied and complex. For instance, it is entirely plausible that lower connection strength may result from fewer feedback connections from frontal cortex to caudate, which could then explain more positive than negative coupling between such regions at rest.

The study has its limitations. First, the diffusion-weighted acquisition paradigm was not optimized for tractography, although it was satisfactory for estimating many tracts. While 30 directions is common for diffusion tensor imaging, it is not optimal for probabilistic tractography. Thus, it will be essential to test the present hypotheses using acquisition protocols that yield data capable of estimating complex crossing of fibers along pathways of interest.

Second, as is true of most studies in connectomics, results are dependent on the parcellation scheme one uses. Future studies should use the most functionally-specific parcellation; for instance, ones that segment the amygdala into its distinct nuclei, if possible with the resolution of one's data. This is evident in some extant contradictory findings in which some have found a negative correlation between the structural connectivity of the medial OFC-amygdala pathway and trait anxiety (e.g., Greening and Mitchell, 2015), whereas others found a positive correlation between the structural connectivity of the ventral prefrontal cortex-amygdala pathway and trait anxiety (Clewett et al., 2014). Because the ventral prefrontal cortex in the latter study covers a large swath of cortex, it included parts of rostral cingulate cortex that is likely functionally separable from medial OFC as evidenced by the present study. Moreover, an issue of cross-study comparisons is the disjunction between the nomenclature (e.g., vmPFC) and the structural boundaries defining such anatomical regions (Roy et al., 2012), in which different public or manually-drawn atlases differ in their definitions of regions. Advances in spatial resolution of neuroimaging are centrally important to translate findings from non-human optogenetics and other more fine-grained work in ethology to the study of human neurodevelopment.

Third, we did not conduct clinical interviews, and only excluded participants at screening if they had a history of clinically diagnosed mental health problems, were currently taking psychotropic mediations, or had developmental disorders or learning disabilities. Thus, it may be the case that on the dimensional measure used in the present study of trait anxiety, those at the high end may have met criteria for clinical anxiety but were not yet diagnosed. Alternatively, a strength of the study is that it is based on a community sample, which may be more generalizable than clinical studies in regards to sampling a wider spectrum of adolescents with varying levels of trait anxiety.

In sum, findings here advance theory regarding the neurobiological features associated with trait anxious arousal, which may inform an understanding of signs of risk that emerge prior to clinical presentation of disease. Results demonstrate how circuit-based findings in ethology may be applied to study the structural organization of the human connectome from diffusion-weighted imaging. Future research should endeavor to flesh out a more mechanistic understanding of how such structural correlates of anxiety are related to functional neural dynamics, as well as a more precise computational explanation of the information processing underlying various forms of anxiety.

Conflict of interests

None to Declare.

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