A preliminary study of functional connectivity in comorbid adolescent depression

Kathryn R. Cullen a,∗, Dylan G. Gee c, Bonnie Klimes-Dougan a, Vilma Gabbay c, d, Leslie Hulvershorn c, Bryon A. Mueller a, Jazmin Camchong a, Christopher J. Bell a, Alaa Houria a, Sanjiv Kumra a, Kelvin O. Lima b, F. Xavier Castellanos c, d, Michael P. Milham c, **

a University of Minnesota Medical School, Department of Psychiatry, Minneapolis, MN, United States
b Veterans Administration Medical Center, Minneapolis, MN, United States
c New York Child Study Center, New York University School of Medicine, Department of Child and Adolescent Psychiatry, New York, NY, United States
d Nathan Kline Institute for Psychiatric Research, Orangeburg, NY, United States

ARTICLE INFO

Article history:
Received 18 March 2009
Received in revised form 8 May 2009
Accepted 10 May 2009

Keywords:
Adolescence
Brain imaging
Depression
Functional connectivity
Resting state functional MRI
Subgenual anterior cingulate cortex

ABSTRACT

Major depressive disorder (MDD) begins frequently in adolescence and is associated with severe outcomes, but the developmental neurobiology of MDD is not well understood. Research in adults has implicated fronto-limbic neural networks in the pathophysiology of MDD, particularly in relation to the subgenual anterior cingulate cortex (ACC). Developmental changes in brain networks during adolescence highlight the need to examine MDD-related circuitry in teens separately from adults. Using resting state functional magnetic resonance imaging (fMRI), this study examined functional connectivity in adolescents with MDD (n = 12) and healthy adolescents (n = 14). Seed-based connectivity analysis revealed that adolescents with MDD have decreased functional connectivity in a subgenual ACC-based neural network that includes the supragenual ACC (BA 32), the right medial frontal cortex (BA 10), the left inferior (BA 47) and superior frontal cortex (BA 22), superior temporal gyrus (BA 22), and the insular cortex (BA 13). These preliminary data suggest that MDD in adolescence is associated with abnormal connectivity within neural circuits that mediate emotion processing. Future research in larger, un-medicated samples will be necessary to confirm this finding. We conclude that hypothesis-driven, seed-based analyses of resting state fMRI data hold promise for advancing our current understanding of abnormal development of neural circuitry in adolescents with MDD.

© 2009 Elsevier Ireland Ltd. All rights reserved.

Adolescent major depressive disorder (MDD) is associated with severe consequences including suicide, the third leading cause of death in adolescence [31]. Further, adolescent MDD is a strong predictor of MDD in adulthood, which carries its own burden of disadvantage [37]. Because of the substantial brain maturation that takes place during adolescence within the neural networks that mediate emotion processing [9,38], pathophysiology in adolescents should be examined separately. However, such research has been scarce.

Fronto-limbic neural circuitry has been implicated in MDD [27,33]. Within this network, converging lines of evidence suggest that the subgenual ACC (ACC) region plays a critical central role in the neural circuitry that underlies MDD [12,29]. A core function of subgenual ACC is to regulate amygdala activity, preventing excessive emotional reactivity and stress responses [32]. In a recent investigation of functional connectivity in healthy adults, Stein et al. delineated a circuit linking subgenual ACC ventrally to amygdala and rostrally to supragenual ACC (Brodmann area (BA) 32) [34]. Disruption of this regulatory circuit is thought to underlie depression [11], and provides a useful starting point for examination of neural circuitry in adolescents with MDD.

