

Introduction

Overview

Early life stress (ELS) during childhood, characterized by serious or repeated abuse, neglect, trauma, and/or exposure to family dysfunction, has been linked to psychopathology in adulthood, including Major Depressive Disorder (MDD), Post-Traumatic Stress Disorder (PTSD), and Obsessive-Compulsive Disorder (OCD) (Heim & Binder, 2012). According to the National Comorbidity Survey, at least 32% of psychiatric disorders appear to be connected to ELS, and 44% of childhood-onset disorders are related (Green et al., 2010). ELS is also associated with poor treatment outcome, even when controlling for severity of depression (Nanni, Uher, & Danese, 2012; Spatz et al., 2007).

Conservative estimates by the Center for Disease Control and Prevention (CDC) indicate that more than 15% of U.S. children between 2 and 17 years have experienced serious maltreatment (Middlebrooks & Audage, 2008). Moreover, the Adverse Childhood Experience (ACE) Study found that two-thirds of its 17,000 adult participants reported at least one experience of childhood abuse or neglect (CDC, 2006). Therefore, ELS is a very serious problem that appears to have a *pathogenic* impact on mental health conditions that continues across the lifespan for a significant proportion of the population.

There is significant evidence that ELS overwhelms a child's ability to cope adaptively, producing long-lasting problems (Pechtel & Pizzagelli, 2011). Children with ELS are more likely to have "a low threshold for stress, thereby becoming overly reactive to adverse experiences throughout life" (Middlebrooks & Audage, 2008).

The dysfunctions in self-reflection, cognition, affect, behavior, and information processing associated with ELS suggest that they may be mediated by dysregulated brain development. In individuals with ELS, neural networks related to stress adaptation remain more dissociated, typical of an early stage of maturation (Daniels, McKinnon, & Lanius, 2011; Pechtel & Pizzagelli, 2011). This altered course of neural development may leave individuals more vulnerable to further stress and to adult psychopathology.

Research is clearly needed to determine the specific relationship between ELS and alterations in neural circuitry related to stress and emotional processing, which may be responsible for treatment-resistant psychopathology and an impaired ability to handle stress often seen well beyond childhood into adulthood. This study is designed to investigate the relationship between ELS and psychopathology in adulthood, specifically MDD. This study assesses the degree and type of dysregulation in functional neural networks, critical to adaptive emotional, cognitive, and behavioral functioning, which appear to underlie MDD. In particular, this study is focused on the dysfunctional impact of ELS on these neural networks as well as the mediating role of neural network dysregulation on the development of MDD in adulthood.

Functional Neural Networks

Recent functional neuroimaging research has focused on the discovery of large-scale canonical neural networks comprised of disparate regions across the brain, which are functionally connected and tend to selectively activate in organized patterns (Chen et al., 2013). These functional neural networks appear to be critically

related to adaptive emotional, cognitive, and behavior functioning. Moreover, these neural networks organize developmentally during the critical childhood years when ELS appears to have the most impact on functioning across the lifespan. Therefore, a brief overview of these neural networks would be helpful in order to understand the potential dysfunctional impact of early life stress on the developing brain.

In affectively healthy adults who are not engaged in a task, the default mode network (DMN) is activated, reflecting adaptive self-referential activities including evaluating the current situation, remembering the past, and planning the future (Mason et al., 2007). The DMN includes the medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC), precuneus, anterior cingulate cortex (ACC) and lateral parietal cortices (Greicius, Krasnow, Reiss, & Menon, 2003). During the first twelve years of life, however, the components of the DMN are “only sparsely connected” (Daniels et al., 2011). These regions of the brain appear to connect and organize into a self-referential network in a stepwise sequence during childhood, providing a developing sense of self and ability to reflect and process information and emotions. Therefore, the DMN may be highly vulnerable to dysregulated connectivity when a child experiences ELS during this critical stage of brain maturation, thereby interfering with the development of adaptive self-awareness and reflection.

During goal-directed and working memory tasks that require active attention, decision-making, and conflict resolution, the DMN tends to deactivate in healthy adults, at which time the executive control network (ECN) activates and takes control (Corbetta & Shulman, 2002). The ECN includes the dorsolateral prefrontal cortex

(dlPFC), medial frontal gyrus, and the anterior cingulate cortex (ACC). When adaptively functioning, the ECN provides “top-down” higher cortical cognitive modulation of other networks, including the limbic system, in order to maximize effective functioning and regulation of emotions, attention, and behaviors. As with the DMN, the ECN appears to develop systematically throughout childhood, providing an increasing ability to adaptively modulate emotions and focus attention on tasks and problems into adulthood. As a result, the development of an integrated well-connected ECN, like the DMN, may be limited due to the effects of stress during childhood (Arnsten, 2009). An ECN that is abnormally connected with other networks, such as the DMN, due to ELS, could impair an individual’s ability to shift adaptively in order to cognitively regulate emotions as well as effectively focus attention on tasks and problems, without emotional intrusions and other distractions.

The salience network (SN) appears to function by identifying stimuli relevant to maintaining health and stability in a changing environment (Seeley et al., 2007). In this way, the SN may have a higher-order monitoring role, shifting activation of the other neural networks, as needed, depending on an appraisal of current circumstances and priorities. The SN includes the dorsal anterior cingulate cortex (dACC), anterior insula (AI), superior temporal pole, and orbital frontoinsula (FI). Closely related to the SN is the dorsal attention network (DAN). The DAN has been shown to activate as a function of attention to the behavioral significance of stimuli (Vossel, Geng, & Fink, 2014). Core regions of the DAN are in the intraparietal sulcus and frontal eye fields. Traumatic stress during childhood may sensitize the SN and DAN to be chronically hyper-vigilant to perceived threat even in response to minor

stress decades after the original abuse and neglect (Wingen, Geuze, Vermetten, & Fernandez, 2011). Moreover, if ELS dysregulates the development of adaptive connections between the SN and DAN with the DMN and ECN, this reduced connectivity could produce dysfunctional emotional regulation. Therefore, these attentional networks could be highly vulnerable to dysregulation due to ELS and may be responsible for many of the symptoms exhibited by adults who have experienced ELS. Functional neural network dysregulation, including impaired “top-down regulation.” produced by ELS could lead to maladaptive emotional reactivity, including inappropriate fear and anger, anxiety and ruminations, and an inability to concentrate on functional tasks and activities.

ELS and Neural Network Dysregulation Studies

While earlier studies have looked at the relationship between ELS and specific brain region volume and activation as well as task-directed functional magnetic resonance imaging (fMRI) paradigms, this literature review will focus on the more recent studies that have been conducted to investigate the relationship between ELS and functional neural networks using a resting-state fMRI connectivity (rs-fcMRI) paradigm in both patient (e.g., Bluhm et al., 2009; Wang, Paul, Stanton, Greeson, & Smoski, 2013) and healthy populations (e.g., Philip et al., 2013a, 2013b, 2014). The rs-fcMRI paradigm is used to evaluate the functional neural network interactions that occur when the participant is not engaged in an explicit task. This paradigm appears to have greater significance than regional activation and volume studies given the importance of large-scale neural networks to adaptive emotional, cognitive, and

behavioral functioning and, therefore, on the implications for the development of psychopathology (Wang, Hermens, Hickie, & Lagopoulos et al., 2012).

