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Anxiety Modulates Insula Recruitment in Resting-State Functional Magnetic Resonance Imaging in Youth and Adults

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Abstract

Research on resting-state functional connectivity reveals intrinsically connected networks in the brain that are largely consistent across the general population. However, there are individual differences in these networks that have not been elucidated. Here, we measured the influence of naturally occurring mood on functional connectivity. In particular, we examined the association between self-reported levels of anxiety and connectivity in the default mode network (DMN). Healthy youth ($n = 43$; ages 10–18) and adult participants ($n = 24$, ages 19–59) completed a 6-min resting-state functional magnetic resonance imaging scan, then immediately completed questionnaires assessing their mood and thoughts during the scan. Regression analyses conducted separately for the youth and adult samples revealed brain regions in which increases in connectivity differentially corresponded to higher anxiety in each group. In one area, the left insular cortex, both groups showed similar increased connectivity to the DMN (youth: -30, 26, 14; adults: -33, 12, 14) with increased anxiety. State anxiety assessed during scanning was not correlated with trait anxiety, so our results likely reflect state levels of anxiety. To our knowledge, this is the first study to relate naturally occurring mood to resting state connectivity.

Key words: anxiety; default-mode; development; DMN; fMRI; functional connectivity; ICA; ICN; insula

Introduction

Traditional functional magnetic resonance imaging (fMRI) studies compare neural activation during a task to a baseline state, often viewing a fixation cross. The discovery of low-frequency fluctuations (~ 0.01–0.1 Hz) in the baseline blood oxygen level dependent (BOLD) signal that are synchronized across brain regions belonging to known functional networks (Biswal et al., 1995) has inspired new lines of inquiry in neuroscience research and demonstrated that our concept of a "baseline" might need to be redefined. By investigating resting-state fMRI (rs-fMRI), researchers have discovered that fluctuations in brain activity organize into coherent, temporally correlated networks involving distributed brain regions (Damoiseaux et al., 2006; Fox et al., 2005). Rs-fMRI research examines the activity of these intrinsic connectivity networks (ICNs) during task-free periods. These networks have been found to be generalizable across individuals (Beckmann et al., 2005), have high test-retest reliability (Thomason et al., 2011), and change over the course of development (Dosenbach et al., 2010). These ICNs persist even during sedation (Greicius et al., 2008) and during the onset of sleep (Larson-Prior et al., 2009).

One of the best-characterized ICNs is the default mode network (DMN), which consists of the posterior cingulate cortex, bilateral aspects of the ventral and dorsal medial prefrontal cortices, inferior parietal lobules, lateral temporal cortices, and the hippocampal formation (Buckner et al., 2008; Raichle et al., 2001). The regions comprising the DMN are more active during rest blocks than during task blocks; consequently, they have been called the "default mode," or "task negative," network (Fox et al., 2005). Many possible roles have been ascribed to the DMN, ranging from supporting mind wandering and internal thought to monitoring the external environment (Buckner et al., 2008; Fransson, 2005; Gusnard and Raichle, 2001). Theories concerning the role of the DMN, however, are predominantly based on functional attributes of the brain regions it includes, and on the fact that it is less active during tasks (Buckner et al., 2008). Thus, there is a need to further define fundamental properties of the DMN,

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including how psychological processes alter connectivity of the DMN during rest.

To elucidate the role of resting-state functional connectivity, some investigators have examined the regional overlap between areas included in the DMN and areas activated for tasks that are potentially supported by the DMN, such as autobiographical memory, prospection (imagining one's future), and theory of mind (understanding the independent thoughts and feelings of others) (Spreng and Grady, 2010). The overlap between DMN regions and those activated by these tasks suggests that the DMN plays a supporting role in these behaviors. Other researchers propose that the DMN supports "mind wandering," as evidenced by increased DMN activity during self-reported periods of stimulus-independent thought (Mason et al., 2007). Researchers have theorized that while "at rest," the brain is actually busy monitoring the environment and the self, processing emotions and stimuli, and maintaining memories (Binder et al., 1999; Fransson 2005; Gusnard and Raichle, 2001; McKiernan et al., 2006).

Recently, some studies have related functional connectivity to psychopathology (Bluhm et al., 2009; Cao et al., 2006; Greicius, 2008; Greicius et al., 2007; Hamilton et al., 2011a; Liu et al., 2005; Rombouts et al., 2005; Tian et al., 2006; Wang et al., 2006; Weng at al., 2010; Zhou et al., 2007), mood (Eryilmaz et al., 2011; Harrison et al., 2008), and the presence of psychoactive substances (Khalili-Mahani et al., 2011). Harrison and colleagues (2008), for example, induced a sad mood in healthy subjects and found increased recruitment of the dorsal anterior cingulate and anterior insular cortices to the salience network (SN) as sadness increased. Eryilmaz and colleagues (2011) examined rs-fMRI after mood induction by movies and found that resting activity in the insula was significantly increased after movies designed to induce anxiety or fear. Importantly, both these investigations manipulated the participants' emotions. To date, no studies have examined how unconstrained state emotions are related to connectivity in the DMN.

The current study was designed to gain a better understanding of naturally occurring (spontaneous) psychological processes that may alter connectivity of the DMN in youth. We hypothesized that there would be specific functional connectivity changes in the brain associated with individual psychological states. Since we were examining anxiety, we expected to observe differences in regions known to be involved in emotion-related processing, such as the limbic lobe. Since little is known about how connectivity is altered by natural variance in mood during the scan experience, we also recruited a sample of adult participants as a comparison group. A secondary advantage of this approach is that we can differentiate effects that appear to persist across development from those present only in specific age groups. Much of the research conducted on participants from different age groups is designed to perform comparisons between groups. In contrast, here we chose to identify connectivity characteristics that were consistent across the lifespan. This helps identify factors present early in development that may be foundational in the organization of brain circuitry. We examined how normal variation in anxiety-related effects in a healthy population is reflected in the DMN ICN. Thus, we gave participants minimal instructions before and immediately after the resting-state scan. While participants were still in the scanner, we administered a questionnaire assessing their anxiety levels during the scan.

