UNIVERSITY OF CALIFORNIA, SAN DIEGO

Altered Functional Connectivity of Subgenual Anterior Cingulate Cortex during Negative Emotion Processing in Adolescent Depression

A thesis submitted in partial satisfaction of the

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in

Biology

by

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2013

The thesis of Guang Yang is approved, and it is acceptable

in quality and form for publication on microfilm and electronically:

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Chair

University of California, San Diego

2013

DEDICATIONS

I dedicate this thesis to Rachel, Jacky, and Andy

for their love, support and encouragement.

I would also like to dedicate this thesis to my family:

To my father, Yanfeng Yang and my mother Huijuan Li

For being the inspirations for my life.

To my brother, Michael, and my sister, Dana, for filling my life with joy.

EPIGRAPH

"I do not know what I may appear to the world, but to myself I seem to have been only a boy playing on the sea-shore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me"

Isaac Newton

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ABSTRACT OF THE THESIS

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by

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Functional neuroimaging studies have advanced our understanding of the network dysfunctions related to major depressive disorder (MDD) in adults. However, our current understanding of these network alterations in adolescents very limited, especially in relation to the subgenual anterior cingulate cortex (ACC). The main goal of this study is to further our understanding of the role of the subgenual ACC in adolescents with depression during fear processing by examining how MDD affects subgenual ACC connectivity with other brain regions. Nineteen unmedicated adolescents diagnosed MDD and nineteen well-matched controls ages 13-17 years underwent a gender discrimination task during fMRI, where the participants viewed images of different intensities of fear. Whole brain analysis demonstrated that adolescents with depression showed decreased

activation in the left precuneus, left ACC, and right precentral gyrus compared to normal controls when viewing faces showing stronger fearful emotions. Functional connectivity analysis showed: (1) increased connectivity between the right subgenual ACC and the left amygdala, (2) decreased connectivity between the right subgenual ACC and the left fusiform gyrus, right precuneus, right insula, and (3) decreased connectivity between the left subgenual ACC and the bilateral insula.

Overall, our findings show that the subgenual ACC plays a key role in modulating perceptual and cognitive processes, and that, like adult depression, adolescent depression involves a disruption of networks involving the subgenual ACC. Our findings build towards a model of a disrupted network in clinically depressed adolescence such that low fear/neutral faces are deemed threatening and a wider network is recruited.

Introduction

Depression is considered one of the most common mental disorders in the adult population. According to studies performed by the National Institute on Mental Health, major depressive disorder (MDD) is the leading cause of disability in the United States for ages 15-44 (NIMH). Major Depressive Disorder affects 6.7 percent of the adults in the United States, which pertains to 21.2 million adults as of 2013 in the U.S. alone. The Centers for Disease Control and Prevention (CDC) estimate that 1 in 10 U.S. adults report depression at any given time. A recent cost-of-illness study, which quantifies the total cost related to a specific disease, found that workers with depression had significantly greater lost productive time (LPT) compared to healthy workers (5.6hr/week compared to 1.5hr/week), with 81% being attributed to impaired performance during work (Stuart et al. 2003). The implications of this study was that depressed employees result in an estimated total of 44 billion dollars of LPT per year in the United States, an excess of 33 billion compared to those without depression (Stewart et al. 2003).

It is well established that MDD is a great source of concern in adults. However, depression is also prevalent in adolescents. It is estimated that 4 to 8 percent of the adolescent population is depressed (Lewinsohn et al. 1994). One in five adolescents has reportedly experienced depression at some point (NAMI 2013). According to Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV-TR), adult MDD is "diagnosed clinically by the occurrence of depressed mood and/or loss of interest or pleasure in life activities for at least two weeks, as well as at least five of the following symptoms that cause significant impairment in social, work, or other important areas of daily life: depressed mood most of the day, diminished interest or pleasure in all or most

activities, significant unintentional weight loss or gain, insomnia or sleeping too much, agitation or psychomotor retardation noticed by others, fatigue or loss of energy, feelings of worthlessness or excessive guilt, diminished ability to think or concentrate, or indecisiveness and recurrent thoughts of death" (Center for Substance Abuse Treatment 2008). Symptoms of depression in adolescents, however, appear to differ, and include increased irritability, outbursts of anger, and social withdrawal with increased risk of suicide. Studies by Pine et al. (1999) suggest that symptoms of depression in adolescents could be early markers for adult depressive disorder (Pine et al. 1999). Since MDD typically begins during adolescence (Kessler, Avenevoli, and Merikangas 2001) and confers a high risk of recurrence in adulthood (Lewinsohn, Rohde, Klein, and Seeley 1999), it is of utmost importance that we understand the development and progression of this mental illness during adolescence, when the risk of developing depression and impact on quality of life is extremely high.

Although the National Institute of Mental Health (NIMH) ranked MDD as the leading cause of disability in the United States for ages 15-44, very little is known about the pathogenesis of depression. This is partly attributed to the difficulty in developing an animal model that perfectly replicates the symptoms of depression or the recurrence of depressive episodes. However, recent advances in neuroimaging technologies spurred the advancement of scientific research in depression by allowing investigators to examine the chemical, anatomical and physiological correlates of depression in vivo (Drevets et al. 2008).

Recently, functional magnetic resonance imaging (fMRI) has become an invaluable tool in the study of various neurological and psychiatric disorders. fMRI

involves placing the subject inside a large homogenous magnetic field and measuring changes in blood oxygenation and flow that occur in response to neural activity. Since its inception in the early 90s, fMRI has rapidly risen in popularity in the neuroimaging field due to its noninvasiveness and superior spatial and temporal resolution. Unlike positron emission topography (PET), fMRI does not require subjects to undergo injections of a radioactive tracer. And unlike electroencephalography (EEG), fMRI is not restricted to capturing only the signal in the peripheral regions of the brain, thus making fMRI the ideal tool for studying regions deep beneath the cortex, including the limbic regions involved with emotional regulation, processing and memory. Therefore fMRI is the ideal noninvasive neuroimaging tool for studying the brain circuitry related to depression, particularly in adolescents.

The primary method by which investigators utilize fMRI in the study of mental illnesses is through the blood-oxygen-level-dependent (BOLD) contrast (Huettel 2009). The BOLD contrast, first proposed by Seiji Ogawa in 1990, provides a platform by which investigators can examine difference in neural activity in different tissues of the brain by exploiting activity-related differences in magnetic susceptibility (Ogawa et al. 1990). The brain utilizes blood glucose as the primary source of energy. When neurons change from a depolarized (active) to hyperpolarized (inactive) state, there is a need for the neurons to maintain their electrochemical gradient, a process that requires an expenditure of energy. Therefore, neuronal activity is associated with a regional increase in blood flow and glucose levels, which subsequently leads to a regional change in oxygen levels via oxygenated hemoglobin cells in the blood. Higher neuronal activity leads to an increase in consumption of oxygen, thus increasing the population of deoxyhemoglobin in the

same region. In contrast with hemoglobin, deoxyhemoglobin is paramagnetic (Pauling & Coryell 1936), and will produce a local distortion of the magnetic field within the large homogenous magnetic fields produced by the MRI machine. The local distortions are localized within 2 or 3 mm of where the neural activity takes place (Huttel 2009). By measuring the location and magnitude of these distortions, researchers are able to infer the directionality and intensity of neural activity in different regions of the brain.

Recently, fMRI has been used by investigators to examine not only localized changes in brain activation but also differences in brain activation on the network level. One increasingly popular method is functional connectivity analysis (Friston et al. 1994), which is defined as temporal correlations between spatially distinct brain areas. The pathophysiology of MDD has been thought to involve dysfunctions on the network level. Specifically, altered functional connections between the frontal and the limbic regions have been shown to play a role in depression. A study by Mayberg et al. (1999) performed in adult populations with induced transient sadness have reported an increase in activation of the subgenual anterior cingulate and anterior insula and decrease in activation of the neocortical regions (right dorsolateral prefrontal and inferior parietal). Further investigations have also reported a reciprocal relationship between cortical and limbic regions in diagnosed depression, showing that a decrease in cortical activation is accompanied by an increase in limbic activation in MDDs (Mayberg et al. 1999). Additionally, recovery from depression showed a reversal of these effects, with an increase in cortical regions and a decrease in the limbic regions.