Prior rs-fcMRI ELS studies have produced a variety of results, depending on the type of analysis (e.g., region-of interest versus independent component); however, all of them point to the long-term impact of ELS on neural network *dysregulation* into adulthood. There is growing evidence from studies using an rs-fcMRI paradigm that ELS is associated with more dissociated and disconnected functional neural networks, similar to the loosely organized neural networks seen in children (Daniels, et al., 2011). For instance, Wang et al. (2013) found that MDD was associated with a *specific reduction* in functional connectivity between the ventral medial pre-frontal cortex (vmPFC) and the ventral anterior cingulate cortex (vACC), suggesting reduced “top-down” cortical regulation of emotions. However, MDD patients with ELS (specifically childhood neglect) “displayed more *widespread reduction* of functional connectivity strength” across a wide variety of regions of the brain using an rs-fcMRI paradigm and independent component analysis (ICA) (Wang et al., 2013, p. 1). While specific functional neural network dysregulation patterns were not examined in this study, their findings support the idea that ELS impairs the development of normal neural connectivity among various regions of the brain, even more than MDD without ELS.

Consistent with Wang et al.’s findings, Bluhm et al. (2009) reported that female patients with PTSD due to ELS had reduced DMN connectivity as compared to healthy controls, using an rs-fcMRI paradigm and region-of interest (ROI) analysis. In the ROI paradigm, key regions within relevant networks are used as “seed” regions

within the analysis, measuring the strength of association between seed regions and other relevant regions of the brain. Specifically, they found that ELS patients had less connectivity between the PCC and other DMN regions as well with the amygdala and hippocampus. Similarly, Herring et al. (2013) found that adolescents with ELS had lower hippocampus-subgenual cingulate rs-fcMRI in both males and females in addition to lower amygdala-subgenual cingulate connectivity in females only. Burghy et al. (2012) also found that female adolescents with ELS had reduced functional connectivity during the resting-state between the vmPFC and amygdala.

Together, these studies support the hypothesis that ELS may be associated with the development of *reduced* connectivity and communication within the DMN and between the DMN and other regions of the brain. Specifically, the neural connectivity dissociation between higher cortical areas, such as the PFC and PCC, and emotional centers, such as the vACC and amygdala, is suggestive of limited “top down” cognitive regulation of emotions and attention as well as an impaired ability to reflect and solve problems effectively due to intrusive emotional reactivity. They also provide evidence that this neural network dysregulation may create a neuropathologic vulnerability to the development of serious psychopathology including MDD and PTSD. Furthermore, if the neural network dysregulation produced by ELS is more extensive than MDD or PTSD without ELS, these findings may account for the poor treatment outcomes observed in prior clinical studies (Nanni et al. 2012).

Philip et al. (2013a, 2013b, 2014) have done an interesting set of exploratory studies using an rs-fcMRI paradigm with adults who experienced ELS, but with no

history of psychiatric disorder. Using ROI analyses, they found that ELS participants had decreased DMN intra-connectivity between the PCC seed and the mPFC and inferior temporal cortex (Philip et al., 2013a). Using a dlPFC seed, they found greater dissociation between the ECN and DMN in individuals with ELS but no psychiatric history (Philip et al., 2014). These studies provide evidence that ELS may produce reduced functional neural network connectivity, both within the DMN and between the DMN and other networks, even in those without overt psychopathology. In this way, ELS may make children's brains more vulnerable to developing adult psychopathology, which may become manifested in response to specific triggers, such as stressful circumstances. Obviously, further research is needed to explore why certain individuals may be more resilient, thereby avoiding manifest psychopathology, despite ELS and related functional neural network dysregulation.

Overall, prior neural imaging studies suggest that ELS is associated with reduced adaptive neural connectivity across a variety of regions of the brain, particularly between the DMN and the ECN and other neural areas. This dissociated neural connectivity may be responsible, at least in part, for the impaired ability for individuals with ELS to handle life's stresses, due to reduced "top-down" cognitive regulation of emotions and attention as well as adaptive self-reflective and problem-solving processing, resulting in maladaptive emotional reactivity, rumination, anxiety, and depression (Daniels et al., 2010, 2011). This appears to be particularly evident with childhood neglect (Wang et al., 2013), females with ELS (Herringa et al., 2013; Burghy et al., 2012; Bluhm et al., 2009), and for individuals suffering from PTSD related to ELS (Bluhm et al., 2008; Daniels et al., 2010, 2011).

MDD and Neural Network Dysregulation Studies

There have been a growing number of studies investigating the neural network dysregulation associated with MDD. In contrast to the functional neural network *hypo*-connectivity found in ELS studies, this research has revealed specific patterns of *both hyper*-connectivity and *hypo*-connectivity in MDD patients (Crowther, 2015). A recent meta-analysis of 27 rs-fcMRI studies by Kaiser et al. (2015) revealed that MDD was associated with *hypo*-connectivity *within* the frontoparietal network (FN), the “hub” of cognitive control, as well as *between* the FN, and the parietal regions of the DAN. This hypo-connectivity is suggestive of impaired “top-down” cognitive regulation of emotions and attention and may be responsible for the mood instability and distractibility seen in MDD. However, Kaiser et al. also found a pattern of *hyper*-connectivity *within* the DMN as well as *between* the DMN and both the ECN and the FN (Sheline, Price, Yan, & Mintun, 2010; Greicius et al., 2007, Burghy et al., 2012). Of particular interest, Sheline et al. (2010) discovered that a specific region, the dorsal medial prefrontal cortex (dmPFC), which they termed the “dorsal nexus,” had *increased* rs-fcMRI with large regions within the DMN and ECN. The dmPFC is a central information-processing region that normally enables adaptive communication between the functional neural networks. Sheline et al. (2010) suggest that MDD may involve “hot wiring” hyper-connectivity among these networks through the “dorsal nexus,” that may be responsible for the pervasive depressive symptoms that appear to involve multiple neural networks. They argue that this dysfunctional “dorsal nexus” network may help explain the decreased ability to focus on cognitive tasks, rumination, excessive self-focus, increased vigilance, and

emotional, visceral, and autonomic dysregulation seen in MDD and related disorders (Sheline et al 2010). These patterns of functional neural network *hypo-* and *hyper-*connectivity in MDD may reflect the depressive biases toward internal thoughts instead of engaging adaptively with the world as well as impaired mood regulation (Kaiser et al., 2015).