Materials and Methods

Participants—youth

Forty-three healthy children and adolescents (16 women, mean age = 13.2, SD = 2.0, range 10–18 years) participated in this study. Participants were 22 Caucasian (51%), 4 Hispanic (9%), 8 Biracial (19%), 3 Asian American (7%), and 2 African American (5%), with 4 (9%) declining to list race/ethnicity. Participants were recruited through their parents with fliers at Stanford University, postings in the San Francisco Chronicle (local paper), and postings on Craigslist (a community based, free advertisement website), and were paid $25/h for their participation. Children with any DSM-IV Axis I psychopathology, learning disabilities, attention disorders, head trauma, or any contraindications for MR scanning were excluded from the research study. We also excluded children if their parents had a history of substance abuse or dependence, psychosis, or bipolar disorder. All were right handed, fluent in English, and indicated no learning disabilities. Participants completed the Schedule for Affective Disorders and Schizophrenia for School-Age Children: Present and Lifetime version (K-SADS-PL; Kaufman et al., 1997). Parents and youth gave informed consent and assent, respectively, as approved by the Stanford University Institutional Review Board.

Participants–adults

Twenty-four adults (16 women, mean age = 35.3, SD = 11.6, range 19–59) participated in this study. Participants were 17 Caucasian (71%), 5 Asian American (21%), 1 (4%) Hispanic, and 1 (4%) declining to list race/ethnicity. Two participants were left handed, all were fluent in English and indicated no learning disabilities. Participants had no history of head injury or mental impairment, and had no past or present Axis 1 disorders. Participants completed the Structured Clinical Interview for DSM (SCID; First et al., 1995). Participants gave informed consent as approved by the Stanford University Institutional Review Board.

Participants were recruited, through their parents if they were minors, through fliers at Stanford University, postings in the San Francisco Chronicle (local paper), and postings on Craigslist (a community-based, free advertisement website), and were paid $25/h for their participation. All protocols followed institutional and national rules for human research and complied with the ethical guidelines set forth by the Helsinki declaration.

Clinical interviews—youth

Trained interviewers assessed the diagnostic status of the participants by administering the K-SADS-PL, which has been shown to be a reliable assessment of psychiatric diagnostic status in youth (Kaufman et al., 1997). None of the participants included in this analysis had any past or present psychiatric diagnoses. To prepare for the MRI session, parents and youth viewed a video introduction to the process. The youth also filled out additional measures at this visit, including the Screen for Childhood Anxiety Related Emotional Disorders (SCR-C) and the Anxiety Sensitivity Index Child

(ASIC). The SCR-C (formerly abbreviated, SCARED) is a measure of trait anxiety in youth and assesses different types of anxiety such as separation anxiety, social anxiety, and panic (Birmaher et al., 1997). The ASIC is a measure of sensitivity to the feeling of being anxious and involves questions about how anxious a youth becomes while experiencing the physical symptoms of anxiety (heart beating rapidly, can't catch breath, etc.). The ASIC has both 12 and 16-item versions, both valid for assessing anxiety sensitivity in children (Laurent et al., 1998); we administered the 16-item version in this study.

Clinical interviews–adults

Participants were administered clinical interviews (SCID) by trained interviewers. None of the participants included in this analysis had any past or present psychiatric diagnoses. Participants were recruited as part of a larger study, and also completed other measures during these sessions, including the State Trait Anxiety Inventory–Trait (STAI-T) and the State Trait Anxiety Inventory–State (STAI-S). The STAI-S and STAI-T are two 20-item scales designed to assess transient state anxiety (STAI-S) and more stable trait anxiety (STAI-T) (Spielberger, 1983).

Tasks and measures

During the 6-min rs-fMRI scan, participants were instructed to keep their eyes closed and to remain awake. Immediately after the rs-fMRI scan, while the participants were still in the scanner, they completed a survey consisting of a list of 14 emotions or thoughts they might have had during the scan, and answered on a 7-item Likert-type scale, in which participants rate their agreement or disagreement to a series of statements, how strongly that emotion or thought applied to them (1 = not at all, 7 = very much). The survey questions were projected into the scanner with instructions at the beginning to think back to what they were thinking about during the previous, eyes-closed scan. For a given question, there were circles marked 1 through 7 on the screen. Participants had a button box with buttons 1, 2, 3, and 4 in their right hand and were instructed and given practice on using button 4 to move the cursor toward the "7" and button 1 to move the cursor toward the "1." Button 2 or 3 entered their answer and advanced them to the next survey item. The questions were as follows: "how happy did you feel, how anxious did you feel, how uncomfortable did you feel, how calm did you feel, how discouraged did you feel, how much did you think about things you need to do later today, how much did you think about things you did recently, how alert did you feel, how positive did you feel, how much did you think about your relationships with other people, how nervous did you feel, how sad did you feel, how content did you feel, how much did you think time was passing very slowly." Participants also completed the same scale after leaving the scanner; the two measures were highly correlated, $r(21) = 0.90$, $p < 0.0001$. In the adult group, three subjects were missing the in-scanner questionnaire, so we instead used the after-scan questionnaire for the adults, and the in-scanner questionnaire for the youth. Since the two measures were significantly and highly correlated, this was the most reasonable way to obtain the most representative data from the most participants.

fMRI data acquisition

MRI was performed on a 3.0 T GE whole-body scanner. Participants were positioned in a purpose-built single channel T/R head coil and stabilized by padded clamps and a bite bar formed with dental impression wax (made of Impression Compound Type I, Kerr Corporation, Romulus, MI) to reduce motion-related artifacts during scanning. High-resolution T2-weighted fast spin echo structural images (TR = 3000 ms, TE = 68 ms, ETL = 12) were acquired for anatomical reference. A T2*-sensitive gradient echo spiral in/out pulse sequence (Glover and Law, 2001) was used for all functional imaging (TR = 2000 ms, TE = 30 ms, flip angle = 77°, FOV = 22 cm, 64°–64, 29 slices, 4.0 mm slice thickness). An automated high-order shimming procedure, based on spiral acquisitions, was used to reduce B0 heterogeneity (Kim et al., 2002). Spiral in/out methods can increase signal-to-noise ratio and BOLD contrast-to-noise ratio in uniform brain regions, and reduce signal loss in regions compromised by susceptibility-induced field gradients generated near air-tissue interfaces such as prefrontal cortex (PFC) (Glover and Law, 2001). Compared with traditional spiral imaging techniques, spiral in/out methods result in less signal dropout and greater task-related activation in PFC and medial temporal regions (Preston et al., 2004). A high-resolution anatomical scan (140 slices, 1 mm slice thickness) was collected for every participant using a spoiled GRASS gradient recalled (SPGR) sequence for T1 contrast (TR = 3000 ms, TE = 68 ms, TI = 500 ms, flip angle = 11°, FOV = 25 cm, 256 × 256).