Further studies in neuroimaging have suggested a model of depression based on limbic-cortical dysfunction (Drevets et al. 2008). Using PET, Drevets et al. proposed that

abnormalities in the circuits involving the prefrontal cortex, amygdala and areas of the striatum, pallidum and medial thalamus may be abnormally regulating cognitive and emotional processing in depression (Drevets & Raichle 1992). Further studies showed that the limbic-cortical-striatal-pallidal-thalamic circuits (LCSPT) were involved in emotional behavior, and that dysfunctions of the LCSPT circuit can produce pathological emotional symptoms similar to those found in depression (Drevets 2004). A study that examined the relationship between stroke lesions in brain regions involved in the LCSPT and incidence of major depressive episode found that post-stroke depression was correlated with lesion volume of the left LCSPT circuit (Terroni et al. 2011). Further analysis showed that incidence of major depressive episode was associated with specific regions of the left LCSPT circuit, including the ventral anterior cingulate cortex, subgenual anterior cingulate cortex, subiculum, amygdala, and the dorsal anterior cingulate cortex (Mayberg et al. 1999).

Two particular regions, the amygdala and the subgenual anterior cingulate cortex (ACC), have received special attention in the study of adult depression. The amygdala has been considered significant due to its role in emotional processing and evaluation of external stimuli. Positron emission studies have repeatedly shown significantly increased amygdala activity in adults with MDD (Robertson-nay et al. 2006; Drevets et al. 1992). This finding has also been consistently replicated in functional MRI studies (Fales et al. 2008). These findings were used to develop some of the current models of depression involving the amygdala, such as the model proposed by Drevets and Mayberg. It has been hypothesized that the hyperactivation of the amygdala and related limbic regions leads to increased attention and processing of emotional information in individuals with MDD,

with a bias towards negatively valenced stimulus (Sliz & Hayley 2012). Furthermore, studies have shown that increased amygdala activity in depressed individuals is associated with rumination, a contributing factor in depression (Siegle 2006). Indeed, a large number of behavioral studies have demonstrated that individuals diagnosed with depression present biased emotional processing, with an attentional bias away from positive (e.g. happy faces) emotional cues and a propensity to focus on negative emotional cues (e.g. fearful faces). Consistent with the LCPST model, activity in emotion-related brain regions (amygdala, ventral striatum) were found to be increased in response to sad faces (Lappanen JM 2006). Further behavioral studies have found that depressed individuals were less sensitive to the identification of emotional faces, and a bias for interpreting happy faces as neutral, and neutral faces as sad (Bourke et al. 2010). These findings demonstrate impairment in attention and emotional processing circuits in individuals with depression, perhaps due to heightened limbic activity.

The subgenual ACC plays an important role in the regulation of amygdalar activity and the prevention of excessive emotional and stress-related responses (Phillips et al. 2003). Adult depression studies have shown that connections between subgenual ACC and dorsolateral prefrontal cortex (Hamilton et al. 2011) and dorsomedial prefrontal cortex (Sheline et al. 2010) are strengthened in depressed individuals. A preliminary study by Mayberg et al. using deep brain stimulation (which involves implanting an artificial pacemaker to stimulate certain structures of the brain) of the subgenual ACC found that electrical stimulation of the subgenual ACC can effectively reverse depression symptoms in individuals with treatment-resistant depression (Mayberg et al. 2005). This

major finding solidifies the significance of the subgenual ACC as a potential target of future therapeutic techniques in the treatment of depression.

Although the neural circuitry of adult depression is studied extensively, research and literature on pediatric depression is severely lacking (Hulvershorn et al. 2011; Drevets et al. 1992). Furthermore, Functional MRI studies in pediatric depression reflect inconsistencies and the need for further investigation. In adults, facial recognition tasks show that adults with MDD exhibit increased amygdala activation compared to normal controls (Sheline et al. 2001). However, these findings were not consistently reproduced in children and adolescent studies of depression. For example, a study by Thomas et al. used a facial recognition task comparing 5 girls with MDD with normal healthy girls and reported a hypoactive amygdala response, in contrast with other studies demonstrating hyperactive amygdala response (Thomas et al. 2001, Robertson-nay et al. 2006). A recent novel study by Yang et al. demonstrate decreased activity of the medial frontal regions and increased activity in the anterior cingulate cortex in adolescents with depression, both consistent with adult literature (Yang et al. 2009).

Although the study of functional connectivity in adults with depression is studied extensively, very few studies have been performed in adolescents with depression. Given the importance of the subgenual ACC as a target of potential treatment options such as deep brain stimulation, as well as the lack of consistent literature in the field of adolescent depression, it is imperative to examine the role of subgenual ACC and its connectivity with other brain regions. In a preliminary study by Jin et al., adolescents with depression were found to exhibit atypical functional connectivity in regions of the prefrontal cortex as well as an increase in the strength of connections involving the

amygdala (Jin et al. 2011). A preliminary study by Cullen et al. found decreased resting state functional connectivity between the subgenual ACC and bilateral cortical regions, left superior temporal cortex, and the insular cortex (Cullen et al. 2009). However, the group mentioned the prevalence of psychotropic medications and high rates of comorbidity as a potential confound, and calls for the need of research using treatment-naïve adolescents with MDD. A more recent publication by Davey et al. found increased resting state functional connectivity between the subgenual ACC and the dorsomedial frontal cortex (Davey et al. 2012). In children, one study has shown reduced functional connectivity in individuals with preschool-onset MDD (Luking et al. 2011). As mentioned by Cullen et al., one potential explanation for the difference in functional connectivity differences in the subgenual ACC between adults, adolescents and children is the medication status of the subjects under study. Another possibility is that difference in connectivity may be attributed to the developmental differences between childhood, adolescence and adulthood. This theory is supported by a longitudinal study showing differences in functional connectivity from childhood to adulthood (Fair et al. 2008), as well as a developmental differences specific to the anterior cingulate (Kelly et al. 2009). A recent study by Connolly et al. found elevated connectivity between the subgenual ACC and the insula, and decreased connectivity between the subgenual ACC and the left precuneus, which also correlated significantly with depression severity (Connolly et al. 2013)

The study of facial emotions is important in studying a variety of mental disorders. This is especially important in the study of depression because depressed individuals were shown to possess aberrant emotional processing, with an attentional bias

towards negative stimuli (e.g. fear). Furthermore, abnormal emotional processing has been shown to be correlated with various affective and social symptoms in MDD, and that measurement of facial expression perception may potentially aid in predicting and monitoring treatment response for individuals with depression (Ekman & Friesen 1976). However, to date, no studies have examined the functional connectivity of the subgenual ACC during the processing of emotionally negative stimuli in the adolescent population.

The aim of the present study is to use BOLD and functional connectivity fMRI techniques to examine the reactivity and functional connectivity of the subgenual ACC and related limbic and paralimbic regions in adolescents with depression during fear processing. 19 medication-naïve adolescents (13-17 years old) with a primary diagnosis of MDD and 19 well-matched healthy controls (CTL) underwent fMRI scanning while performing a gender recognition task of faces exhibiting varying degrees of fear (Fear-Weak, Fear-Moderate, Fear-Strong). We examined the functional BOLD differences between MDD and CTL groups, as well as using a pathophysiological interaction modeling technique (PPI; Friston et al., 1997) using *a priori* anatomical seeds centered in the bilateral subgenual ACC in order to ascertain which functionally connected regions alter with adolescent depression (Connolly et al. 2013). Depression severity was measured with the Beck Depression Inventory (BDI-II), the Children's Depression Inventory (CDI), and the Children's Depression Rating Scale-Revised (CDRS-R).

Based on reviewed literature in adolescent depression, we hypothesized that abnormal functional connectivity in regions of the brain involved in emotional processing will be observed in the MDD group relative to healthy controls. More specifically, we hypothesize that attentional bias towards the negative stimuli presented in the task would

lead to altered connectivity between the subgenual ACC and the amygdala. We also hypothesize that this altered connectivity may be significantly correlated with clinical measures of depression severity.

Methods and Materials

Subject Recruitment and Participation

Adolescents with major depressive disorder (MDD) ages 13-17 years from all races, ethnical backgrounds, and genders in the San Diego, California area were recruited for this study. MDD subjects were recruited from adolescent psychiatric outpatient clinics in San Diego County, including PsyCare, Psychiatric Centers at San Diego (PCSD), Kaiser Clinic locations. A full screening was conducted, and exclusionary criteria for adolescents with MDD include: (1) scoring 80 or lower on the performance section of the Wechsler Abbreviated Scale of Intelligence (WASI; 44); (2) being colorblind or having less than 20/40 correctable vision as determined by the Ishihara color plates (8 plate, 2005 edition) and standard Snellen eye chart, respectively; (3) any contraindication to MRI imaging (e.g. ferrometallic implants, braces, claustrophobia); (4) any history of neurological disorder (e.g., meningitis, migraine, HIV), head trauma with loss of consciousness > 2 min, learning disability, serious medical health problem, or a complicated or premature birth < 33 weeks of gestation (exclusionary due to potentially abnormal neurodevelopment); (5) being pregnant or suspected being pregnant; (6) any evidence of illicit drug use, misuse of prescription drugs, or more than 2 alcohol drinks per week either currently or within the past month as determined by the Customary Drinking and Drug Use Record (Brown et al., 1998); (7) left-handedness, as determined by the Edinburgh handedness scale (Oldfield, 1971); (8) prepubertal status (Tanner stages 1 or 2; Tanner, 1955); (9) inability to fully understand and cooperate with the study procedures; (10) a psychiatric diagnosis other than MDD; and (11) use of medication

with central nervous system effects within the past 2 weeks prior to scanning. In addition, a full K-SADS-PL (Kiddie Schedule for Affective Disorders and Schizophrenia) is conducted in order to establish a diagnosis of the adolescent on affective, psychotic, anxiety, behavioral, or substance abuse disorders at the time of the study, as well as establish mental health history for the study. All participating subjects with MDD presented with a current diagnosis of MDD at the time of scanning.