To-date there has been very limited research demonstrating the impact of ELS on the development of this *hyper-*connectivity either within or between functional neural networks. While there is prior evidence that ELS is associated with extensive *hypo-*connectivity among various regions of the brain, suggestive of limited communication and coordination within and among neural networks, it is likely that ELS may also lead to *hyper-*connectivity, as found in MDD, potentially producing the inflexible and rigid maladaptive coping patterns observed in victims of ELS. One reason for these limited findings is that prior studies have not *systematically* examined the impact of ELS on the inter-connectivity *within* and *between* the major functional neural networks, including the DAN and SN as well as the DMN and ECN. They also have not simultaneously controlled for the extent of both ELS and psychopathology and their distinct contributions to neural network dysregulation. Therefore, more controlled studies are needed to clarify the potential mediating role of functional neural network dysregulation on the impact of childhood trauma and abuse in the development of psychopathology in adulthood. The present study was designed to address these issues.

Purpose and Design of Study

This study is based upon the theory that childhood abuse and neglect impairs the normal adaptive development of functional neural networks, especially the DMN, ECN, SN, and DAN, which appears to require a long-term safe environment for normal maturation. As a result of ELS, these neural networks are expected to be relatively disconnected during adulthood, more similar to the limited neural communication found in children. At the same time, a child forced to handle abuse and neglect prior to the development of fully functional neural networks is also expected to develop specific hyper-connectivity patterns as an immature coping response. This network dysregulation may rigidly connect specific brain regions, leaving the individual with an immature and inflexible neural network to handle life's stresses. If so, these neural connectivity dysregulations could explain the neuropathic development of symptoms such as maladaptive ruminations, emotional reactivity, stress response, cognitive functioning, and self-reference seen in MDD due to ELS (Daniels et al., 2010, 2011).

The first goal of the study was to determine whether ELS is predictive of the development of MDD in adulthood. It was hypothesized that adults with high levels of ELS are more likely to suffer from MDD than those with less ELS. The second goal was to determine the specific functional neural network dysregulation patterns associated with MDD in adults. It was hypothesized that patients with MDD are more likely to have both *hypo-* as well as *hyper-connectivity within* and *between* functional neural networks, as compared with relatively healthy adults. The third goal of this study was to assess the specific impact of ELS on the impaired development of functional neural network connectivity including both *hypo-* and *hyper-connectivity* dysregulation, independent of MDD. Finally, the study was designed to examine the

interaction between ELS and MDD in determining specific functional neural network dysregulation patterns, in order to explore the mediating role of neural dysregulation in the impact of childhood trauma on the development of psychopathology.

It was hypothesized that ELS is associated with more significant *hypo-* and *hyper-*connectivity both *within* and *between* functional neural networks than participants without ELS, including patients suffering from MDD but with low levels of ELS. In particular, it was predicted that ELS may be associated with dorsal nexus “hot wiring,” as described by Sheline et al. 2010. If findings are consistent with these hypotheses, they may support the theory that ELS is a significant factor leading to more serious psychopathology in adulthood, such as treatment-resistant depression, which is mediated by the impact of childhood abuse and neglect on chronic functional neural network dysregulation.

To meet these research objectives, this study was designed to assess resting state fMRI connectivity within and between four primary functional neural networks: DNM, ECN, DAN, and SN. These connectivity patterns were compared between adult participants with MDD and healthy non-depressed controls, crossed with the extent of self-reported retrospective ELS.

Materials and Methods

Overview of Study

The Duke-UNC MDD neuroimaging research program is a collaborative project focused on the genetic contributions to the development of MDD and functional neural network dysregulation, and their impact on treatment outcome (Crowther et al., 2014; Dichter & Smoski, unpublished research plan). The present study used

demographic, clinical assessment, and resting state fMRI data from this project to investigate the impact of *environmental* factors, specifically early life stress (ELS), in contrast to the *genetic* factors explored in the program. Using this database, together with a retrospective assessment of ELS, this study: (1) assessed the extent of retrospective self-reported ELS in 26 adult MDD outpatients as compared with 17 non-depressed healthy control participants; (2) compared rs-fcMRI patterns of 35 adult MDD outpatients with 20 control participants; and (3) analyzed the impact of ELS on rs-fcMRI in 13 MDD patients with high ELS as compared to 13 MDD patients with low ELS.

Participant Recruitment

The Institutional Review Boards at Duke University Medical Center and the University of North Carolina at Chapel Hill approved the study protocol. All enrolled participants provided written informed consent. Participants with MDD were recruited via internet-based advertisements and a subject registry at the Cognitive Behavioral Research and Treatment Program at Duke University Medical Center. Non-depressed healthy control participants were recruited via listservs at Duke University and UNC-Chapel Hill. Potential participants completed an initial brief phone screen, and those who passed the phone screen were clinically evaluated, including administration of the structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID) (First et al. 2002) conducted by licensed clinical psychologists or trained clinicians to assess for Axis I disorders, and completed the Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1960) and Beck Depression Inventory-2

(BDI) (Beck et al. 1996). If still eligible, they were invited to participate in the fMRI scan session.

Participant Characteristics

Participants in the MDD group met DSM-IV criteria for a current episode of MDD and scored 15 or above on the HAMD. Participants in the control group scored six or lower on the HAMD and did not meet criteria for a current or lifetime episode of a mood disorder. Exclusion criteria included: (1) In the MDD group: current mood, anxiety, psychotic, or substance abuse disorder beyond unipolar MDD or dysthymia, (2) history of psychosis or mania; (3) active suicidal ideation, (4) evidence of organicity, (5) magnetic resonance imaging contraindication (e.g., metal in body), (7) history of neurological injury or disease, and (8) current pregnancy.

Participants were paid for participating in the assessment and neuroimaging sessions. 39 outpatients with MDD and 21 non-depressed controls (CON) enrolled in the parent project and completed the initial assessment. The final sample for this study included all participants in the parent project who successfully completed all assessment and provided acceptable fMRI scans. This criterion resulted in 35 (24 female/11 male) outpatients with MDD and 20 (14 female/6 male) non-depressed control participants, for a total of 55 (38 female/17male) participants in the initial MDD vs. CON rs-fcMRI analyses. Of these participants, 26 (19 female/7 male) MDD and 17 (12 female, 5 male) control participants completed the Childhood Trauma Questionnaire (CTQ) and were included in the MDD-ELS analyses. Demographic information is included in Table 2. As seen in Table 2, there was no significant difference in the age of the MDD and CON participants. While there were greater than

twice the number of female as compared to male participants in the study, the gender ratio remained approximately the same in all groups, as seen in Table 2.

Childhood Trauma Questionnaire

The Childhood Trauma Questionnaire (CTQ) was used to assess early life stress (ELS) experienced as a child or teenager (Bernstein, Ahluvalia, Pogge, & Handelsmann, 1997; Bernstein & Fink, 1998; Bernstein et al., 2003). This questionnaire is a self-reported, retrospective inventory that screens for five different types of childhood maltreatment: emotional, physical and sexual abuse, as well as emotional and physical neglect. The short-form of the CTQ includes 28 items to be answered using a 5-point Likert scale ranging from “never true” to “very often true.” Total CTQ scores range from 28 to 140. For this study, participants were divided into High vs. Low ELS groups. High ELS was defined as the top one-third of the participant pool, with the rest placed in the Low ELS group.