Physiological data correction

Heart rate and respiratory rate were collected during the scan. Since physiological data can confound the rs-fMRI signal, we used the method developed by Chang and Glover (2009) to minimize the influence of respiratory and heart rates. Chang and Glover's tool does this by convolving the independently measured physiological signals with appropriate filters, and then regresses them out of the time series for each voxel.

fMRI preprocessing

fMRI data were processed using Statistical Parametric Mapping software (SPM8; www.fil.ion.ucl.ac.uk/spm/software/spm8/). Preprocessing involved realignment, co-registration of functional and structural images, and normalization to the Montreal Neurological Institute (MNI) template by using a participant-specific transformation generated by fitting the mean functional images to the echo-planar imaging (EPI) standard SPM template. Data were not resampled, but instead retained their original dimension (3.44 × 3.44 × 4 mm). Data were visually inspected at multiple points throughout the analysis to make sure all steps had been correctly completed. Images were smoothed by using a 6 mm Gaussian kernel to decrease spatial noise.

Movement

Movement plots were generated for each participant and inspected for peaks. Time frames corresponding to peaks in movement greater than 1 mm were removed, along with the first three frames for signal stabilization. No >10% of the time frames were removed for any participant for both motion and signal stabilization. If >10% of time frames merited removal, then that participant was not included in

the analysis. The participants whose data were used in this article were those whose motion was low enough to be acceptable.

Independent component analysis

Group independent component analysis (ICA) was implemented by using the GIFT ICA toolbox (http://icatb.sourceforge.net/; Calhoun et al., 2001) run in MATLAB (www.mathworks.com). In GIFT, the Infomax algorithm was used to estimate 31 ICA maps. Our decision to derive 31 components was made by using a number intermediate among those recently published in similar studies of children (Thomason et al., 2011a, 2011b). To determine the component corresponding to the DMN, we used the template-matching strategy described by Greicius and colleagues (2004). Group ICA was conducted on both groups together to generate components that could be easily compared. Thus, we chose to mitigate the possibility that ICA could perform differently within the two groups. The DMN template used is available on www.nitrc.org/projects/r-spit/, along with a description of how the templates were created. The DMN template used, along with the resultant best-fit group ICA component for the DMN, are shown in Figure 1. To address any concern that the template we chose may be more or less appropriate for either child or adult participants, using a method that has been applied in previously published template-matching studies, we tested the goodness of fit of our templates for each subject (Greicius et al., 2004). This involved taking the average z score of voxels falling within the template and subtracting the average z score of voxels falling outside the template. Independent sample t-tests and Pearson correlations were used to test for possible group differences on goodness of fit and to test the relation between participant age and goodness of fit, respectively.

Regression analysis

Using methods described in (Calhoun et al., 2001; Stevens et al., 2009), individual participant DMN maps were extracted by following the group ICA. The resultant individual participant DMN maps were submitted to standard regression analysis implemented in SPM8. Regression analysis was performed by using the anxiety scores reported by participants on the 7-item Likert-type scale given in the scanner in response to the question, "how anxious did you feel?" Regressions were conducted separately within each age group (youth and adults) as well as across all respondents. The products of regression analyses are 3D brain volumes in which significant effects denote areas where functional connectivity to the participant's own mean timecourse across all DMN areas was related to self-reported anxiety during that scan. Thus, in areas with positive correlations, spontaneous function was tightly coupled to the DMN timecourse; conversely, negative correlations denote inverse temporal coupling. Results for the whole brain are given, $p < 0.001$, uncorrected $k > 3$.

AlphaSim

In an effort to mitigate concerns about multiple comparisons, an issue in fMRI research where a set of statistical hypotheses is simultaneously tested at tens of thousands of voxels, we

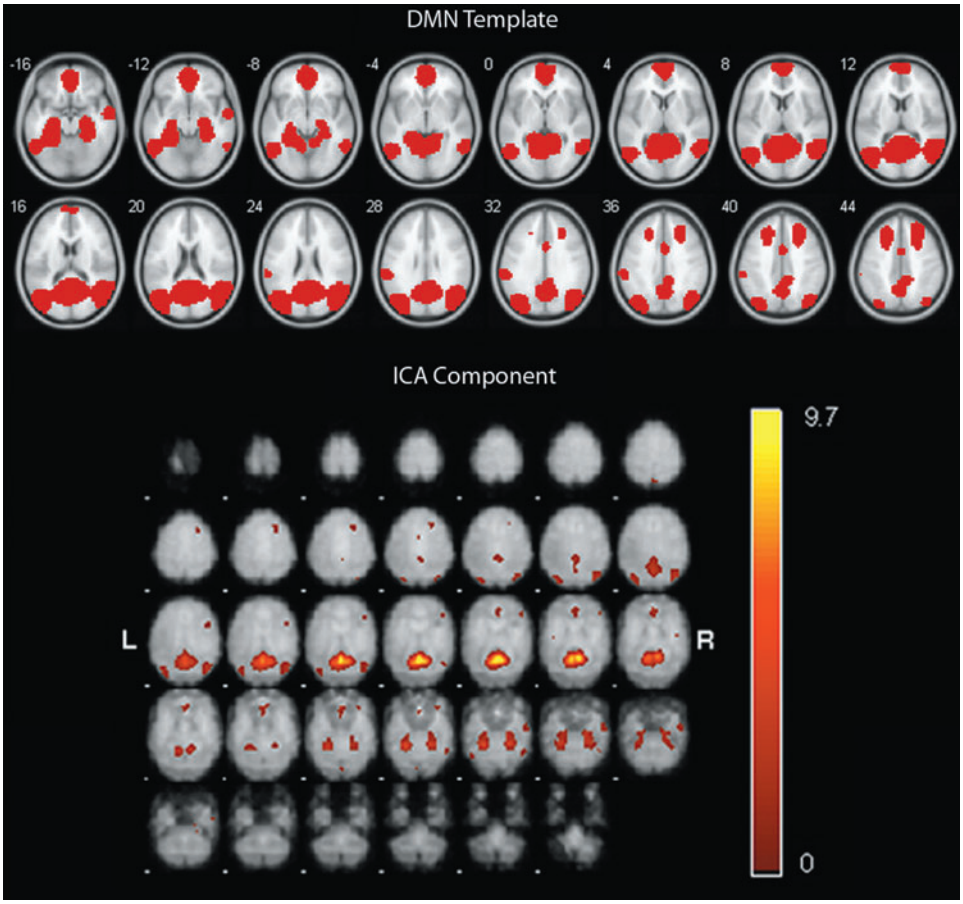

FIG. 1. Axial views of default mode network (DMN) template used all participants. A description of how the templates were made and the templates themselves are available for download at www.nitrc.org/projects/r-spit/, as well as the component from the GIFT analysis that was selected, using template matching, as the component representing the DMN.