Healthy controls ages 13-17 years from all races, ethnic backgrounds, and genders were recruited for this study from various regions in San Diego through email, recruitment flyers, and Internet advertisements. Controls (CTLs) were excluded from the study if they were assessed on the Family Interview for Genetics Studies (Maxwell, 1992) to have a family history of mood (Axis I) or psychotic disorders in first- or second-degree relatives, a CDRS-R T-score over 54, or if they met any of first 11 exclusion criteria listed above for the adolescents with MDD. Healthy adolescents were screened with DPS (Diagnostic Predictive Scales) in order to exclude the possibility of mental health disorders.

Clinical Interviews

Clinical scales were used in order to quantify depression and anxiety in adolescents enrolled in the study. In particular, the Children's Depression Rating Scale – Revised (CDRS-R), the Beck's Depression Inventory (BDI), Children's Global Assessment Scale (CGAS), Children's Depression Inventory (CDI), Montgomery-Asberg Depression Rating Scale (MADRS), and Pediatric Anxiety Scale (PARS). Each participant and a parent were interviewed by a bachelor's level research specialist trained

according to local diagnostic reliability standards. A child and adolescent psychiatrist provided best-estimate diagnosis for verification purposes.

Experimental Task

A gender identification task was used in this study to examine the implicit visual processing of differing fear levels in adolescent with MDD compared to normal controls using fMRI. Subjects were trained to perform the task using a laptop prior to performing the task during the fMRI scan. During the scan, participants were shown a total of sixty validated facial expressions showing various degrees of fearful emotion displayed by 10 volunteers (5 male, 5 female, Eckman 1976). A morphing software was used depict the expression of 20% fearfulness (Fear-Weak), 50% fearfulness (Fear-Moderate) and 80% fearfulness (Fear-Strong). There were a total of 20 Fear-Weak expressions, 20 Fear-Moderate expressions, and 20 Fear-Strong expressions (Figure 1a). Each facial expression was presented twice at each level of the three fear intensities. A total of 12 fixation crosses were also presented for a total of 72 trials for a total time of six minutes. All trials were presented in a pseudorandomized order. Each trial was presented for 3000ms, with the inter-trial interval (ITI) randomly varying according to a Poisson distribution (mean ITI = 2000ms), with average trial time being 5000ms. Thus the total scan lasted 360 seconds. During the task, subjects were asked to identify the gender of the face by pressing either leftmost “Male” or rightmost “Female” buttons on a button box. The faces remain on the screen for the entire duration of the trial, and the “Male” and “Female” boxes disappear when the participant makes a decision for that trial (Figure 1b). The reaction time (RT) and accuracy of each trial was collected for each participant

as behavioral data scanning were recorded for each trial. However, several behavioral files were lost during data transfer. Thus, we report behavioral data from only 16 MDD and 13 CTL. After the scan session, subjects were asked to rate each face.

Image Acquisition

Images were acquired on a 3T GE MR-750 scanner (General Electric, Milwaukee, WI) with Twin Speed gradients using the GE 8-channel head coil. Each session consisted of a 3-plane scout scan (10 seconds), a high-resolution anatomical scan, a series of T2*-weighted echoplanar imaging scans to measure the BOLD response, and echoplanar imaging-based field maps to correct for susceptibility-induced geometric distortions. Functional scans covering the entire brain were acquired parallel to the anterior and posterior commissure (T2*-weighted echoplanar imaging; repetition time, 2,000 ms; echo time, 32 ms; flip angle: 90^{o}; 64 × 64 matrix; 30 2.6-mm oblique slices with a 1.4-mm gap; 192 repetitions). During the same experimental session, a T1-weighted image with an inversion time of 450 ms to null the CSF (fast spoiled gradient echo sequence; repetition time: 8.0 ms; echo time: 3.1 ms; flip angle: 12°; field of view: 22 cm; matrix, 256 × 256; 0.98- × 0.98- × 1.0-mm^3 voxels) was collected in the sagittal plane for anatomical reference. During the scan, subjects were asked to remain relaxed but awake, to be as still as possible, and to follow the directions for the task. Visual stimuli was projected onto a screen and viewed through a small, angled mirror mounted above the participant's head.

Behavioral Data

Information about the time between trials and amount of time between onset of stimulus and the first button press was recorded during the scan. Each rating for faces shown was also recorded in order to assess subject response time and response accuracy. Post-scan analysis was performed to assess average trial response time and overall task accuracy for each subject. Only sixteen depressed and thirteen controls retained behavioral data due to technical difficulties during data transfer.

Processing of Imaging Data

All functional and structural image processing and analysis were conducted with the Analysis of Functional NeuroImages (AFNI) software (Cox 1996). Echo-planar imaging (EPI) DICOM acquisition slice data were reconstructed into 3D+time series volumes with slice-time correction. The first two volumes of each echo-planar scan were excluded from analysis due to effects of magnetic saturation. To minimize motion artifact, an AFNI 3D-coregistration algorithm (3dvolreg) of a 9-variable rigid body alignment was used to realign all echoplanar images to a baseline time showing the least amount of head motion. The anatomical data was then aligned to the echoplanar images using AFNI 3dAllineate alignment software *(12 variable alignment).* Both the anatomical and echoplanar images were then converted to stereotaxic Talairach coordinates across all subjects for comparison. The echoplanar images were then despiked by replacing outliers (2.5 standard deviations from the mean) in the time series with the interpolated value of neighboring voxels (3dDespike). Subjects were excluded from the dataset if more than 15% of the time series showed excessive outliers or motion. The data was then

blurred using a Gaussian filter kernel until the measured full-width half-maximum (FWHM) reached 4mm in order to account for individual variations in anatomical landmarks. A multiple regression model (3dDeconvolve) was then used to fit the acquired signal according to the task stimuli regressors. Six motion parameters (roll, pitch, yaw, dx, dy, dz) were used as nuisance regressors to account for motion artifacts. The three trial types (Fear-Strong, Fear-Moderate, and Fear-Weak) were used as regressors-of-interest. Linear trend was modeled in the time series of each voxel in order to account for correlated drift. Finally, the data were converted to percent signal change by dividing the time series of each voxel by the mean global signal and transformed to stereotaxic Talairach coordinates. Thus, all subsequent analyses were conducted at 4x4x4 mm resolution in Talairach space.

Region-of-interest (ROI) Seed Definitions

We defined anatomical bilateral subgenual ACC seeds based on a prior study examining subgenual ACC connectivity in adolescent depression during resting state (Connolly et al., in press). These anatomical seeds were transformed from MNI to Talairach space and resampled to 4x4x4mm.

Group Analysis

A voxel-based two-sample t-test was used to examine group differences between the three conditions (Fear-Weak, Fear-Mod, Fear-Strong). Due to the ambiguity of the "Moderate Fear" condition and the large variation in brain activity between subjects,

subsequent analysis of the “Moderate Fear” condition was dropped. Significant voxels were required to pass a voxelwise statistical threshold of t_{38}=2.024 (p=0.05, uncorrected). Monte Carlo simulations were used to control for multiple comparisons, where the minimum number of contiguous voxels passing the voxelwise threshold that would result in a clusterwise 5% probability of being due to chance in 10,000 simulation iterations of based on an average skullstripped whole brain mask created from all subjects (downsampled to 4x4x4 mm) and the applied FWHM values of the functional data were computed using AFNI Alphasim. This cluster threshold was 11 voxels.

Functional Connectivity Analysis

Functional connectivity analysis was performed on both MDD and CTL adolescents. Anatomical bilateral subgenual ACC seeds used by Connolly et al. were used as our regions of interest. Before conducting the functional connectivity analysis, anatomical data was aligned to the echoplanar images. Furthermore, individual datasets underwent slice-time, motion correction (See “Processing of Imaging Data above”). Time points were censored if they were 2.5 standard deviations away from the mean. Functional connectivity analysis was performed using methods based on the psychophysiological interactions method adapted for AFNI (Chen, 2012) Separate functional connectivity was performed for each seed region. We then deconvolved the resultant time course to derive the underlying neural time course in the seed. This estimated neural time course was then multiplied by the condition regressors (Fear-Strong minus Fear-Weak) to create an interaction time course at the neuronal level. To estimate the interaction time course at the BOLD level, the neuronal interaction time

course was convolved with a modified gamma variate function. Next, a multiple regression model was run separately for each seed to examine connectivity of all voxels for the condition as measured by Pearson's correlations. Since the output from this model is R^2 values, we square-rooted the values and then multiplied each by their respective sign of the estimated beta weights to obtain directionality of association. The correlation coefficients of the interaction time series were then converted to z-scores using Fisher transformation. Group analysis was then performed by running a voxel-based t-test on the z-values of the interaction effect.