There are five CTQ subscales: Emotional Abuse (EA), Physical Abuse (PA), Sexual Abuse (SA), Emotional Neglect (EN), and Physical Neglect (PN). Clinical validation studies have been done to develop cut-off scores that reliability correlate to other assessment measures including clinical interviews (e.g., Bernstein et al., 2003). Cut-off CTQ subscale scores used in other recent neuroimaging ELS studies (e.g., Chaney et al., 2014) can be applied: Emotional Neglect > 14, Physical Neglect > 9, Emotional Abuse > 12, Physical Abuse > 9, Sexual Abuse > 7. While there were insufficient participants in this sample to analyze rs-fcMRI data by CTQ subscales, these cut-offs were used to assess the type of abuse and neglect experienced by the participants with ELS.

Imaging Methods

A stimulus-free baseline resting state fMRI paradigm was used since it is particularly conducive to investigating functional neural connectivity patterns (Wang et al., 2012). Initial region-of-interest (ROI) analyses were done using a priori seed regions since this approach is generally considered easier to interpret resting-state fMRI data (Zhou et al., 2010). For this purpose, 29 ROIs from the Raichle mask (Raichle, 2011) were used including 9 ROIs from the default mode network (DMN), 8 ROIs from the dorsal attention network (DAN), 5 ROIs from the executive control network (ECN), and 7 ROIs from the salience network (SN). A complete list of the ROIs used in the initial rs-fcMRI analyses is listed in Table 1.

Functional neural images were acquired at the Duke-UNC Brain Imaging and Analysis Center (BIAC) on a General Electric (Waukesha, WI, USA) MR750 3.0 T scanner equipped with 50 mT/m gradients (200 T/m/s slew rate) and an 8-channel head coil for parallel imaging. High-resolution T1-weighted anatomical images were acquired with 162 axial slices using a FSPGR pulse sequence (TR = 7.584 ms; TE = 2.936 ms; FOV = 256 mm; image matrix = 256 × 256; voxel size = 1 × 1 × 1 mm; flip angle = 12°) and used for normalization and coregistration with the functional data. This structural image was aligned in a near axial plane defined by the anterior and posterior commissures. Whole-brain functional images were acquired using a spiral-in SENSE sequence (TR = 1500 ms; TE=30 ms; FOV=240 mm; image matrix, 64 x 64; flip angle = 60°; voxel size, 3.75 × 3.75 × 4.0 mm; 34 axial slices) to reduce susceptibility artifacts and recover signal in orbital frontal regions (Pruessmann, Weiger, Bornert, & Boesiger, 2001; Truong & Song, 2008). The resting state

functional scan was 300 seconds long and participants were instructed to rest comfortably with their eyes open while viewing a gray fixation cross. A semi-automated high-order shimming program ensured global field homogeneity.

Imaging Data Preprocessing

The first four volumes of each functional imaging dataset were discarded to allow for magnetic field stabilization. Heart rate and respiration were acquired from each participant during the scan, and retrospective correction for physiological motion were performed using AFNI 3dretroicor (Glover, Li, & Ress, 2000), and signal outliers were removed from the data using AFNI 3dDespike. Brain extraction, motion correction, spatial smoothing, and slice-timing correction were then performed using FSL version 5.0.1 (FMRIB Software Library, FMRIB Centre, Oxford University, U.K.) as previously described (Schiller, Minkel, Smoski, & Dichter, 2013). Data was affine-registered to MNI152 standard space using MCFLIRT in FSL using an intermodal registration tool (Jenkinson, Bannister, Brady, & Smith, 2002; Smith et al., 2004). Next, white matter and cerebrospinal fluid were regressed out using FMRIB's Automated Segmentation Tool (FAST) in FSL. Voxel-wise temporal autocorrelation were estimated and corrected using FMRIB's Improved Linear Model (Jenkinson & Smith, 2001), and data was bandpass filtered between 0.008 and 0.1 Hz using custom python scripts. Volumes that exceeded framewise displacement of 0.5 or DVARS of 0.5% (mean global intensity of a single volume over brain mask intensity) were removed prior to connectivity analyses (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012).

Functional Connectivity Analyses

Functional connectivity within and between resting state networks was analyzed via a seed-based approach. Standard seed regions were used to analyze each of the four canonical resting state networks (Schmidt, Akrofi, Carpenter-Thompson, & Husain, 2013; Woodward, Rogers, & Heckers, 2011). These seeds were the insula, dACC, anterior PFC, and lateral parietal for the SN (Elton & Gao, 2013; Seeley et al., 2007); the posterior and anterior IPS, MT, and frontal eye fields for the DAN (Schmidt, Akrofi, Carpenter-Thompson, & Husain, 2013; Vincent, Kahn, Snyder, Raichle, & Buckner, 2008); the dorsal medial and anterior PFC and superior parietal for the ECN (Elton & Gao, 2013; Seeley et al., 2007; Sheline et al., 2010); and the posterior cingulate/precuneus, medial PFC, lateral and inferior temporal, medial dorsal thalamus, and posterior cerebellum for the DMN (Sheline et al., 2010). Seed regions were 5 mm spheres with centers as described in Raichle (2011).

Mean fMRI timeseries were extracted from seed ROIs using FSL `fslmeants` and analyzed as regressors to identify voxels correlated with seed timeseries for each participant in FSL FEAT as a first-level explanatory variable using a general linear model approach with FILM prewhitening (Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012). The resulting parameter estimate maps for each participant were entered into group-level analyses calculated by a mixed effects analysis using Bayesian estimation techniques (FILM, Woolrich, Ripley, Brady, & Smith, 2001) to compare MDD and control groups with respect to seed-based connectivity using FMRIB Local Analysis of Mixed Effects (FLAME 1+2, Beckmann, Jenkinson, & Smith, 2003) using Z statistic images cluster thresholded at $Z > 2.3$ with a corrected cluster significance threshold of $p < 0.05$. Average Z-scores from clusters with significantly

different connectivity between groups were extracted for each participant for ELS analyses using the Total CTQ scores. Cluster localizations were based on Harvard–Oxford cortical and subcortical structural probabilistic atlases in FSLView v3.1.8. Pearson correlational, analysis of variance, and t-tests were used to analyze significant group differences and relationships among study variables.