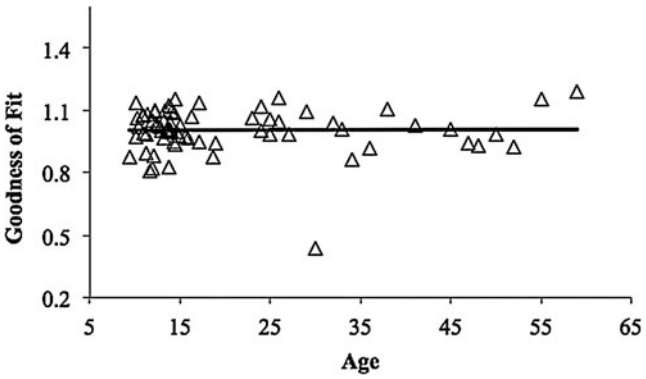

FIG. 2. Scatterplot of goodness-of-fit scores to the DMN template from www.nitrc.org/projects/r-spit/for all subjects. Trend line included showing no association between participant age and goodness-of-fit score.

implemented AlphaSim (http://afni.nimh.nih.gov/afni/AFNI_Help/AlphaSim.html) and determined that the minimal cluster requirement at $p < 0.001$ for the whole brain was 4 voxels, to achieve a family wise error (FWE) correction at $p < 0.05$.

Results

Goodness of fit

To make sure that our template was equally appropriate for both groups, we tested the goodness of fit as described by Greicius and colleagues (2004). The goodness of fit for all subjects to the DMN template from www.nitrc.org/projects/r-spit/is shown in Figure 2. We found no difference between the two groups in their goodness of fit to the template, $t(69) = 0.35$, $p = 0.24$. We also found no correlation between goodness of fit and participant age, $r(71) = 0.007$, $p = 0.95$. These data provide evidence that the template was equally valid in each of our study groups and that its goodness of fit was not related to the age of the participants.

Both groups

The regression analyses examining the areas that were recruited increasingly to the DMN with increasing anxiety scores yielded different results in youth and adults. Even so, both samples showed increased recruitment of the left anterior insula (youth: -30, 26; 14 MNI, Z = 2.85; adults: -33, 12, 14 MNI; Z = 3.78, Table 1, Fig. 3). Results are presented at $p < 0.001$, uncorrected, and a 3 voxel clustering threshold (k) was used. The insula cluster in youth survived FWE correction at $p < 0.05$, but the insula cluster in adults did not survive FWE for the whole brain at this level. Both groups showed other regions that were increasingly correlated with the DMN as anxiety scores increased, but none of the other regions overlapped between the two groups. There were also brain areas in which we observed the opposite effect (increased correlation with the DMN as anxiety score decreased), but none of these were common to both groups. When the analysis was conducted in the two groups combined, the left anterior insula cluster was still present (-37, 8, 14 MNI; Z = 3.13), FWE corrected at $p < 0.05$. MNI coordinates are presented throughout the article.

Youth results

The regions in which the youth specifically showed increasing recruitment to the DMN with increasing anxiety scores, shown in greater temporal correlation between the time courses for these regions and the DMN, were the left anterior insula (MNI -30 26 14, Z = 2.85) and a cluster in the left caudate (MNI -12 12 2, Z = 3.94). The regions showing increased temporal correlation to the DMN as anxiety scores decreased were the right lingual gyrus (MNI 25-64-2, Z = 3.73), right cerebellar vermis (MNI 8-50-6, Z = 3.57), and the right middle frontal gyrus (MNI 42 32 34, Z = 3.94). These results are presented in Table 1 and Figure 4. Only the areas that were positively correlated with anxiety scores are shown in Figure 4; those were of primary interest, as that is where we found overlap between the two groups.

Table 1. Regions Showing Increased Connectivity to the Default Mode Network as Anxiety Scores Increased or Decreased in Youth and Adults

Group	*X*	*Y*	*Z*	*Cluster size*	*Z-score*	*Area*
Common areas of increased connectivity with increasing anxiety score						
Youth	−30	26	14	10	2.85	Left insula (BA 13)
Adults	−33	12	14	3	3.78	Left insula (BA 13)
Areas of increased connectivity with increased anxiety score in youth						
Youth	−30	26	14	10	2.85	Left insula (BA 13)
	−12	12	2	4	3.94	Left caudate (head)
Areas of increased connectivity with decreased anxiety score in youth						
Youth	25	−64	−2	60	3.73	Right lingual gyrus (BA 19)
	8	−50	−6	9	3.57	Right cerebellar vermis
	42	32	34	7	3.94	Right middle frontal gyrus (BA 9)
Areas of increased connectivity with increased anxiety score in adults						
Adults	−33	12	14	3	3.78	Left insula (BA 13)
	49	−2	22	3	3.56	Right inferior frontal gyrus (BA 6)
	−20	−40	−14	6	3.69	Left parahippocampal gyrus (BA 36)
	−20	−60	6	4	3.69	Left posterior cingulated (BA 30)
Areas of increased connectivity with decreased anxiety score in adults						
Adults	49	−36	2	7	4.15	Superior temporal gyrus (BA 22)

Coordinates are presented in Montreal Neurological Institute.

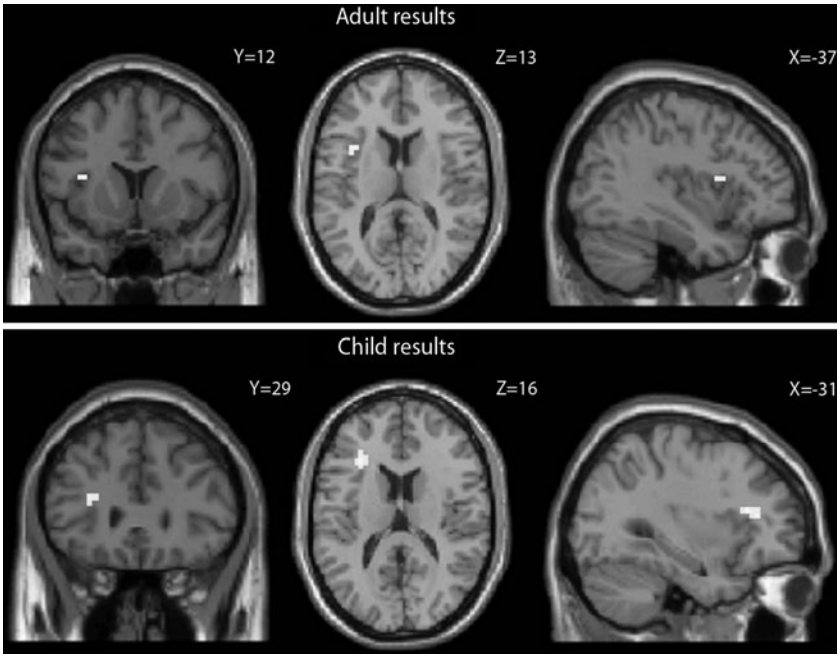

FIG. 3. Common insula recruitment in adults and youth. White clusters indicate increased connectivity to the DMN as anxiety scores increased. $p < 0.001$ uncorrected, cluster threshold = 3 voxels.