Correlational Analysis of Brain Imaging Data

Correlational analysis was performed to assess the association between functional connectivity abnormalities and clinical measures (i.e. CDRS-R, BDI-II) and behavioral measures (accuracy, response time). The analysis was conducted using SPSS (Norusis 1990).

Results

Behavioral Data

The MDD and CTL groups did not differ significantly in response time (t = -1.3074, p=0.203). However, an accuracy difference was observed between the two groups (t = 2.5812, p=0.018).

Sociodemographic and clinical scales

The MDD and NCL groups did not significantly differ in age (t_{36}=0.81, p=0.43), gender (χ^2_1=0, p=1), ethnicity (U=207, p=0.42), Tanner stage (U=195, p=0.15), performance IQ (t_{36}=1.80, p=0.08), income (U=226, p=0.09), mother's education (U=160, p=0.58), or father's education (U=171, p=0.09). MDD adolescents showed significantly greater levels of depression as measured by the BDI-II (t_{36}>3.5, p<0.001), CDRS-R (t_{36}>3.5, p<0.001), and K-SADS diagnosis.

Whole Brain Results

Whole brain analysis of the percent signal change for Fear-Strong vs. Fear-Weak contrast was performed for both the MDD and CTL groups. The MDDs group showed weaker activity in the left precuneus, left anterior cingulate cortex, and right precentral gyrus during the Strong-fear relative to the Weak-fear. (Figure 2, Table 1).

Functional Connectivity Results

The Strong-Fear and Weak-Fear conditions as well as the contrast between these two conditions were examined in a functional connectivity analysis based on the pathophysiological interactions method using seeds from the bilateral subgenual ACC (Table 2).Our findings show that in the Strong-Fear - Weak-Fear contrast, depressed adolescents showed a stronger connection between the right subgenual ACC seed and the left amygdala/striatum. (Table 2), and a weaker connection between the right subgenual ACC seed and the left fusiform gyrus, right precuneus, right medial frontal gyrus, left cingulate, right insula region, and right medial temporal gyrus. Furthermore, depressed adolescents showed a stronger connection between the left subgenual ACC seed and the left insula, left cingulate, right insula, left medial frontal gyrus and the left medial frontal gyrus.

Brain-Behavioral Correlation Analysis

Functional connectivity differences between the right subgenual ACC and the right precuneus were found to inversely correlate with the Beck Depression Inventory (BDI-II; r=-0.623, p=0.004) and the Children's Depression Inventory (CDI; r=-0.562, p=0.012). Right subgenual ACC to right medial frontal gyrus connectivity difference between the two groups was found to positively correlate with the PARS (r=0.532, p=0.034). Connectivity differences between the right subgenual ACC and the right medial temporal gyrus was found to positively correlate with the MADRS (r=0.469, p=0.043). Finally, connectivity differences between the left subgenual ACC and the right insula was found to positively correlate with the PARS (r=0.535, p=0.033).

Discussion

This study had several major findings. First, depressed adolescents, in contrast to age-matched healthy adolescents, showed decreased activation to a fearful face processing task in the anterior cingulate and left precuneus. Second, while brain activation did not differ significantly during emotional face processing, the connective networks between the subgenual ACC and the amygdala, precuneus, insula and fusiform gyrus was significantly disrupted. Overall, the depressed group showed greater connective recruitment in conjunction with the subgenual anterior cingulate during low fear faces, but less recruitment during high fear faces. This suggests adolescent depression may, in part, be characterized by a poorly modulated affective circuit.

Whole brain:

Our whole brain analysis revealed that MDD exhibited decreased activity regions part of the default mode network, specifically the left precuneus and the left anterior cingulate cortex. The default mode network includes regions such as medial prefrontal cortex (MPFC), the anterior cingulate cortex (ACC), the posterior cingulate cortex (PCC), the precuneus, and the dorsomedial thalamus (Greicius et al. 2003). The precuneus is posited as a brain region responsible for passive acquisition of external and internal information during resting state (Raichle et al. 2001). Our finding suggests that MDDs may exhibit a failure to recruit the precuneus during acquisition of increasingly negative emotions, and that a different mechanism is responsible for the abnormal suppression of these regions.

Adult studies have shown an increase in ACC in depressed individuals compared to normal controls (65). However, other studies show that there appears to be a distinction between different regions of the ACC. Specifically, studies have shown hyperactivity of the subgenual ACC (Mayberg 2003) but hypoactivity of the dorsal ACC (Davidson et al. 2002). Visual analysis of our whole brain ACC findings show that they are part of the dorsal ACC (Figure 2b), making this finding consistent with findings of dorsal ACC abnormalities in adult depression studies.

Emotional faces have been shown to activate the precentral gyrus (62). Our findings show that MDDs show a deactivation in the precentral gyrus in response to emotional faces compared to normal controls (Figure 2c). One explanation for this difference in findings is that the study by (Carvajal 2013) used happy, sad, and angry faces, whereas in this task we used faces with differing degrees of fear. This suggests that various types of emotions are processed through different pathways and that abnormalities in the precentral gyrus are associated with abnormal fear processing in adolescents with depression. Additional studies should attempt to elucidate the role of precentral gyrus during processing of different emotions in depression.

Functional Connectivity

PPI analysis show that there is a substantial difference in networks relating to the subgenual ACC between MDD and CTL groups in response to an emotional stimulus, consistent with the overwhelming majority of studies demonstrating the significance of subgenual ACC as a key factor in depression-related network alterations (Connolly et al. 2013, Yang et al. 2009). As predicted in our hypothesis, we observed increased

functional connectivity between the subgenual ACC and the amygdala in response to increasing levels of fear emotion (Table 2, Figure 3A, Figure 4A), which is consistent with prior studies that implemented an independent components analysis and reported greater coactivation of the sgACC, thalamus, precuneus, and orbitofrontal cortex in MDD relative to CTL subjects (Greicius et al. 2007). The subgenual ACC has shown to play a role in the regulation of amygdala activity in adults (Yang et al. 2009). Here we demonstrate that this regulation is abnormal in adolescents with depression during the processing of negative stimuli, and that perhaps in adolescents with depression, the subgenual ACC is abnormally recruiting amygdala activity, leading to altered emotional processing and increased ruminative thoughts in response to negative emotional stimulus.

Our findings extend prior work and support the notion that function within an emotion processing circuit that includes the sgACC is aberrant in MDD both at rest and during emotion processing (Connolly et al. 2013). Converging results suggest that the sgACC is hyperactive in MDDs at rest and in healthy volunteers during induced sadness (Mayberg et al. 1999). Previous studies in both adult and adolescent depression literature demonstrate increased activity in the amygdala during emotional processing (Yang et al. 2010; Perlman et al. 2012; Greening et al. 2013; Drevets et al. 2008). Our findings suggest that the amygdala hyperactivity may be modulated by increased connectivity between the amygdala and the subgenual ACC during processing of negative emotional stimuli. It is possible that this increased connectivity between the subgenual ACC and the amygdala could play a role in depressed individuals' bias towards negative stimuli. Furthermore, abnormal connectivity in MDDs between the subgenual ACC and the

amygdala may suggest a ruminative coping mechanism in adolescents with depression.

Our findings also demonstrate negative connectivity between the right subgenual ACC and the right precuneus in MDDs compared to normal controls (Table 2, Figure 3B, Figure 4A) in the Strong-Fear minus Weak-Fear condition. The precuneus has shown to play a significant role in self-related awareness and stimulus processing and is part of the default mode network. A negative connectivity between the precuneus and the subgenual ACC suggests that MDDs show alterations in the default mode network that may affect internal awareness and processing of negatively valenced stimulus. Furthermore, connectivity to the right precuneus negatively correlated with depression scores, suggesting that adolescents with the most severe cases of depression exhibit almost no connectivity between the right precuneus and the subgenual ACC, whereas those with the least severe depression show high negative connectivity between the two regions. Interestingly, healthy adolescents show a strong positive connectivity between the right precuneus and the subgenual ACC. These results put together suggest that depressed adolescents show decreased connectivity between the subgenual ACC and the right precuneus, and that those who are less depressed develop a coping mechanism in response to a negative stimulus through an unknown pathway that decreases depressive symptoms and form an inverse connection between the subgenual ACC and the right precuneus, whereas those who did not develop this coping mechanism (and subsequently show little or no connectivity between the two regions) were more severely depressed. Perhaps the interactions of these two regions can be further explored in future studies as a possible predictor of depression severity and a measure of treatment prognosis.