Results

Childhood Trauma Questionnaire Analyses

Of the 55 participants in the study, 26 in the MDD and 17 in the CON group completed the CTQ, for a total of 43 participants. There was a significant difference in Total CTQ scores between these groups, with an average score of 49.7 for MDD and 31.0 for CON ($p < .0001$), as seen in Table 2. This difference in the distribution of Total CTQ scores is depicted visually in Figure 1. This discrepancy between MDD and CON in CTQ scores is further highlighted when the participants were classified into High vs. Low ELS, using a cut-off of the top one-third of Total CTQ scores. As seen in Table 3, there were 14 MDD participants with High ELS (range 44-106) and 13 with Low ELS (range 27-43). However, there were *no* CON participants with High ELS; all 17 in the CON group had low ELS (range 25-41).

Given the confound between clinical status and ELS scores, ELS rs-fcMRI analyses were restricted to the MDD group. One participant's CTQ score was > 3 SD from the MDD group mean and was subsequently removed from analyses. As a result, ELS rs-fcMRI analyses were performed with 13 High ELS and 13 Low ELS MDD participants.

Regarding ELS subtypes, Table 3 summarizes the number of participants who reported clinically significant levels of each form of abuse and neglect. (Note that several participants reported more than one form of ELS.) All of these participants were in the MDD group. The predominant forms of ELS were emotional abuse and neglect (12 each), with 9 participants reporting physical neglect, 7 participants reporting sexual abuse, and 5 participants reporting physical abuse. Again, it is striking that *all* participants with clinically significant levels of childhood abuse and neglect were suffering from clinical depression, in this sample. Since the number of participants in each subgroup was fairly small, no further analyses were done and the remaining analyses used Total CTQ scores.

MDD vs. CON Resting State Connectivity Analyses

Preliminary rs-fcMRI analyses were done comparing all MDD and CON participants using 29 Raichle mask regions. These analyses revealed 17 significant (and one trend-level) differences in standardized correlation (r) connectivity between the two groups ($p < .05$). Table 5 summarizes the 12 regional pairs for which the MDD group was significantly *less* connected than the CON group. This analysis revealed the widespread *hypo-connectivity between* the DMN regions and the other three networks, especially among the parietal, temporal, insula, and prefrontal regions, in the MDD group. Similarly, there was extensive *hypo-connectivity between* the DAN regions and the other three networks, especially among parietal, temporal, cingulate, cerebellar, and prefrontal regions, in the MDD group. There was also *reduced within-DAN* connectivity between the right anterior IPS and frontal eye field, in the MDD group.

In contrast, these rs-fcMRI analyses also revealed 4 significant (and one trend-level) pairs that revealed *greater* connectivity in the MDD group as compared with the CON group, as summarized in Table 6. Most of this *hyper*-connectivity was *within* networks. Specifically, there was significant *within*-DMN *hyper*-connectivity between the left inferior temporal and both the medial prefrontal and (trend-level) right posterior cerebellum in the MDD group. In addition, the MDD group had greater *within*-DAN *hyper*-connectivity between the bilateral posterior IPS, right middle temporal, and left frontal eye field. Finally, significant *inter*-network connectivity was seen between the superior parietal (ECN) and left frontal eye field (DAN).

Therefore, these rs-fcMRI analyses revealed widespread *hypo*-*inter*-network and *hyper*-*intra*-network connectivity in the MDD group as compared to the CON group. Therefore, the MDD group appeared to have *greater* local nodal connectivity *within* networks, especially within the DMN and DAN, with extensive *reduced* communication and regulation *among* the networks. This neural network dysregulation was particularly evident in the DMN and DAN, across the parietal, temporal, prefrontal, cingulate, insula, cerebellar, and visual regions of the brain.

ELS Resting State Connectivity Analyses in MDD Patients

The 18 regional pairs for which the *z*r scores were significant (or trend-level) in the MDD-CON rs-fcMRI analyses were used in the correlational and group ELS analyses to determine the impact of ELS on rs-fcMRI within the MDD group. There was a significant Pearson correlation between the Total CTQ score and the *z*r score for the left inferior temporal and right posterior cerebellum, both within the DMN ($R=.575, p<.003$). The scatter plot of this relationship is depicted in Graph 2, and

visually captures the significant correlation. Therefore, the greater the ELS, as measured by the Total CTQ score, the stronger the connectivity between these two DMN regions. This finding is also reflected in the significant difference between the High ELS participants within the MDD group and the Low ELS participants ($p < .05$), as seen in Table 7.

These results suggest that ELS significantly increases the *intra-DMN hyper-connectivity*, while controlling for the diagnosis of MDD. In other words, while MDD is typically associated with intra-DMN hyper-connectivity, ELS appears to potentiate this connection. The additive impact of ELS on DMN dysregulation is visually depicted in Table 8, in which the significantly increased connectivity between the left inferior temporal and right posterior cerebellum due to ELS is included in the MDD-CON connectivity table.

Discussion

The findings from this study confirm the initial hypotheses and provide meaningful contributions to our understanding and articulation of the serious impact of early life stress on the developing brain of the child, which appears to lead to specific functional neural network dysregulation patterns that may mediate the development of major psychopathology, including depression, decades later. A major finding from this study is that *only* participants with Major Depressive Disorder reported higher, clinically relevant levels of early life stress, including emotional, sexual, and physical abuse as well as emotional and physical neglect. Control participants, who were selected based on the absence of current or past psychiatric disorders, reported at most low subclinical childhood abuse and neglect. Though the

methodology of this study was not designed to examine population differences in ELS between individuals with or without MDD, this finding suggests that there is a strong connection between early life stress and psychopathology, including depression, in adulthood.

While a small sample, this skewed distribution is striking and should be replicated with a larger population. Recruitment of participants with childhood abuse and neglect, yet without a history of psychopathology, would be an interesting method to investigate psychological resilience. This study used a retrospective report of ELS, which may sensitize individuals suffering from current depression to emphasize life problems, including childhood maltreatment. Nevertheless, this finding is consistent with prior research, which points to the potential pathogenic impact of early life stress decades later. Future research, including longitudinal studies, will be needed to clarify the relationship of childhood maltreatment and the development of psychopathology, which include other contributing factors, such as genetic vulnerability.

By using a systematic analysis of the critical regions within each of the major functional neural networks, this study uncovered a remarkably clear pattern of neural dysregulation, consistent with but more articulate than prior studies.

Widespread *hypo-inter-network* and *hyper-intra-network* connectivity was revealed in the brains of patients diagnosed with Major Depressive Disorder. The functional neural networks of these patients, especially the default mode and dorsal attention networks, were widely dissociated from other networks. Adaptive communication and regulation among the functional neural networks, essential for healthy effective

psychological functioning, appear to be impaired in patients with MDD. Without this coordination between networks, individuals are unable to shift effectively from adaptive self-reflection to task-focused problem solving to emotional processing to attention to critical aspects of the environment. Therefore, the important functions of these neural networks, including healthy self-reference, attention to relevant stimuli, adaptive emotional regulation, and “top-down” cognitive regulation of “fight-flight” impulses appear to be impaired (Crowther et al., 2015; Kaiser et al., 2015).