To examine whether these anxiety scores reflected state or trait anxiety, we correlated the in-scanner anxiety scores with the ASIC and the SCR-C, both administered before the scan session. Neither the SCR-C total composite score nor any of its subscale scores were correlated with the in-scanner anxiety scores, $r(35) = 0.18$ total; 0.04 panic; 0.11 general anxiety; 0.07 separation anxiety; 0.24 social anxiety; 0.25 avoidance; $p = 0.31$, 0.82, 0.52, 0.69, 0.17, and 0.18, respectively. The ASIC 16 scores was significantly correlated with in-scanner anxiety scores, $r(35) = 0.38$, $p = 0.02$.

Adult results

The regions where the adults specifically showed increased temporal correlation with the DMN with increasing anxiety scores were the left anterior insula (MNI -33 12 14, Z = 3.78), right inferior frontal gyrus (MNI 49-2 22, Z = 3.56), left parahippocampal gyrus (MNI -20-40-14, Z = 3.69), and left posterior cingulate (MNI -20-60 6, Z = 3.69). The superior temporal gyrus (MNI 49-36 2, Z = 4.15) showed increased temporal correlation to the DMN as anxiety scores decreased (Table 1 and Fig. 5). Again, only the areas that were positively correlated with anxiety scores are shown in Figure 5, as those were of primary interest.

We conducted a similar analysis with the adult anxiety scores to address whether these results reflected state or trait anxiety. In an earlier session, most participants completed the STAI-T, a measure of trait anxiety. The postrest anxiety measure was not correlated with the STAI-T, $r(17) = 0.23$, $p = 0.37$, thus suggesting that our findings are due to state anxiety.

Discussion

The number of studies using MRI analysis of ICNs to examine brain connectional architecture is sharply increasing. An important question raised by a number of investigators is how mood or psychological state influences the systems interrogated in functional connectivity analyses. Arguably the best characterized of ICNs at this time is the DMN, which may demonstrate altered connectivity as a function of individual differences in mood. The DMN is posited to be implicated in introspective thought, often concerned with remembering or imagining the self in the past and future (Buckner et al., 2008; Raichle et al., 2001). Since a few memories are completely free of emotional valence, we expect that the participant's natural mood will influence how this introspective narrative proceeds. In a recent study, Harrison and colleagues (2008) measured the impact of inducing a negative mood on ICN connectivity in adults and found altered connectivity with the anterior insula. Here, we extended this to investigate naturally occurring individual differences in mood in both adults and youth. The areas temporally coupled to the DMN differed according to

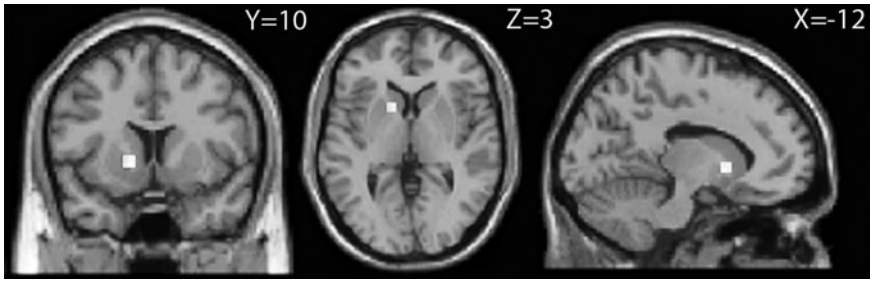

FIG. 4. Youth-specific results. White clusters indicate increased connectivity to the DMN as anxiety scores increased. $p < 0.001$ uncorrected, cluster threshold = 3 voxels.

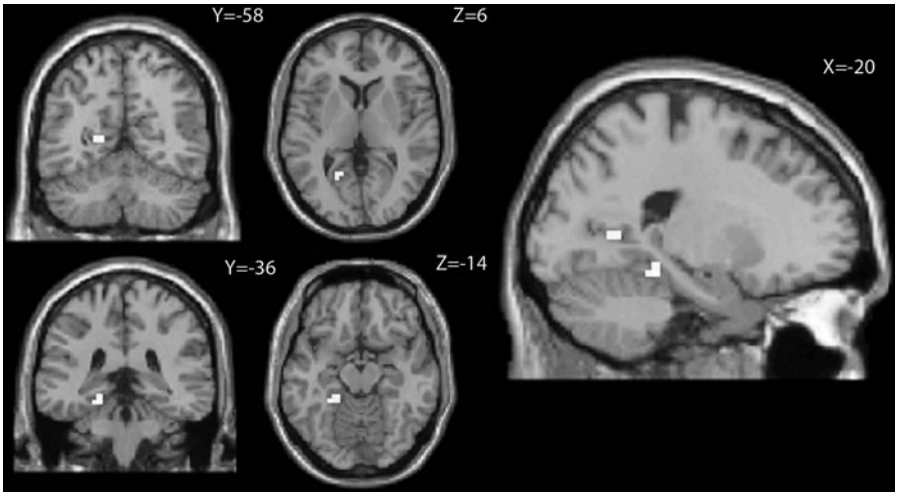

FIG. 5. Adult-specific results. White clusters indicate increased connectivity to the DMN as anxiety scores increased. $p < 0.001$ uncorrected, cluster threshold = 3 voxels.

self-reported, naturally occurring levels of anxiety during the scan session.