Our findings demonstrate negative functional connectivity between the bilateral subgenual ACC and its ipsolateral insula region (Table 2, Figure 3C, 3D, 3E). The insula has been understood as being involved in the switching mechanism between the default and saliency network (Menon 2011). Adult depression studies have shown that MDDs exhibit an increase in functional connectivity between these two regions during sad mood induction. (Anand et al. 2005) The connectivity between the subgenual ACC and the insula has been implicated to provide the "input" and "output" components for a system based on self-awareness. A recent study has demonstrated co-activation of the subgenual ACC and the insula in response to pain (Medford et al. 2010), which is considered an interoceptive feeling (Craig 2009). Cullen et al. (2009) proposed that decreased connectivity between the subgenual ACC and the insula may be related to adult depressive symptoms such as somatic complaints and negative bias in interpreting interpersonal feedback. The study by Connolly et al. (2013) showed that adolescents with MDD exhibited increased connectivity between the subgenual ACC and the bilateral insulae during resting state. However, this is the first study that showed negative functional connectivity between the two nodes in response to a negative emotion (Table 4A, 4B). Furthermore, we found that this pathway correlated positively with the PARS, suggesting its possible contribution in producing negative bias processing in anxiety disorders. This finding suggests that the subgenual ACC – insula dysfunction in adolescent depression contributes to abnormalities in processing and perception of a negative emotional visual input.

A previous study by Stella & Chan (2009) found a correlation between increased fusiform gyrus activation in those with high-risk for developing depression and

increasingly fearful facial emotional stimulus, suggesting that individuals high-risk for depression may perceive negative expressions as more self-threatening compared to normal individuals. Our whole-brain analysis did not reveal differences in fusiform gyrus between groups. This may be due to the fact that Stella & Chan (2009) did not use a clinically depressed sample, but rather a sample of individuals with high-risk for depression. This may also be attributed to a developmental difference between adolescent and adult populations. However, our connectivity findings show that the connection between subgenual ACC and fusiform gyrus was diminished in depressed adolescents compared to normal healthy controls in the viewing of increasingly fearful faces (Table 2), suggesting that the subgenual ACC may provide an inhibitory effect on the fusiform gyrus, such that a decrease in the subgenual ACC to fusiform gyrus connection may produce sub-threshold levels of increase fusiform gyrus activity. Further studies are needed to examine this relationship in greater detail.

Dysfunctions in the dorsolateral prefrontal cortex are highly implicated in the study of both adult and adolescent depression (Mayberg et al 1999). We did not find connectivity between subgenual ACC and dlPFC in response to negatively valenced stimuli. Frontocingulate dysfunctions was found in studies of adult depression (Mayberg et al. 1999) but were not replicated in this study. This may suggest a developmental difference between adult and adolescent depression and further research is required to assess the significance of this difference and its clinical manifestations.

Further analysis into our functional connectivity results reveals an interesting trend: there is an overall strengthening in connectivity between the bilateral subgenual ACC and limbic regions during the Weak-Fear condition only (Figure 4E, 4F). However,

connectivity between the bilateral subgenual ACC and limbic regions is decreased during the Strong-Fear condition (Figure 4C, 4D). These results suggest that the MDD may be overreactive to faces close to neutral/low fear expressions, and abnormally reactive to strongly fearful faces. Further studies should probe this network for other emotive faces and investigate if these deficits are ameliorated by treatment.

Conclusion

Overall, our findings show that the subgenual ACC plays a key role in modulating perceptual and cognitive processes, and that, like adult depression, adolescent depression involves a disruption of networks involving the subgenual ACC. We confirmed our hypothesis that emotional processing to fear will evoke communication between the subgenual ACC and the amygdala in adolescents with depression. However, we were not able to correlate this finding to clinical scales. Finally, we provided a mechanism by which altered subgenual ACC to precuneus connectivity may lead to and be used as a predictor of depression severity and a measure of treatment prognosis. Overall, our results build towards a model of a disrupted network in clinically depressed adolescence such that low fear/neutral faces are deemed threatening and a wider network is recruited. However, when strong fear faces are processed the MDD do not effectively recruit these regions in contrast to healthy controls. This suggests that MDD may be over reactive to mildly emotive faces but may not effectively modulate arousal for strongly emotive faces. Further studies should probe this network for other emotive faces and investigate if these deficits are ameliorated by treatment.

Figures and Tables

Table 1. Whole brain analysis of Strong-Fear minus Weak-Fear contrast between MDDs and CTLs. The direction refers to a numerical comparison between the percent signal change between the two groups. The cluster size refers to the number of voxels of significant difference between the two groups. Each voxel refers to a block of 4x4x4mm cube of brain tissue. Location is given by the X,Y, and Z coordinates in standardized (Talairach) space.

Whole-Brain Analysis
Strong-Fear –Weak-Fear contrast

Brain Region	**Direction**	**Cluster Size (number of voxels)**	**X**	**Y**	**Z**
L Precuneus	CTL > MDD	22	-7	64	40
L ACC	CTL > MDD	11	-19	-33	21
R Precentral Gyrus	CTL > MDD	11	49	11	30

Table 2. Functional connectivity analysis of Strong-Fear minus Weak-Fear between MDDs and CTLs using the bilateral subgenual ACC as *a priori* seeds. The direction refers to a numerical comparison between the percent signal change between the two groups. The cluster size refers to the number of voxels of significant difference between the two groups. Each voxel refers to a block of 4x4x4mm cube of brain tissue. Location is given by the X,Y, and Z coordinates in standardized (Talairach) space. Significant regions under discussion are highlighted in yellow.

Functional Connectivity Analysis
Strong-Fear – Weak-Fear contrast

Seed	**Direction**	**Cluster**	**Location (x,y,z)**	**Cluster Size (number of voxels)**
R-sgACC	MDD > CTL	L Amygdala/Striatum	-16,10,-8	11
R-sgACC	CTL > MDD	L FFA	-31,74,-18	32
R-sgACC	CTL > MDD	R Precuneus	23,64,49	30
R-sgACC	CTL > MDD	R MFG	21,-10,54	26
R-sgACC	CTL > MDD	L Cingulate	-3,45,36	17
R-sgACC	CTL > MDD	R STG/Insula	53,45,21	12
R-sgACC	CTL > MDD	R MTG	57,16,-9	11
L-sgACC	CTL > MDD	L Putamen/Insula	-28,-14,6	18
L-sgACC	CTL > MDD	L Cingulate	-4,-20,26	14
L-sgACC	CTL > MDD	R Insula/Putamen	31,-16,2	13
L-sgACC	CTL > MDD	L MFG/Cingulate	-5,-13,41	13
L-sgACC	CTL > MDD	L MTG	-49,46,11	12

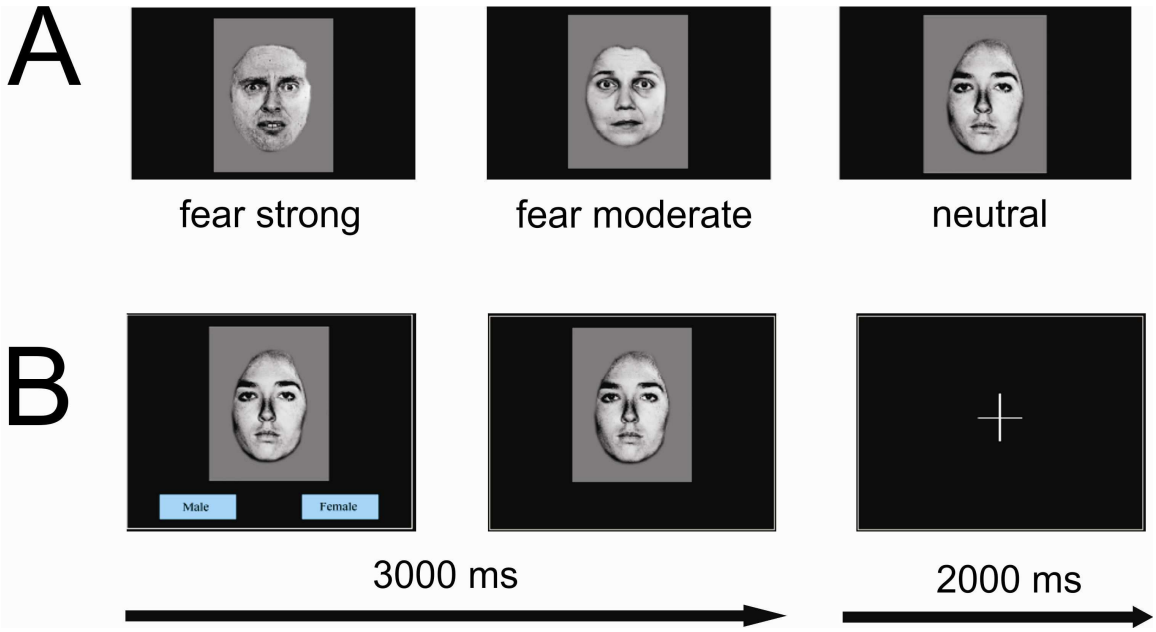

Figure 1. Task Description. A. A visualization of faces exhibiting different degrees of fear (Fear-Strong, Fear-Moderate, Fear-Weak/Neutral). B. Gender discrimination task lasts 3000ms, with an average of 2000ms of inter-trial period where the subject is shown a fixation cross.