Resting state functional magnetic resonance imaging (fMRI) has emerged as a novel approach to examine neural connections in vivo. This method measures the spontaneous, slow-wave (0.01–0.1 Hz) fluctuations in blood oxygen level dependent (BOLD) signal that are observed at rest; “functional connectivity” is indicated by inter-regional correlations of these temporal patterns [17]. Resting state fMRI has now been used to map connectivity patterns in healthy adults [10,25], normative development [15,23], and disease processes [4]. Resting state fMRI techniques in adults with depression have begun to identify group differences in functional connectivity within brain networks, although analysis strategies have varied...
Wechsler Abbreviated Scale of Intelligence [36], significant med-
all participants consisted of intelligence quotient (IQ) < 80 on the
ing from 15 to 19 years of age in this study. Exclusion criteria for
posed by Stein et al. [34], we analyzed functional connectivity
nectivity in adolescents with depression compared with healthy
examine neural circuitry in adolescents with MDD.
[1,19]. No prior studies have reported using resting state fMRI to
aligned axial slices; matrix = 64
talic Resonance Research at the University of Minnesota. We
Siemens Trio 3 Tesla scanner located at the Center for Mag-
nessive echo planar imaging (EPI) whole-brain functional volumes
achieved by the institutional review board of the University of Minnesota. Participants age 18 and over pro-
vided signed informed consent; those under age 18 provided assent,
and a parent or guardian provided signed informed consent.
For each participant, DSM-IV Axis I diagnoses were estab-
ish a consensus between independent parent and
child interviews conducted by a child and adolescent psychiatrist
and a developmental clinical psychologist using the Schedule for
fective Disorders and Schizophrenia for Children – Present and
Lifetime Version [22]. Severity was assessed using (a) the Beck
Affective Disorders and Schizophrenia – Children and
[22]. Severity was assessed using (a) the Beck
Affective Disorders and Schizophrenia for Children – Present and
Lifetime Version [22]. Severity was assessed using (a) the Beck