In our analysis, we found differences and similarities in the regions that showed altered connectivity to the DMN. Differences between age groups are interesting for examining how functional networks are fine tuned over development, but we found the similarities to be equally compelling. Since the similarities are already present at an early stage of development, they may represent foundations on which the functional networks are built. The insula was the only brain region in which self-reported anxiety altered DMN connectivity in both adults and youth, thus suggesting that insula connectivity is organized in early life and continues through development. Our insula result did not survive correction for multiple comparisons in adults, but it did in children and when both groups were combined. We observed altered left anterior insula connectivity to the DMN independently in both groups, which corroborates the results. This observation of similar insula connectivity across age groups is consistent with previous analyses of human cortical development. The insular cortex develops earlier than the frontal cortex (Benes, 1994); evolutionary studies also document the early origin of the insular cortex (Mega et al., 1997). The insula, which is phylogenetically older and earlier to develop during brain maturation, might, thus, be already able to participate in the DMN in the same way in youth as it is in adults. Although not a primary hypothesis in the present analysis, we did observe that anxiety was negatively correlated with increased connectivity in a number of regions. These results suggest that there are areas of the brain in which changes in connectivity moderate the effects of anxiety. Regions that moderate the effects of anxiety may also differ between youth and adults. Even in early life, negative emotional states may alter insula connectivity to the DMN, thus suggesting an early role for the insula as a critical hub for influencing emotional processing at the neural systems level.

Central functions of the insula include emotional processing and the experience of pain. Numerous studies report increased insula activity in individuals with higher levels of trait anxiety (Stein et al., 2007) and in individuals with anxiety disorders such as post traumatic stress disorder (PTSD), social phobia, and specific phobia (Etkin and Wager, 2007). This anxiety response can be beneficial: Baumgartner and colleagues (2009) recently found that patterns of anterior insula activation predicted when a partner in a game was going to be deceptive. The insula is active during both pain perception and while watching a loved one in a painful situation (Singer et al., 2004). The anxiety response ascribed to the insula may be evolutionarily adaptive for the individual and his/her love ones, but this response can become maladaptive when it is too active (Etkin and Wager, 2007). Our data suggest that functional connectivity of the insula is dynamic, and that functional involvement of the insula in activity of other structures may be a key mechanism by which the insula operates to alter emotional experience.

Although it is most widely known for its role in emotional processing, the insula is a heterogeneous structure with other functions, such as interoception, monitoring of external sensory processes, and autonomic regulation (Augustine, 1996). Given these many putative roles, Kurth and colleagues (2010) proposed that the insula is an integrative structure. In their review, Wager and Barrett (2004) found that the left anterior insula was preferentially activated during emotional tasks compared with more superior insular regions or to the right anterior insula. Using two different approaches, both Wager and Barrett and Kurth and colleagues concluded that these varying roles are localized in different subdivisions of the insula. Wager and Barrett examined past anatomical studies to divide the insula into an anterior ventral aspect responsible for emotion, a dorsal anterior aspect responsible for motivation and goal directed behavior, a posterior aspect responsible for pain perception, and a mid-insula for which they assigned no unique roles. Kurth and colleagues completed a meta-analysis of available functional imaging data and hypothesized a similar division: a dorsal anterior region responsible for cognitive tasks, an anterior ventral region responsible for social-emotional tasks, a mid-insula aspect responsible for smell and taste, and a mid-posterior insular region responsible for sensorimotor tasks. Given the specific functions ascribed to the insula and its probable integrative role, it is well positioned as a brain region responsible for integrating sensory awareness and higher cognition.

Recent fMRI research using functional connectivity (Hamilton et al., 2011b; Sridharan et al., 2008) suggests a privileged role for the insula in regulating brain dynamics. The concept of multiple dynamic resting networks with different

functions was first presented by Fransson (2005); determining exactly what modulates the dynamics of those networks, however, is an ongoing process. Although the insula is generally considered a primary node of the SN (Seeley et al., 2007; Thomason et al., 2011b; White et al., 2010), recent research has also established the insular cortex as a hub that facilitates switching between the DMN and executive control network (ECN) (Sridharan et al., 2008). Downar and colleagues (2000) suggested that, as a sensitive detector of novel stimuli, the insula is responsible for determining which stimuli are salient. Sridharan and colleagues (2008) extended this to propose that the insula then facilitates switching between the DMN and the ECN, thus allowing the brain to actively concentrate on relevant stimuli. Clinical groups may differ in this aspect of insula function as well. Hamilton and colleagues (2011b) found that both healthy control participants and those with Major Depressive Disorder (MDD) had increased signal change in the insula during the *onset* of a switch in dominance between task-positive and default-mode networks. Thus, again, signaling in the insula preceded function in other brain networks. Further, Hamilton et al. also observed that in MDD participants, DMN dominance was associated with higher levels of maladaptive, depressive rumination, and they suggested that the DMN undergirds representation of negative, self-referential information in depression. Together with our data, these results support a formulation in which the insula serves as a detector that monitors emotional state and, based on a close functional relation with the DMN, may be well suited to initiate adaptive engagement of this and other neural systems. In this article, we show that even subclinical levels of state anxiety in healthy individuals influence how the insula is functionally linked to activity in the DMN.

There were brain regions that showed connectivity alterations that were group specific. Two of the structures that showed increased connectivity to the DMN with increased anxiety in adults but not in youth were the parahippocampal gyrus and the posterior cingulate. Since this connectivity was present in adults but not in children, it may reflect how the DMN and its interactions with other networks change over development. These areas do develop later than the insula. Myelination in the hippocampus continues throughout adolescence (Benes et al., 1994), and the cingulate cortex is one of the last brain areas to reach peak thickness (Shaw et al., 2008). Taken together, one explanation for the parahippocampal and posterior cingulate results in adults is that these are areas which have yet to mature in youth and, therefore, are not yet able to participate in the DMN in an adult capacity. The fact that we observed select areas in which functional connectivity was altered with increased levels of anxiety only in adults supports the formulation that the DMN and connectivity of the DMN to other brain areas continue to develop throughout adolescence.

A critical question when interpreting mood-related results is what bearing state versus trait anxiety has on the data. In this study, we attempted to examine how mood states, not traits, affect functional connectivity. The participants were all healthy, with no past or current anxiety disorders. Nevertheless, to be confident we were not assessing effects of subthreshold levels of trait anxiety, we examined the relation between the measures of state and trait anxiety. The measure of anxiety we administered after rest in the scanner did not correlate with either the SCR-C in youth or the STAI-T in adults, thus supporting the conclusion that our findings were due to state, rather than trait, anxiety. Interestingly, there was a significant correlation between the measure of anxiety administered in the scanner and the ASIC, a measure of the sensitivity of children to the physical feelings of anxiety, thus suggesting that individuals who were more anxious in the scanner have a greater sensitivity to the physical symptoms of anxiety. To support the validity of our approach, we conducted a goodness-of-fit analysis and found our DMN template to be equally valid for adults and youth. Although research practices for making ICN comparisons between children and adults are in a nascent stage, we were able to show that goodness of fit to our chosen network template did not differ between groups or as a function of age.