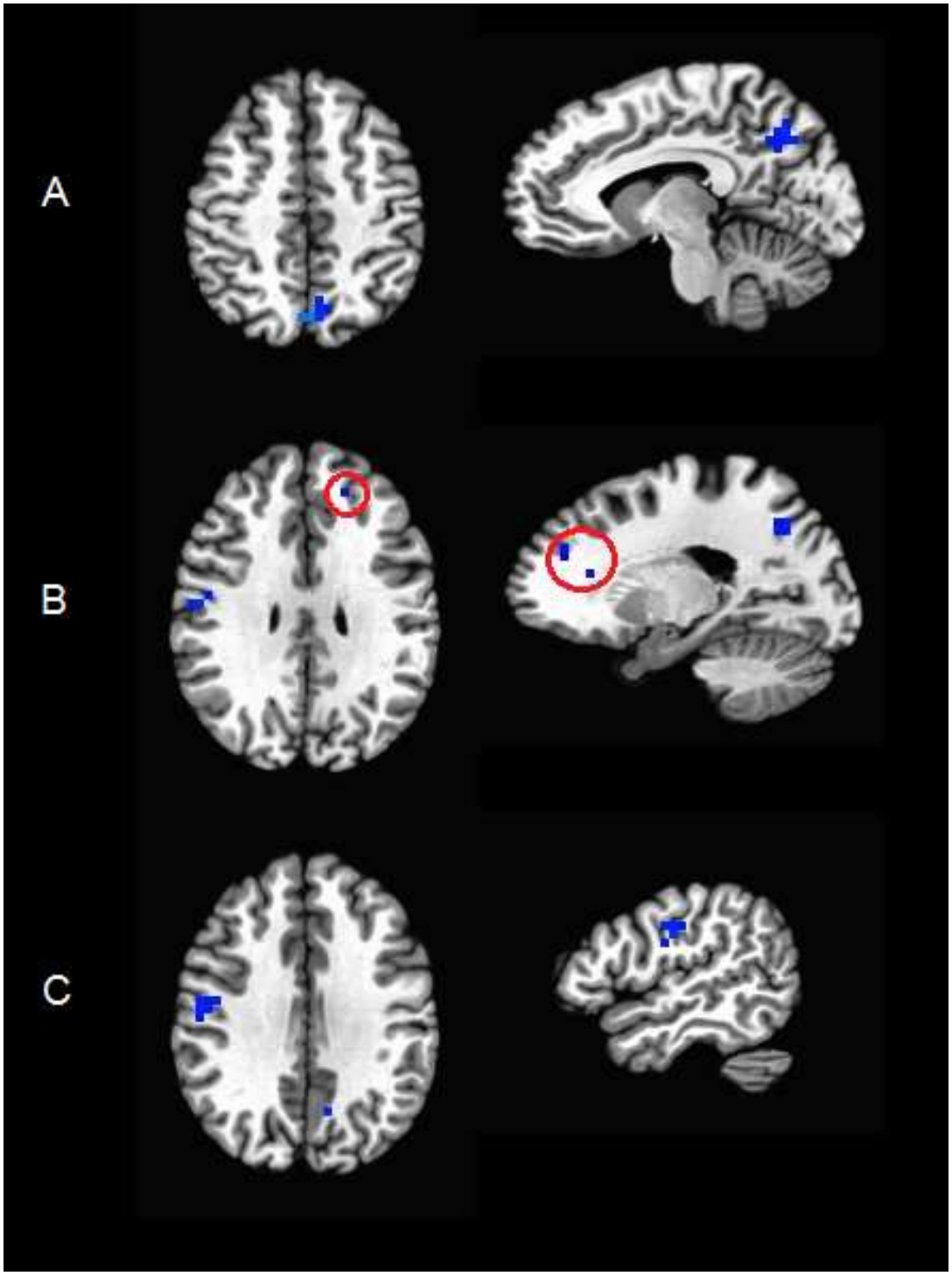

Figure 2. A visual of the whole brain BOLD results. Images are in standardized (Talairach) space and are generated using AFNI. Regions showing decreased activity in MDDs compared to normal controls are displayed in blue. A. Decreased activity in the left precuneus. B. Decreased activity in the left ACC. C. Decreased activity in the right precentral gyrus.

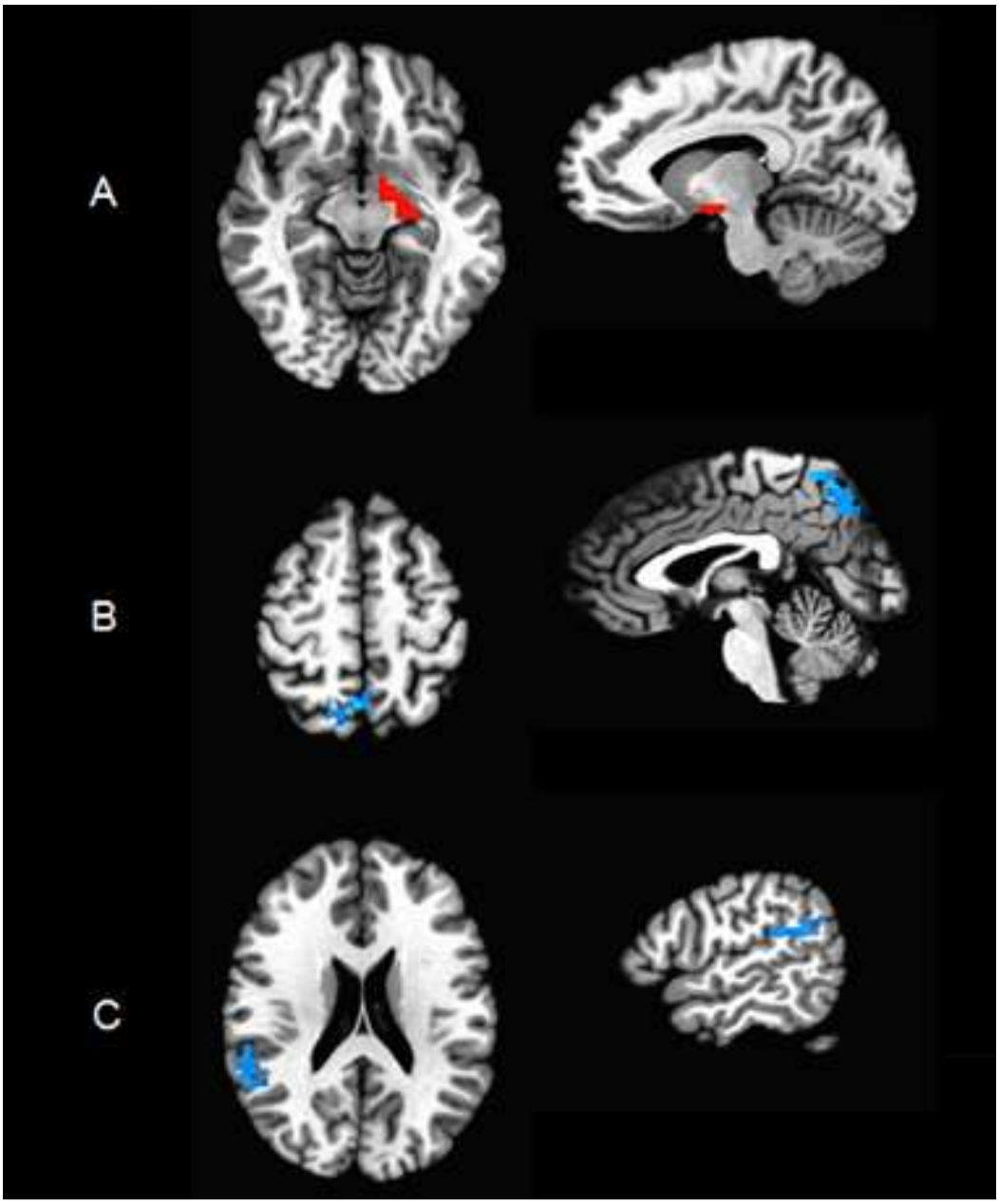

Figure 3. A visual of the functional connectivity results using PPI. Images are in standardized (Talairach) space and are generated using AFNI. Regions showing increased connectivity in MDDs compared to normal controls are displayed in red, and regions showing decreased connectivity in MDDs are displayed in blue. A. Increased connectivity between the right subgenual ACC and Left Amygdala. B. Decreased connectivity between the right subgenual ACC and the right precuneus. C.Decreased connectivity between the right subgenual ACC and the right insula. D. Decreased connectivity between the left subgenual ACC and the left insula/putamen. E Decreased connectivity between the left subgenual ACC and the right insula/putamen