At the same time, patients with Major Depressive Disorder were found to have marked *hyper-connectivity within* the default and dorsal attention networks. This finding is striking since it suggests that these networks are excessively rigid, unable to shift adaptively to other critical functions of the brain. It is almost as if the brain becomes divided into overly distinctive neural networks, less able to integrate and collaborate with each other, perhaps reflecting the “divided self” described by many people suffering from depression. As a result, MDD patients may be locked into emotional self-rumination due to hyper-connectivity within the DMN, unable to transition adaptively to effective problem solving by the ECN. Similarly, hyper-connectivity within the DAN may lead patients to become hyper-vigilant, compulsively attentive to irrelevant emotionally driven stimuli in their body and environment. Together, this functional brain imbalance may have a marked clinical impact on these patients, including excessive attention to emotional stimuli, obsessional self-focus, emotional ruminations, depressive biases toward internal thoughts instead of engaging adaptively with the world, an inability to shift to

adaptive tasks, and impaired mood regulation (Crowther et al., 2015; Kaiser et al., 2015).

While patients with Major Depressive Disorder generally appear to suffer from these dysfunctional brain networks, the experience of childhood abuse and neglect appears to potentiate this pattern of neural dysregulation. The more patients reported the experience of childhood trauma, the greater their *within*-default mode *hyper*-connectivity, specifically between the left inferior temporal and right posterior cerebellum. The posterior cerebellum is associated with working memory, planning, and behavior, while the temporal lobe plays a critical role in the subjective experience of emotion (Beatty et al., 2014). Therefore, the increased hyper-connectivity between these default mode network regions may reflect and be responsible for the exaggerated preoccupation with negative emotions from the ELS survivor's past as well as emotional flashbacks and memories triggered by relatively minor events in adult life, all of which intrude upon the individual's perspective about their past, present, and future. Furthermore, this hyper-connectivity within the DMN may produce an inability to cope adaptively with life stresses, overwhelming their ability to relax, reflect, plan, and behave adaptively in their current adult life. Therefore, this functional neural network dysregulation may be responsible, at least in part, for the clinical finding that ELS often leads the individual to have "a low threshold for stress, thereby becoming overly reactive to adverse experiences throughout life" (Middlebrooks & Audage, 2008).

This very serious long-term neurotoxic impact of early childhood stress, combined with neural imbalances associated with Major Depressive Disorder, may

play a significant role in producing the more pervasive and serious emotional dysregulation, impulsivity, rumination, phobias, obsessive-compulsive symptoms, seen in patients who have experienced childhood abuse and neglect (Pechtel & Pizzagelli, 2011). Therefore, this widespread neural network dysregulation may underlie the mediating impact of early life stress on the development of more serious treatment-resistant Major Depressive Disorder, Post-Traumatic Stress, and Obsessive-Compulsive Disorder (Heim & Binder, 2012).

A deeper understanding and assessment of the dysfunctional neural networks that underlie psychopathology, such as Major Depressive Disorder, as well as the impact of early life stress can be useful in predicting treatment outcome and selection. Crowther et al. (2015) reported treatment outcomes from the parent study of this dataset, which found that the outcome of Behavioral Activation Treatment for Depression (BATD) was best predicted by the relative *normalcy* of the functional neural networks for patients with Major Depressive Disorder, not the *severity* of the depressive symptoms. In fact, the treatment outcome was more successful for patients with more serious symptoms, in contrast to clinical expectations. Therefore, an analysis of functional neural networks may be a better predictor of treatment outcome than clinical symptomatology, and could guide more sophisticated choices of treatment. The Crowther et al. (2015) study may also explain how the increased default mode network hyper-connectivity, found in the current study for individuals with childhood abuse and neglect, may contribute to the poor treatment outcome observed in many patients with ELS in clinical studies (Nanni, Uher, & Danese, 2012; Spatz et al., 2007).

Therefore, it is increasingly clear that clinicians need to assess both the extent of early life stress and functional neural network dysregulation prior to developing an appropriate treatment plan and realistic prognosis. This study, together with Crowther et al. (2015), suggest that patients with major psychopathology, such as depression, especially those with childhood abuse and neglect, should be assessed using an rs-fcMRI paradigm in order to assess the degree of functional neural network dysregulation that may complicate treatment outcome.

Furthermore, by identifying the specific networks dysregulated by early life stress, these findings may help guide the development and choice of treatments that target and normalize adaptive functional neural network connectivity. For instance, studies have shown that specific anti-depressant medication, particularly SSRIs, appear to normalize default mode network connectivity in depressed patients (Posner et al., 2013). Innovative treatments have also been found to improve neural network functioning including repetitive transcranial magnetic stimulation (rTMS) (Salomons et al., 2014), deep brain stimulation (Mayberg et al., 2005; Mayberg, 2007), meditation (Brewer et al., 2011; Xu et al., 2014), and mindfulness (Farb et al, 2012). For instance, Froeliger et al. (2012) found that the regular practice of meditation produced meditation-state functional connectivity (msFC), which strengthened the functional connectivity between the dorsal attention network with the default mode and salience networks, while decreasing functional connectivity within the default mode network. Similarly, Taylor et al. (2012) found that experienced meditators had weaker intra-network default mode connections than beginning meditators, especially the regions associated with self-referential processing and emotional

appraisal. Daily meditation could be particularly therapeutic to reduce the intra-network default mode hyper-connectivity observed in individuals with early childhood abuse and neglect observed in this study.

Therefore, meditation and mindfulness practices, as well as other innovative treatments, may restore healthy balanced connectivity in these dysfunctional neural network patterns, triggered by early life abuse and neglect and other contributing factors, in order to enhance more adaptive self-reflection, cognition, attention, and behavior as well as top-down higher cortical emotional and stress regulation. In this way, increased understanding of the specific neural network dysregulation associated with early life stress, which contributes to treatment-resistant adult psychopathology, together with an increased knowledge about the differential impact of various treatments on the restoration of normal functional neural network connectivity, may facilitate the choice of specific interventions that target these underlying neural dysfunctions.

Study Limitations and Future Research Directions

This study has clear limitations, which should be addressed in future research. The limited number of participants reduced the sample size of various subgroups and restricted the analyses that could be performed. In particular, there were no participants in the control group with clinically relevant levels of childhood abuse and neglect. Future studies could more systematically recruit for non-depressed participants with a history of early life stress. In addition, with more participants, analyses could be done regarding the differential neural dysregulation of individuals with various forms of early life stress, including emotional, sexual, and physical

abuse, as well as emotional and physical neglect. Since the Childhood Trauma Questionnaire (CTQ) is a self-reported retrospective inventory, individuals suffering from depression could have biased responses. While the CTQ has been clinically validated, future studies of early life stress could benefit from more objective measures including longitudinal study designs.