All participants were scanned using a research-dedicated
Siemens Trio 3 Tesla scanner located at the Center for Mag-
netic Resonance Research at the University of Minnesota. We
obtained a 6-min ‘resting-state’ scan, comprising 180 contigu-
ous echo planar imaging (EPI) whole-brain functional volumes
(TR = 2000 ms; TE = 30 ms; flip angle = 90°; 34 contiguous AC–PC
aligned axial slices; matrix = 64 × 64; FOV = 22 cm; acquisition voxel
size = 3.4 mm × 3.4 mm × 4 mm). During this scan participants were
structed to rest without moving. To increase scanning tolerability,
participants listened to their choice of music during the acquisition
of all neuroimaging data. Given concerns about the potential impact
of this baseline, we limited our examination to positive connections,
which show less sensitivity to choice of baselines than negative
connections [35]. A high-resolution T1-weighted anatomical image
was also acquired for purposes of anatomical localization.
AFNI [71] was used for image preprocessing (http://www.afni.
nimh.gov/afni), performing slice timing correction for inter-
leaved acquisition (using Fourier interpolation), motion correction
(by aligning each volume to a “base” image [middle vol-
ue] using Fourier interpolation) and de-spooling (detection and
reduction of extreme time series outliers using a hyperbolic
tangent function). All other data processing was carried out
using FSL (http://www.fmrib.ox.ac.uk), including spatial smoothing
(FWHM = 6 mm), mean-based intensity normalization of all vol-
umes by the same factor, temporal bandpass filtering (highpass
temporal filtering; Gaussian-weighted least-squares straight line
fitting, with sigma = 100.0 s; Gaussian lowpass temporal filtering
FWHM = 2.8 s) and pre-whitening. Each individual’s time series was
 spatially normalized by registration to the MN152 (Montreal Neu-
rological Institute) template with 1 mm³ resolution, using a 12
degree of freedom affine transformation.
Following the methods of Castellanos et al. [4], we examined
functional connectivity using a seed-based approach. Using coord-
inates recently validated in a path-analysis of amygdala circuitry
[34], spherical seeds with a radius of 8 mm (257 voxels) were cre-
at ed in 2 mm × 2 mm × 2 mm space for subgenual ACC (Montreal
Neuroimaging Institute (MNI) coordinates 0, 15, −14), amygdala
(−26, 0, −20) and supragenual ACC (0, 34, 30). For each partici-
ent, we calculated the mean time series of each seed by averaging
across all voxels within the seed. Individual participant analyses
were carried out for each seed using the general linear model imple-
mented in FSL program FEAT. Nine nuisance covariates (time series
for global signal intensity, white matter, cerebrospinal fluid, and
six motion parameters) were included in regression analyses to
minimize the contributions of artificial physiological signals (e.g.,
cardiac and respiratory cycles).
Group-level analyses were carried out using a mixed-effects
model implemented in the FSL program FLAME. We included age
and sex as statistical covariates to ensure that observed group
effects would be independent of age-related changes or differen-
tial gender risk. IQ was also included as a covariate to account for
group differences (t(24) = 2.03, p = 0.054) (see Table 1). Corrections
for multiple comparisons were carried out at the cluster level for the
statistical map produced with each seed ROI using Gaussian random
field theory (min Z > 2.3; cluster significance: p < 0.05, corrected).
For each seed, this group-level analysis produced thresholded
Z-score maps for functional connectivity observed in the direct
comparison of MDD versus controls. Correlational analyses were
conducted to assess the impact of clinical variables on functional
connectivity. Additionally, data was examined to evaluate whether
individuals with specific clinical variables would represent outliers.
Two participants with MDD were excluded from analyses due to
unusable data leaving 12 depressed participants (9 females) and
14 healthy control participants (8 females) for analyses. This sam-
ple of depressed adolescents had moderate to severe illness, with
mean duration of illness over 2 years, and mean GAF and BDI-II
scores of 45 and 27, respectively. Most (83%) of the MDD group had
current comorbid anxiety disorders, including Generalized Anxiety
Disorder (n = 7), Social Phobia (n = 3), and Post Traumatic Stress Dis-
order (PTSD) (n = 2). A fourth of the sample had comorbid Attention
Deficit Hyperactivity Disorder (ADHD), and approximately a third
had a past diagnosis of a substance use disorder but were free of
any use for at least 2 months. Most depressed participants were
being treated with medication; only one subject was medication-
aive, and another was medication-free for 1 month prior to the
scan. Group characteristics are detailed in Table 1.
Adolescents with MDD exhibited decreased functional connectiv-
ty between subgenual ACC and a network of cortical areas
including supragenual ACC (BA 32), right medial frontal cortex (BA
10), left superior (BA 8) and inferior (BA 47) frontal cortex, left supe-
rior temporal cortex (BA 22), and insular cortex (BA 13) (Z > 2.3,
p < 0.05, corrected) (Fig. 1). No significant group differences were
found in the analyses using amygdala or supragenual ACC seeds.
Within the depressed group, no significant relationships were
identified between measures of functional connectivity and symp-
tom severity (BDI-II scores), duration of illness, medication status,
or presence of an anxiety disorder. Individuals without comorbid
anxiety (n = 2) and those not taking medications (n = 2) did not rep-
resent outliers in the analysis.
In this preliminary study we identified decreased functional
connectivity within a key neural circuit arising from the sub-
genual ACC in adolescents with MDD in comparison to healthy
participants using resting state functional neuroimaging. This find-
ing adds to converging lines of evidence implicating subgenual
ACC as a central component of altered brain networks in depres-
Table 1
Demographic and clinical characteristics of adolescents with major depressive disorder (MDD) and healthy control participants.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MDD (n = 12)</th>
<th>Healthy (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean years ± SD)</td>
<td>16.5 ± 0.95</td>
<td>16.8 ± 1.5</td>
</tr>
<tr>
<td>Gender (male/female, n (%))</td>
<td>3/9 (25/75%)</td>
<td>6/8 (43/57%)</td>
</tr>
<tr>
<td>IQ (mean ± SD)¹</td>
<td>106.0 ± 10.5</td>
<td>114.4 ± 10.4</td>
</tr>
<tr>
<td>Ethnicity – n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>10 (83%)</td>
<td>9 (64%)</td>
</tr>
<tr>
<td>African American</td>
<td>2 (16%)</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Asian</td>
<td>0</td>
<td>2 (14%)</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Illness history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of illness (mean months ± SD; range)</td>
<td>26.5 ± 25.9 (1–96)</td>
<td>0</td>
</tr>
<tr>
<td>Medication-naïve/medication-free/medicated (n)</td>
<td>1/1/10</td>
<td>15/0/0</td>
</tr>
<tr>
<td>Global assessment of functioning (mean ± SD)</td>
<td>45.42 ± 9.7</td>
<td>No information</td>
</tr>
<tr>
<td>Medication class – n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selective serotonin reuptake inhibitors²</td>
<td>9 (75%)</td>
<td>0</td>
</tr>
<tr>
<td>Atypical antidepressants²</td>
<td>1 (8%)</td>
<td>0</td>
</tr>
<tr>
<td>Mood stabilizers²</td>
<td>2 (16%)</td>
<td>0</td>
</tr>
<tr>
<td>Atypical antipsychotics²</td>
<td>2 (16%)</td>
<td>0</td>
</tr>
<tr>
<td>Stimulants²</td>
<td>2 (16%)</td>
<td>0</td>
</tr>
<tr>
<td>Selective norepinephrine reuptake inhibitor²</td>
<td>1 (8%)</td>
<td>0</td>
</tr>
<tr>
<td>BDI-II (mean ± SD)³</td>
<td>277.7 ± 11.3 (15–44)</td>
<td>No information</td>
</tr>
<tr>
<td>Current comorbidity – n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD²</td>
<td>3 (25%)</td>
<td>0</td>
</tr>
<tr>
<td>Any anxiety disorder²</td>
<td>10 (83%)</td>
<td>0</td>
</tr>
<tr>
<td>Past comorbidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance use disorder, partial or sustained remission, no use for at least 2 months</td>
<td>4 (32%)</td>
<td>0</td>
</tr>
</tbody>
</table>