Conclusions

The data presented in this study are the first to suggest that natural levels of mood state are reflected in resting-state functional connectivity. Moreover, we show that this modulation of ICN dynamics by mood state is reliable across different age groups. It is critical to understand individual differences in ICN dynamics, to better understand the role that large-scale brain connectivity plays in overall brain function. Here, we highlight a consistency in DMN and insula connectivity that appears to be present from an early stage in development. We posit that this insula region may serve as a central and foundational node in linking emotional awareness to spontaneous DMN function.

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Author Disclosure Statement

The authors have no competing financial interests.

References

Augustine J. 1996. Circuitry and functional aspects of the insular lobe in primates including humans. Brain Res Rev 22:229–244.

Baumgartner T, Fischbacher U, Feierabend A, Lutz K, Fehr E. 2009. The neural circuitry of a broken promise. Neuron 64: 756–770.

Beckmann C, DeLuca M, Devlin J, Smith S. 2005. Investigations into resting-state connectivity using independent component analysis. Philos Trans R Soc 360:1001–1013.

Benes FM. 1994. Development of the corticolimbic system. In: Dawson G, Fischer KW (eds.) *Human Behavior and the Developing Brain*. New York: Guilford Press.

Benes FM, Turtle M, Khan Y, Farol P. 1994. Myelination of a key relay zone in the hippocampal formation occurs in the human brain during childhood, adolescence, and adulthood. Arch Gen Psychiatry 51:477–484.

Binder J, Frost J, Hammeke T, Bellgowan P, Rao S, Cox R. 1999. Conceptual processing during the conscious resting state: a functional MRI study. J Cogn Neurosci 11:80–93.

Birmaher B, Khetarpal S, Brent D, Cully M, Balach L, Kaufman J, Neer SM. 1997. The screen for child anxiety related emotional disorders (SCR-C): scale construction and psychometric characteristics. JAAC 36:545–553.

Biswal B, Yetkin FZ, Haughton VM, Hyde JS. 1995. Functional connectivity in the motor cortex of the resting human brain. Magn Res Med 34:537–541.

Bluhm R, Williamson P, Lanius R, Théberge J, Densmore M, Bartha R, Neufeld R, Osuch E. 2009. Resting state default-mode network connectivity in early depression using a seed region-of-interest analysis: decreased connectivity with caudate nucleus. Psychiatry Clin Neurosci 63:754–761.

Buckner R, Andrews-Hanna J, Schacter D. 2008. The Brain's default network: anatomy, function, and relevance to disease. Ann NY Acad Sci 1124:1–38.

Calhoun V, Adali T, Pearlson GD, Pekar JJ. 2001. A method for making group inferences from functional MRI data using independent component analysis. Hum Brain Mapp 14: 140–151.

Cao Q, Zang Y, Sun L, Sui M, Long X, Zou Q, Wang Y. 2006. Abnormal neural activity in children with attention deficit hyperactivity disorder: a resting- state functional magnetic resonance imaging study. Neuroreport 17:1033–1036.

Chang C, Glover GH. 2009. Effects of model-based physiological noise correction on default mode network anti-correlations and correlations. Neuroimage 47:1448–1459.

Damoiseaux JS, Rombouts SARB, Barkhof F, Scheltens P, Stam CJ, Smith SM, Beckmann CF. 2006. Consistent resting-state networks across healthy subjects. PNAS 103:13848–13853.

Dosenbach NUF, Nardos B, Cohen AL, Fair DA, Power JD, Church JA, Nelson SM, Wig GS, Vogel AC, Lessov-Schlaggar CN, Barnes KA, Dubis JW, Feczko E, Coalson RS, Pruett Jr., JR, Barch DM, Petersen SE, Schlaggar BL. 2010. Prediction of individual brain maturity using fMRI. Science 329:1358–1361.

Downar J, Crawley AP, Mikulis DJ, Davis KD. 2000. A multimodal cortical network for the detection of changes in the sensory environment. Nat Neurosci 3:277–283.

Eryilmaz H, Ville D, Schwartz S, Vuilleumier P. 2011. Impact of transient emotions on functional connectivity during subsequent resting state: a wavelet correlation approach. Neuroimage 54:2481–2491.

Etkin A, Wager TD. 2007. Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, Social Anxiety Disorder, and Specific Phobia. Am J Psychiatry 164: 1476–1488.

First MB, Spitzer RL, Gibbon M, Williams JBW. 1995. *Structured Clinical Interview for the DSM-IV Axis I Disorders—Patient Edition (SCID-I/P) version 2.0.* New York, NY: New York State Psychiatric Institute.

Fox M, Snyder A, Vincent J, Corbetta M, Essen D, Raichle M. 2005. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. PNAS 102:9673–9678.

Fransson P. 2005. Spontaneous low-frequency BOLD signal fluctuations: an fMRI investigation of the resting-state default mode of brain function hypothesis. Hum Brain Mapp 26: 15–29.

Glover GH, Law CS. 2001. Spiral-in/out BOLD fMRI for increased SNR and reduced susceptibility artifacts. Magn Reson Med 46:515–522.

Greicius M. 2008. Resting-state functional connectivity in neuropsychiatric disorders. Curr Opin Neurol 21:424–430.

Greicius M, Flores B, Menon V, Glover G, Solvason H, Kenna H, Reiss AL, Schatzberg AF. 2007. Resting-state functional connectivity in major depression: abnormally increased contributions from subgenual cingulate cortex and thalamus. Biol Psychiatry 62:429–437.

Greicius M, Kiviniemi V, Tervonen O, Vainionpää V, Alahuhta S, Reiss A, Menon V. 2008. Persistent default-mode network connectivity during light sedation. Hum Brain Mapp 29: 839–847.

Greicius MD, Srivastava G, Reiss AL, Menon V. 2004. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. PNAS 101: 4637–4642.

Gusnard D, Raichle M. 2001. Searching for a baseline: functional imaging and the resting human brain. Nat Rev Neurosci 2: 685–694.

Hamilton JP, Chen G, Thomason ME, Schwartz ME, Gotlib IH. 2011a. Investigating neural primacy in Major Depressive Disorder: multivariate granger causality analysis of resting-state fMRI time-series data. Mol Psychiatry 16:763–772.