The additive neural dysfunctional impact of early life stress found in this study may have been, at least in part, related to increased symptom severity and complexity that was not controlled in this study. Therefore, future studies should be designed to control for severity and complexity of clinical symptoms rather than simply the diagnosis of Major Depressive Disorder. Future research is also needed to investigate the impact of early life stress on the severity and type of clinical symptoms, as mediated by functional neural network dysregulation, not just on psychiatric diagnoses. Finally, treatment outcome studies, such as Crowther et al. (2015), using a variety of targeted intervention methods, could help develop more effective treatments that directly restore healthy balance to functional neural networks impaired by early life stress and other contributing factors.

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TABLE 1: RAICHLEREGIONS OF INTEREST

29 ROI Spheres*		Raichle Mask	
DMN			
1	Posterior cingulate/precuneus	1 post_cing	pstcing
2	Medial prefrontal	2 med_prefrontal	mdpfrt
3	Left lateral parietal	3 lat_parietal_L	latparL
4	Right lateral parietal	4 lat_parietal_R	latparR
5	Left inferior temporal	5 inf_temporal_L	infstemL
6	Right inferior temporal	6 inf_temporal_R	infstemR
7	Medial dorsal thalamus	7 med_dor_thal	mdthal
8	Left posterior cerebellum	8 post_cerebel_L	pstcbxL
9	Right posterior cerebellum	9 post_cerebel_R	pstcbxR
DAN			
10	Left frontal eye field	10 frontal_eye_L	frteyeL
11	Right frontal eye field	11 frontal_eye_R	frteyeR
12	Left posterior IPS	12 post_IPS_L	pstIPSL
13	Right posterior IPS	13 post_IPS_R	pstIPSR
14	Left anterior IPS	14 ant_IPS_L	antIPSL
15	Right anterior IPS	15 ant_IPS_R	antIPSR
16	Left MT	16 MT_L	MTL
17	Right MT	17 MT_R	MTR
ECN			
18	Dorsal Medial PFC	18 dor_mPFC	dormPFC
19	Left anterior PFC	19 ant_PFC_L	antPFCL
20	Right anterior PFC	20 ant_PFC_R	antPFCR
21	Left superior parietal	21 sup_par_L	supparL
22	Right superior parietal	22 sup_par_R	supparR
SN			
23	Dorsal anterior cingulate	23 dor_ant_cing	dorcing
24	Left anterior PFC	24 ant_PFC_L	antPFCL
25	Right anterior PFC	25 ant_PFC_R	antPFCR
26	Left insula	26 insula_L	insulaL
27	Right insula	27 insula_R	insulaR
28	Left lateral parietal	28 lat_parietal_L	latparL
29	Right lateral parietal	29 lat_parietal_R	latparR

*from Raichle, 2011; Sheline et al., 2010; Coricelli & Nagel, 2009; Stein et al., 2007

TABLE 2: PARTICIPANT DEMOGRAPHICS

	GROUP	N	F	M	MEAN	SIGNIFICANCE
AGE	CONTROL	20	(14)	(6)	31.1	n.s.
	MDD	35	(24)	(11)	33.8	
CTQ SCORE	CONTROL	17	(12)	(5)	31.0	p<.0001
	MDD	26	(19)	(7)	49.7	
GENDER			38	17		

FIGURE 1: TOTAL CTQ SCORES – MDD vs. CON

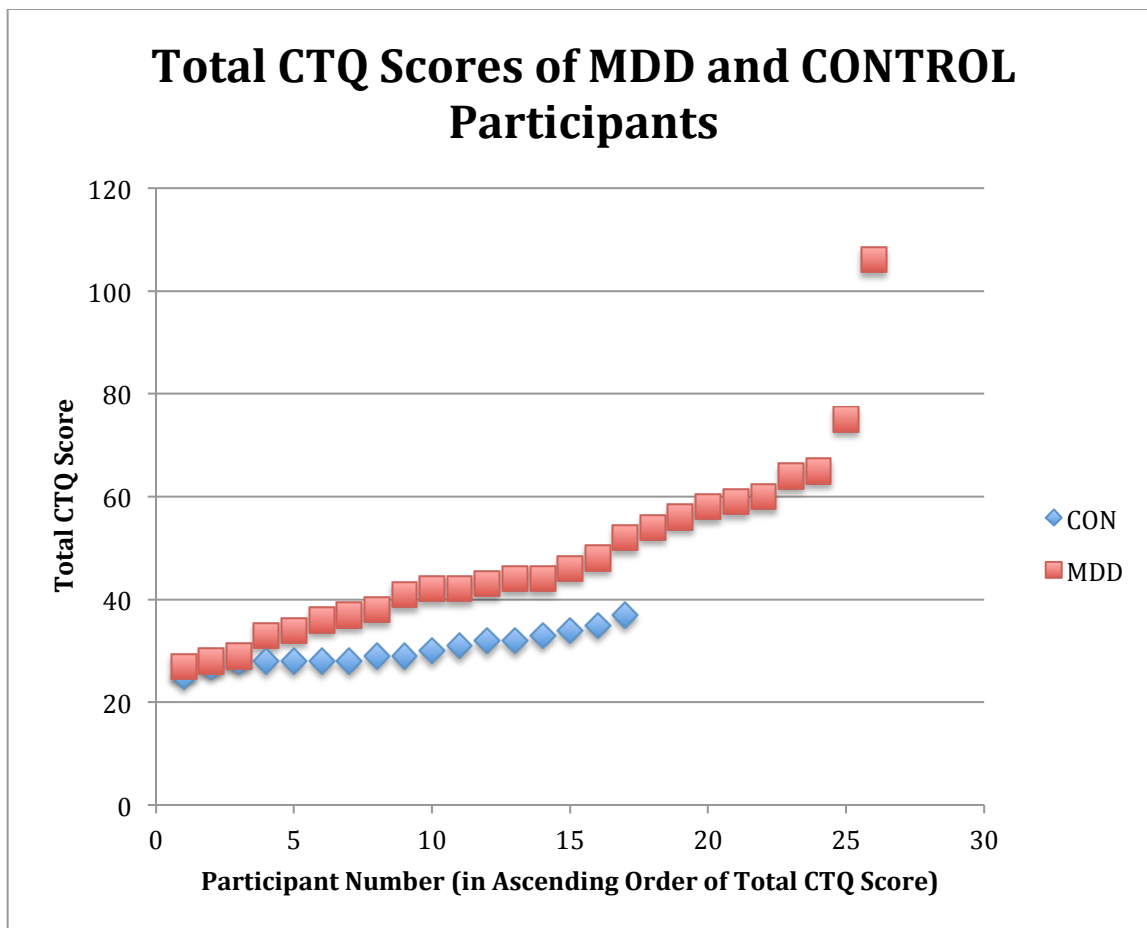


TABLE 3: PARTICIPANTS IN MDD vs ELS CELLS

<u>Group</u>	<u># Participants</u>	<u>Total CTQ Score Range</u>
MDD – High-ELS	14	44-106
MDD – Low-ELS	13	27-43
CON – High-ELS	0	--
CON – Low-ELS	17	25-41