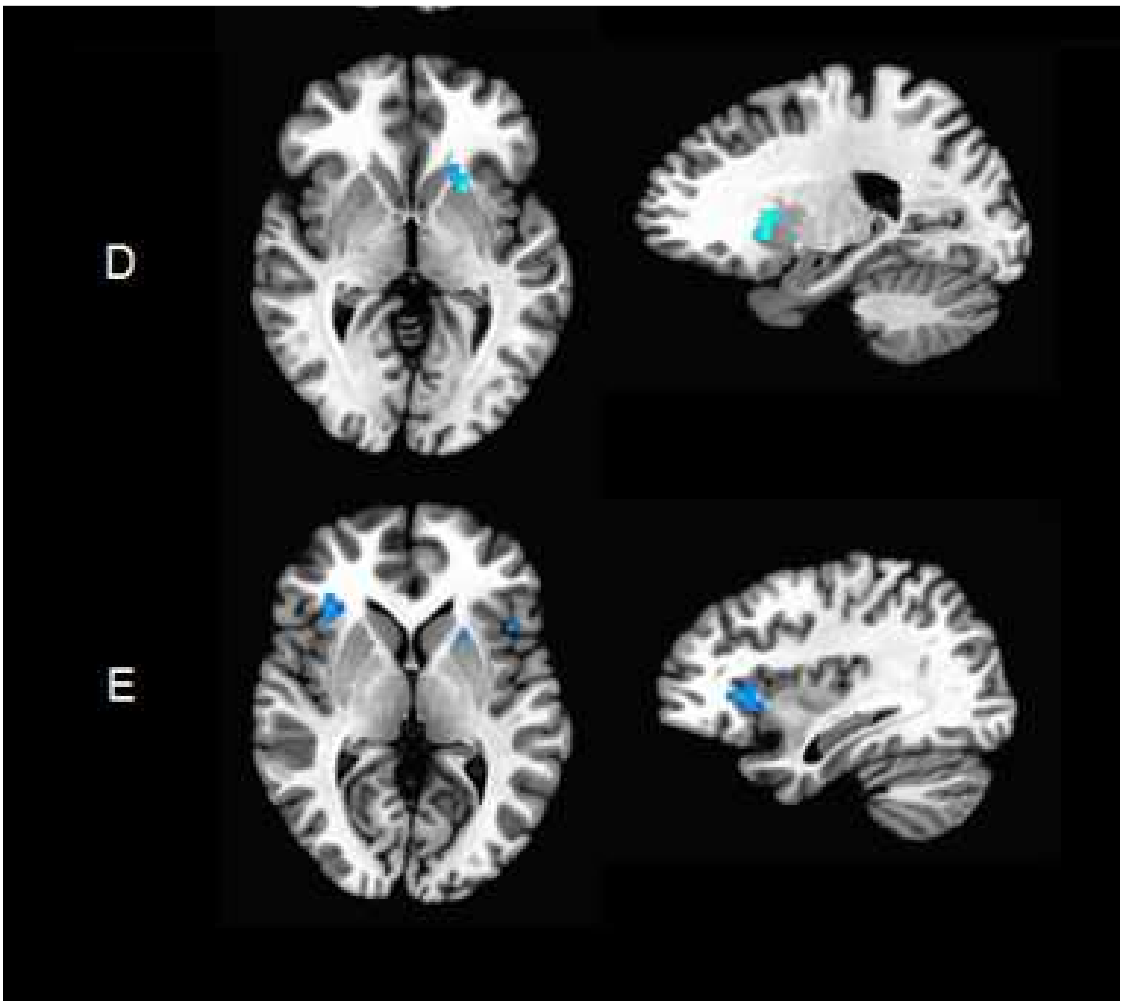

Figure 3. Continued.

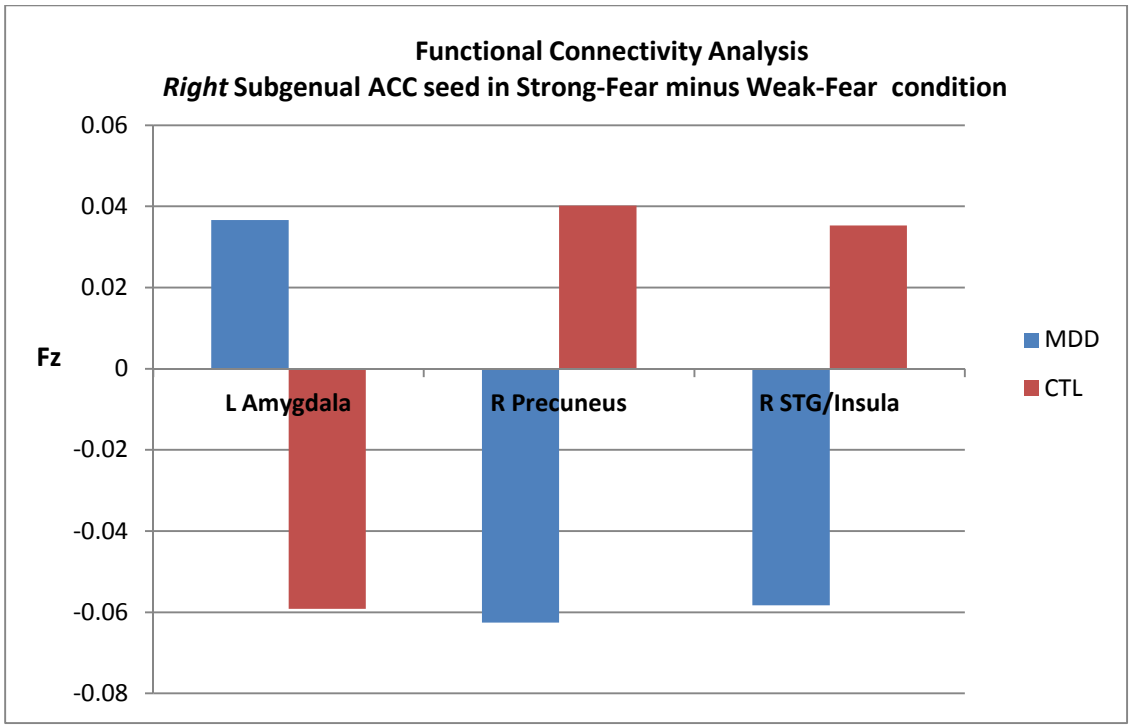

Figure 4A. A bar graph depicting functional connectivity results using the PPI method tailored for fMRI using the right subgenual ACC as the seed region during Strong-Fear minus Weak-Fear contrast. The y-axis displays the Fisher Z-scores.

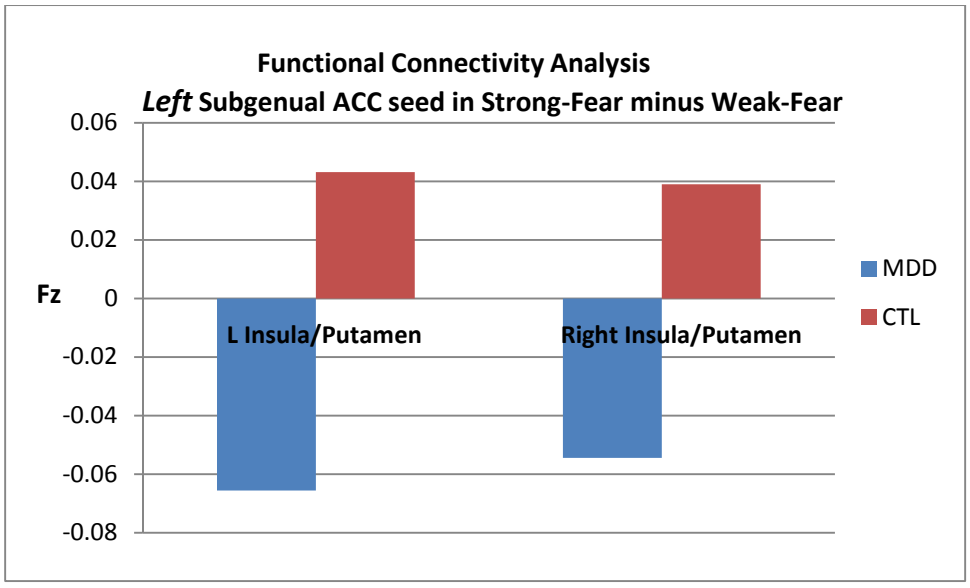

Figure 4B. A bar graph depicting functional connectivity results using the PPI method tailored for fMRI using left subgenual ACC as the seed region during Strong-Fear minus Weak-Fear contrast. The y-axis displays the Fisher Z-scores.