Hamilton JP, Furman DJ, Chang C, Thomason ME, Dennis E, Gotlib IH. 2011b. Default-mode and task-positive network activity in major depressive disorder: implications for adaptive and maladaptive rumination. Biol Psychiatry 70:327–333.

Harrison B, Pujol J, Ortiz H, Fornito A, Pantelis C, Yücel M. 2008. Modulation of brain resting-state networks by sad mood induction. PLoS ONE 3: e1794, 1–12.

Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, Williamson D, Ryan ND. 1997. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry 36:980–988.

Khalili-Mahani N, Zoethout R, Beckmann C, Baerends E, de Kam M, Soeter R, Dahan A, van Buchem MA, van Gerven JMA, Rombouts SARB. 2011. Effects of morphine and alcohol on functional brain connectivity during "resting state": a placebo-controlled crossover study in healthy young men. Hum Brain Mapp [Epub ahead of print]; DOI: 10.1002/hbm.21265.

Kim D, Adalsteinsson E, Glover G, Spielman D. 2002. Regularized higher-order *in vivo* shimming. Magn Reson Med 48: 715–722.

Kurth F, Zilles K, Fox PT, Laird AR, Eickhoff SB. 2010. A link between systems: functional differentiation and integration within the human insula revealed by meta-analysis. Brain Struct Funct 214:519–534.

Larson-Prior L, Zempel J, Nolan T, Prior F, Snyder A, Raichle M. 2009. Cortical network functional connectivity in the descent to sleep. PNAS 106:4489–4494.

Laurent J, Schmidt NB, Catanzaro SJ, Joiner Jr., TE, Kelley AM. 1998. Factor structure of a measure of anxiety sensitivity in children. J Anxiety Disord 12:307–331.

Liu H, Liu Z, Liang M, Hao Y, Tan L, Kuang F, Yi Y, Xu L, Jiang T. 2005. Decreased regional homogeneity in schizophrenia: a resting state functional magnetic resonance imaging study. Neuroreport 17:19–22.

Mason MF, Norton MI, Van Horn JD, Wegner DM, Grafton ST, Macrae CN. 2007. Wandering minds: the Default network and stimulus-independent thought. Science 315:393–395.

McKiernan K, DAngelo B, Kaufman J, Binder J. 2006. Interrupting the "stream of consciousness": an fMRI investigation. Neuroimage 29:1185–1191.

Mega M, Cummings J, Salloway S, Malloy P. 1997. The Limbic System: an anatomic, phylogenetic, and clinical perspective. Anat Neurochem 9:315–330.

Preston AR, Thomason ME, Ochsner KN, Cooper JC, Glover GH. 2004. Comparison of spiral-in/out and spiral-out BOLD fMRI at 1.5 and 3 T. Neuroimage 21:291–301.

Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. 2001. A default mode of brain function. PNAS 98:676–682.

Rombouts S, Barkhof F, Goekoop R, Stam C, Scheltens P. 2005. Altered resting state networks in mild cognitive impairment and mild Alzheimer's disease: an fMRI study. Hum Brain Mapp 26:231–239.

Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, Reiss AL, Greicius MD. 2007. Dissociable intrinsic connectivity networks for salience processing and executive control. J Neurosci 27:2349–2356.

Shaw P, Kabani NJ, Lerch JP, Eckstrand K, Lenroot R, Gogtay N, Greenstein D, Clasen L, Evans A, Rapoport JL, Giedd JN, Wise SP. 2008. Neurodevelopmental trajectories of the human cerebral cortex. J Neurosci 28:3586–3594.

Singer T, Seymour B, O'Doherty J, Kaube H, Dolan RJ, Frith CD. 2004. Empathy for pain involved the affective but not sensory components of pain. Science 303:1157–1162.

Spielberger CD. 1983. *Manual for the State–Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologist Press.

Spreng RN, Grady CL. 2010. Patterns of brain activity supporting autobiographical memory, prospection, and theory of mind, and their relationship to the default mode network. J Cogn Neurosci 22:1112–1123.

Sridharan D, Levitin DJ, Menon V. 2008. A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. PNAS 105:12569–12574.

Stein M, Simmons A, Feinstein J, Paulus M. 2007. Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. Am J Psychiatry 164:318–327.

Stevens MC, Pearlson GD, Calhoun VD. 2009. Changes in the interaction of resting-state neural networks from adolescence to adulthood. Hum Brain Mapp 30:2356–2366.

Thomason M, Dennis E, Joshi A, Joshi S, Dinov I, Chang C, Henry ML, Johnson RF, Thompson PM, Toga AW, Glover GH, Van Horn JD, Gotlib IH. 2011. Resting-state fMRI can reliably map neural networks in children. Neuroimage 55:165–175.

Thomason ME. NITRC: Resting-state pediatric imaging template. www.nitrc.org/projects/r-spit/ Last accessed May 27, 2011.

Thomason ME, Hamilton JP, Gotlib IH. 2011. Stress-induced activation of the HPA axis predicts connectivity between subgenual cingulate and salience network during rest in adolescents. JCPP 52:1026–1034.

Tian L, Jiang T, Wang Y, Zang Y, He Y, Liang M, Sui M, Cao Q, Hu S, Peng M, Zhou Y. 2006. Altered resting-state functional connectivity patterns of anterior cingulate cortex in adolescents with attention deficit hyperactivity disorder. Neurosci Lett 400:39–43.

Wager T, Barrett L. 2004. From affect to control: functional specialization of the insula in motivation and regulation. Psychextra 1–23 (Online).

Wang L, Zang Y, He Y, Liang M, Zhang X, Tian L, Wu T, Jiang T, Li K. 2006. Changes in hippocampal connectivity in the early stages of Alzheimer's disease: evidence from resting state fMRI. Neuroimage 31:496–504.

Weng S-J, Wiggins J, Peltier S, Carrasco M, Risi S, Lord C, Monk CS. 2010. Alterations of resting state functional connectivity in the default network in adolescents with autism spectrum disorders. Brain Res 1313:202–214.

White TP, Joseph V, Francis ST, Liddle PF. 2010. Aberrant salience network (bilateral insula and anterior cingulate cortex) during information processing in schizophrenia. Schizophr Res 123:105–115.

Zhou Y, Liang M, Tian L, Wang K, Hao Y, Liu H, Jiang T. 2007. Functional disintegration in paranoid schizophrenia using resting-state fMRI. Schizophr Res 97:194–205.

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