TABLE 4: PARTICIPANTS IN CTQ SUBTYPE CELLS

<u>CTQ Subtype</u>	<u># Participants</u>
Emotional Abuse	12
Physical Abuse	5
Sexual Abuse	7
Emotional Neglect	12
Physical Neglect	9

TABLE 5: RESTING-STATE NEURAL CONNECTIVITY

MDD < CONTROL

	Medial Prefrontal	L Lateral Parietal	R Lateral Parietal	L.Inferior Temporal	L Posterior Cerebellum	R Frontal Eyefield	Dor Medial PFC	Dor Ant Cingulate	R Lateral Parietal
L Frontal Eyefield				**					
L Posterior Intraparietal Sulcus								**	
R Posterior Intraparietal Sulcus	**						**		
L Anterior Intraparietal Sulcus				**					
R Anterior Intraparietal Sulcus						+			
L Medial Temporal				**			**		
R Medial Temporal									**
L Anterior PFC			**						
R Anterior PFC					**				
L Anterior PFC		**							
L Insula					**				

Default Mode Network (DMN)

Dorsal Attention Network (DAN)

Executive Control Network (ECN)

Saliience Network (SN)

INTRA-NETWORK

INTER-NETWORK

Participants:

MDD: 35

CON: 20

**** significant (p < .05)**

+ trend (.05 < p < .10)

TABLE 6: RESTING-STATE NEURAL CONNECTIVITY

MDD > CONTROL

	Medial Prefrontal	R Posterior Cerebellum	L.Frontal Eyefield	L Medial Temporal
L Inferior Temporal	**	+		
L Posterior Intraparietal Sulcus				**
R Posterior Intraparietal Sulcus			**	
R Superior Parietal			**	

Default Mode Network (DMN)
(DAN)

Dorsal Attention Network

Executive Control Network (ECN)

Salience Network (SN)

INTRA-NETWORK

INTER-NETWORK

Participants:

MDD: 35

CON: 20

**** significant (p < .05)**

+ trend (.05 < p < .10)

**TABLE 7: RESTING-STATE NEURAL CONNECTIVITY
IN MAJOR DEPRESSIVE DISORDER**

HIGH ELS > LOW ELS

	POSTERIOR CEREBELLUM RIGHT (Default Mode Network)
INFERIOR TEMPORAL LEFT (Default Mode Network)	**

# Participants: HIGH ELS: 12 LOW ELS: 13	** significant (p < .05)
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CTQ vs zr (L Inf Temp – R. Post Cerebellum): Pearson Correlation = .575* (df=24)	* significant (p < .003)
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**FIGURE 2: TOTAL CTQ SCORE vs.
zr LEFT INFERIOR TEMPORAL – RIGHT POSTERIOR CEREBELLUM**

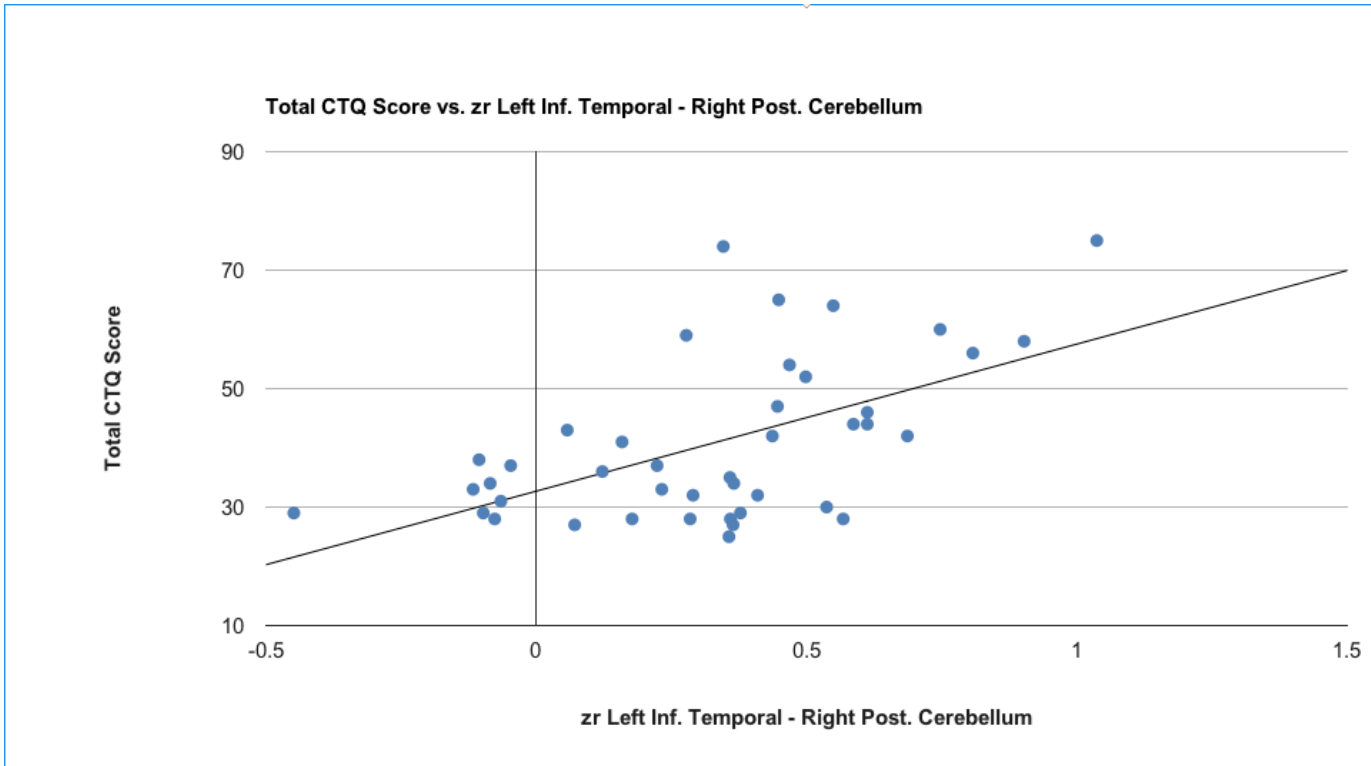


TABLE 8: RESTING-STATE NEURAL CONNECTIVITY

MDD > CONTROL
HIGH ELS > LOW ELS

	Medial Prefrontal	R Posterior Cerebellum	L Frontal Eyefield	L Medial Temporal
L Inferior Temporal	**	** +		
L Posterior Intraparietal Sulcus				**
R Posterior Intraparietal Sulcus			**	
R Superior Parietal			**	

Default Mode Network (DMN)
(DAN)

Dorsal Attention Network

Executive Control Network (ECN)

Salience Network (SN)

INTRA-NETWORK

INTER-NETWORK

HIGH ELS > LOW ELS (MDD)

Participants:

MDD: 35 (HIGH ELS: 13; LOW ELS: 13)

CON: 20

** significant ($p < .05$)

+ trend ($.05 < p < .10$)