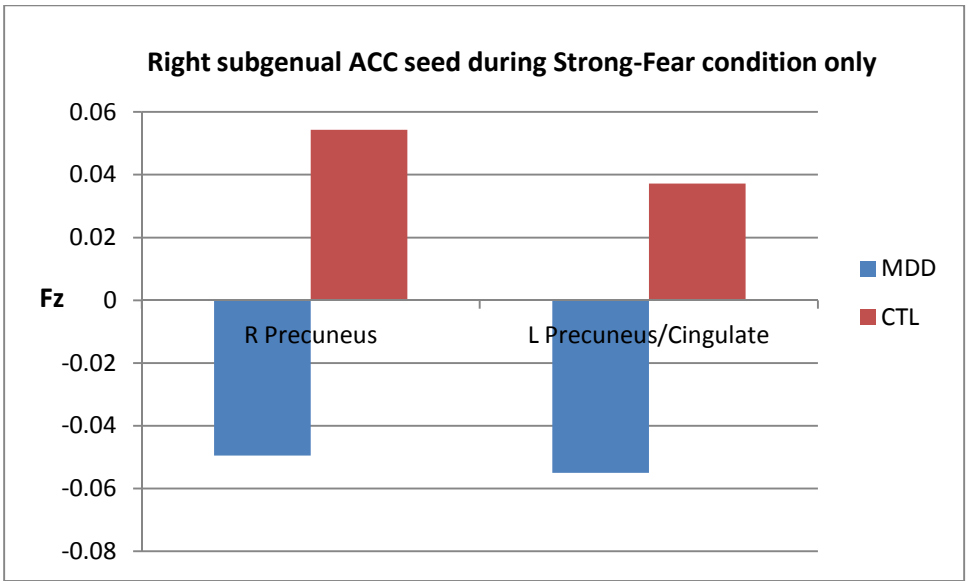

Figure 4C. A bar graph depicting functional connectivity results using the PPI method tailored for fMRI using *right* subgenual ACC as the seed region during *Strong-Fear condition only*. The y-axis displays the Fisher Z-scores.

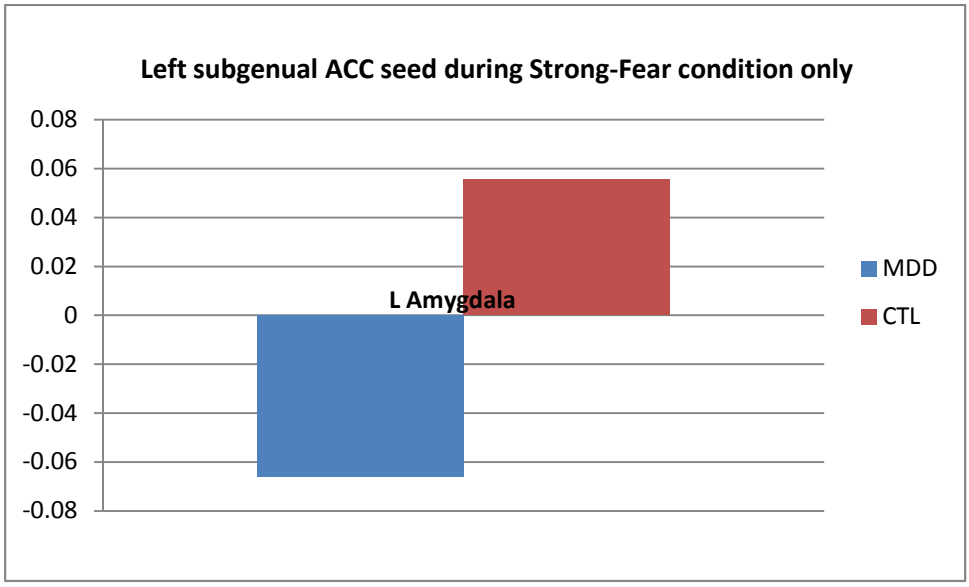

Figure 4D. A bar graph depicting functional connectivity results using the PPI method tailored for fMRI using *left* subgenual ACC as the seed region during *Strong-Fear condition only*. The y-axis displays the Fisher Z-scores.

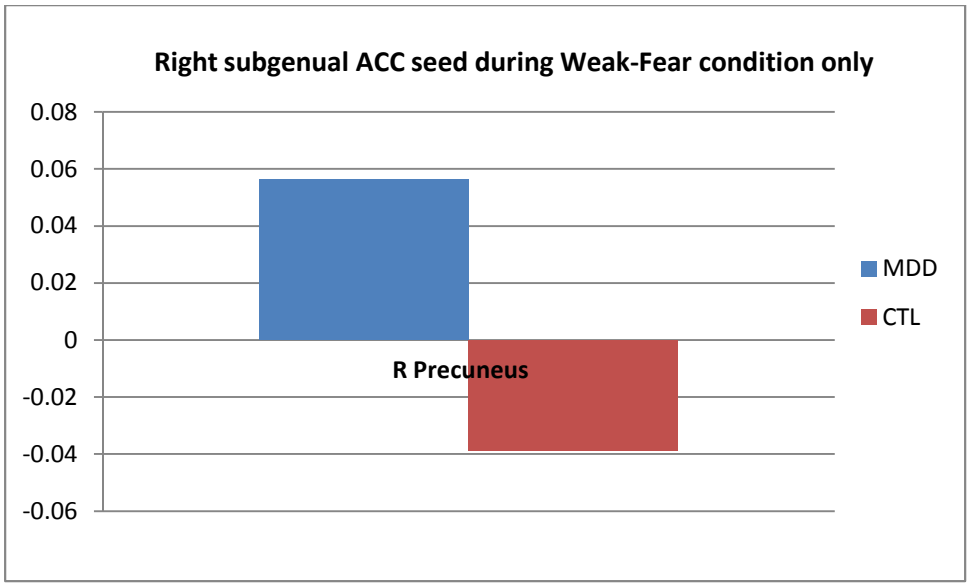

Figure 4E. A bar graph depicting functional connectivity results using the PPI method tailored for fMRI using *right* subgenual ACC as the seed region during *Weak-Fear condition only*. The y-axis displays the Fisher Z-scores.

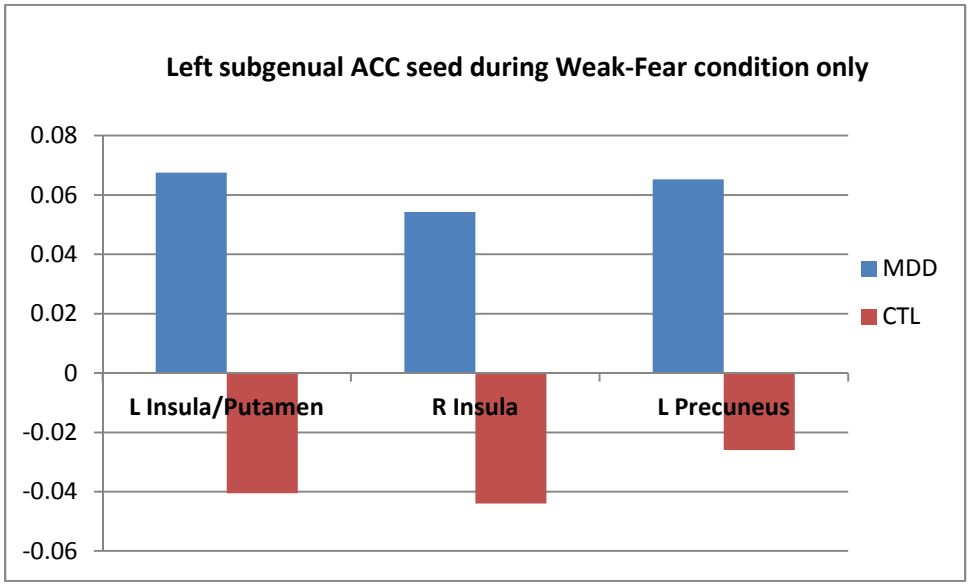

Figure 4F. A bar graph depicting functional connectivity results using the PPI method tailored for fMRI using *left* subgenual ACC as the seed region during *Weak-Fear condition only*. The y-axis displays the Fisher Z-scores.

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