¹ p = 0.054.
² Range.
³ Selective serotonin reuptake inhibitors included fluoxetine (n = 3), sertraline (n = 1), citalopram (n = 4), escitalopram (n = 1).
⁴ Venlafaxine (n = 1).
⁵ Mood stabilizers included lamotrigine (n = 1) and oxcarbazepine (n = 1).
⁶ Atypical antipsychotics included aripiprazole (n = 1) and quetiapine (n = 1).
⁷ Stimulant medications included amphetamine/dextroamphetamine (Adderall) (n = 2).
⁸ Atomoxetine (n = 1).
⁹ Beck Depression Inventory-II.
¹⁰ Attention Deficit Hyperactivity Disorder.
¹¹ Current Anxiety Disorders included Generalized Anxiety Disorder (n = 7), Social Phobia (n = 3), and Post Traumatic Stress Disorder (n = 2).

With treatment-resistant depression leads to altered functional activity within fronto-limbic networks [29,30]. Emerging research using diffusion tensor imaging in healthy adults has begun to delineate the anatomical circuitry connected with this key area [i.e., 20]. However, to our knowledge this represents the first study using seed-based resting state fMRI techniques to specifically measure functional connectivity of subgenual ACC in depressed patients of any age.

The findings presented here add to a previous report from a seed-based analysis of resting state fMRI data in adults with MDD [1]. In a study that examined functional connectivity between supragenual ACC (BA 32) and three limbic targets, Anand et al. reported decreased functional connectivity between supragenual ACC and thalamus in MDD adults [1]. In contrast, our analysis found no group difference in functional connectivity using the supragenual ACC seed. The divergence may reflect methodological differences (i.e., use of targets versus brain-wide search) or may reflect developmental differences.

The refinement of neural connections that transpires in adolescence is the backdrop for the current findings. Preclinical data has documented postnatal maturation of hemodynamic response characteristics in rats, which correlated with maturation of cortical connectivity from infancy to adulthood [5]. Emerging human fMRI research has documented maturation in functional connectivity of neural networks through adolescence [14–16]. One study reported that ACC functional connectivity, especially in networks associated with social and emotional functions, undergoes develop-
opment across adolescence from diffuse patterns in childhood to focal patterns in adulthood [23]. This evidence depicting functional connectivity during adolescence as a “moving target” emphasizes the need for future research to carefully delineate, across multiple developmental time points, how the trajectory of neurodevelopment may be abnormal in depressed individuals.

The subgenual ACC-based network identified in the present study maps on to a set of connections identified in a recent meta-analysis of cortical–subcortical interactions in emotion processing [24]. This analysis identified the “medial PFC group” as “the interface between cognitive context and core affect” [8]. Thus, decreased connectivity in this network may underlie the dysregulation of emotion that occurs in adolescents with MDD. In addition, the network identified here includes the left insular cortex (BA 13). The insula is thought to mediate interpretation of sensory information received from the body (interoception) that contributes to emotional states [8]. Therefore, it is plausible that decreased connectivity in this circuit may underlie depressive symptoms such as somatic complaints and negative bias in interpreting interpersonal feedback. Finally, this network included the superior temporal lobe (BA 22). Although not an area commonly emphasized in MDD research, a recent meta-analysis of MDD fMRI studies noted that this region shows decreased activation during induction of negative affect in MDD patients [26]. We speculate that our finding of reduced connectivity with subgenual ACC may relate to the reported diminished responses of superior temporal cortex in other MDD studies; future studies are needed to further understand this circuit in MDD pathophysiology.

Contrary to predictions, we did not observe a group difference in connectivity between subgenual ACC and amygdala. Prior studies using fMRI data that used emotion-valenced tasks have documented a disconnect or “uncoupling” between amygdala and supragenual ACC or ventromedial prefrontal cortex in depressed patients [21,26]. Notably, Anand and colleagues also failed to identify a disconnect between the ACC and amygdala in their resting state fMRI study of adults with MDD [1]. The failure to identify a group difference in amygdala circuitry in both studies may reflect the specific location used for creating the seeds. Alternatively, detecting group differences in functional connectivity in this circuit may require the use of fear-processing tasks. Future research studies combining resting state and task-based approaches will advance understanding of this circuitry in MDD.

Results from this exploratory study must be interpreted cautiously in light of study limitations. First, most patients were receiving psychotropic medications, a potential confound. However, since successful antidepressant medication treatment in adults has been found to reverse abnormalities of subgenual ACC metabolism [28], the high prevalence of medication treatment in our sample likely diminished group differences rather than representing a source of type I error. Further, this sample of depressed adolescents had moderate to severe levels of depression at the time of the scan despite treatment, indicating poor response. Thus, regardless of medication status, the neural data from this sample is pertinent to understanding the underlying neurobiology of active illness. Future research with treatment-naïve adolescents with MDD is needed to confirm these findings.

Given high rates of comorbidity in this sample of depressed adolescents, future efforts will need to clarify the specificity of the reported findings. Although MDD was the primary diagnosis, most patients in this sample had one or more secondary comorbid diagnoses, most frequently anxiety. Inclusion of anxiety disorders in MDD studies is standard because depression and anxiety are so highly comorbid in adolescents [6] that exclusion of all anxiety disorders would result in a highly atypical sample. Similarly, ADHD precedes depression in many adolescents [i.e., 3], and inclusion of patients with ADHD is common in adolescent MDD studies [i.e., 8]. Finally, although subjects denied all use of substances for 60 days prior to the scan, our sample included 4 subjects with a history of a substance use disorder. Substance use disorders commonly co-occur with depression [18], arguably making our sample more representative. However, the long-term effects of substance use on brain connectivity are poorly studied. While the inclusion of these subjects is a limitation, it was noted that these subjects did not represent outliers in the analysis. Continued efforts to investigate the specific impact of depressive illness versus each of the comorbid secondary disorders on functional connectivity are warranted but will require substantially larger samples.

A methodological limitation of this pilot study pertains to participants continuing to listen to their choice of music during the resting BOLD scan. Anecdotally, it appeared that this approach enhanced relaxation, increased scan tolerability and minimized motion artifacts. However, this approach introduced the potential confound of uncontrolled state-related changes in connectivity. Although spontaneous BOLD fluctuations remain easily detectable across distinct baselines [35], different types of music (happy, sad, neutral) activate distinct neural networks as observed using task-based fMRI. We did not assess the emotional import of participants’ music selections which would have been difficult without limiting choices. Thus we cannot rule out the possibility that systematic between-group differences in music choice may have contributed to our finding of decreased functional connectivity in the subgenual ACC network in depressed adolescents. Replication with independent samples in which data is acquired during varying controlled resting states will be useful in determining the extent to which emotionally meaningful stimuli like music modulate brain connectivity in emotion regulation networks.

In conclusion, the data presented here document decreased functional connectivity in a key network implicated in emotion regulation in adolescents with MDD, and add to converging evidence highlighting the central role of the subgenual ACC in the neural circuitry of depression. These findings in adolescents with MDD are hypothesized to reflect an aberrant developmental process in the formation of these neural connections. Future research is needed in treatment-naïve adolescents (1) to confirm the implication of this circuitry in adolescents with MDD, and (2) to examine the effects of treatment on functional connectivity within this network. Given the presumed increased neuroplasticity during adolescent development, confirmation of our findings should lead to efforts directed toward altering risk trajectories and improving outcomes for youth with MDD.

Acknowledgements

The authors gratefully acknowledge support from the National Institute of Mental Health (Cullen: T32 MH073129; Gabbay: MH077072; Kumra: MH 073150; Lim: MH060662), the Center for Neurobehavioral Development and the Center for Magnetic Resonance Research (BTPRR-P41 RR008079 and P30 NS057091) at the University of Minnesota, the Minnesota Medical Foundation, the Stavros S. Niarchos Foundation, Linda and Richard Schaps, and Jill and Bob Smith.

References
