I, Rupa Radhakrishnan M.D., hereby submit this original work as part of the requirements for the degree of Master of Science in Clinical and Translational Research.

It is entitled:
Altered functional activation and network connectivity underlies working memory dysfunction in adolescents with epilepsy

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Committee member: Jennifer Vannest, Ph.D.
Altered functional activation and network connectivity underlies working memory dysfunction in adolescents with epilepsy

A thesis submitted to the
Graduate School
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Abstract

Executive dysfunction is observed in adolescents with localization-related and generalized epilepsy, and likely contributes to poor outcomes in social, academic, behavioral and quality of life domains. Identification of neuroimaging biomarkers of brain network connectivity could provide insight into the neural mechanisms of executive dysfunction in epilepsy and facilitate more objective assessment of disruptions in executive function related brain networks. Neural correlates of working memory, a specific component of executive function involving short-term retention and manipulation of information, can be assessed by task based functional MRI (fMRI) studies such as the n-back task. N-back tasks are very demanding and require participants to continually adjust the information held in working memory to incorporate the most recently presented stimulus while simultaneously rejecting or ignoring more temporally distant stimuli. As adolescents with epilepsy are at a greater risk of working memory disability, we hypothesized that fMRI activation in brain regions involved in working memory would be significantly different in children with epilepsy relative to healthy controls and that activation patterns would correlate with neuropsychological measures of executive dysfunction. We performed a prospective case-control study of 29 adolescents with MRI non-lesional epilepsy and 20 healthy controls. Both groups performed standardized measures of executive function and questionnaires (parent and child Behavior Rating Inventory of Executive Function - BRIEF) to rate executive function and behavior. All participants performed an n-back fMRI task with 2-back and 0-back components. We performed group analysis of brain functional activation, ROI based analyses of working memory specific brain regions and functional connectivity analyses of the known working memory networks. Adolescents with epilepsy scored poorly on working memory neuropsychological tests Wechsler Intelligence Scale Digit Span, and the working memory composite scores, as well as on the the BRIEF parent report. Analysis of n-back task MRI revealed significantly reduced functional connectivity (p<0.05) between the left frontal operculum to the anterior cingulate gyrus (components of the cingulo-opercular network) in
adolescents with epilepsy when compared to controls. There were significant correlations between the Weschler intelligence scale Digit Span scores and activation in the left frontal pole (r = 0.53, p = 0.013), left frontal operculum/insula (r = 0.53, p = 0.015) and left parieto-occipital area (r = 0.48, p = 0.034) in controls, but not in adolescents with epilepsy. In adolescents with epilepsy, there was significant correlation between the activation in the left temporal lobe and parent BRIEF working memory scores (r = 0.49, p = 0.009). These findings suggest presence of functional reorganization during performance of working memory task. Correlations between brain region activation and specific neuropsychological working memory scores that were significantly worse in adolescents with epilepsy suggests that the altered pattern of working memory processing in epilepsy patients may be inefficient.
Acknowledgements

I would like to first and foremost thank Jennifer Vannest PhD and Avani Modi PhD, who have graciously allowed me to work with data collected through their funded project for my thesis. Dr. Vannest, my primary mentor for my Master’s in Clinical and Translational Research Program and a member of my thesis mentorship committee, has provided me with guidance, training, constant encouragement, and feedback to successfully complete this thesis. Dr. Modi, has provided valuable feedback on data analysis and manuscript preparation, especially with respect to the neuropsychological scores. I am thankful to Shari Wade PhD, who has helped me better understand the implications of the results of this study and has critically reviewed the first draft of this manuscript. I would like to thank Mekibib Altaye PhD and Erin Haynes PhD, also members of my thesis committee, for their review of my final thesis manuscript. Thanks also to Thomas Maloney MS, an excellent research analyst who patiently helped me with imaging data analysis in this project. I am thankful to Angela Combs, who has helped with coordination of data collection efforts. Thank you to Dr. Vannest’s graduate student, Shelby Merder, for helping with MRI response data collection. And last, but not the least, I would like to acknowledge the MRI technologists, who helped to scan these children, as well as the participants and their families.
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Figure 1. Functional ROIs used in the study, based on areas of activation from the n-back task (z>5) in the fronto-parietal and cingulo-opercular executive function networks

Figure 2 (top) reflects brain region activation in controls and and figure 3 (bottom) reflects brain region activation in epilepsy group with the N-back paradigm. For each, two sets of slices are selected and labeled with the z-coordinate in MNI space. Threshold is z>2.3 p<.05 corrected. Warm colors reflect 2-back > 0-back and cool colors reflect 0-back greater than 2-back
INTRODUCTION

Executive functions (EF) are necessary for complex, goal-oriented human behavior, including initiation, problem-solving, planning and organization, self-monitoring, attention, impulse control and working memory.[1, 2] Impairment in executive function can be a component of multiple pediatric neurologic conditions including epilepsy, traumatic brain injury, meningitis, hemorrhage and autism to name a few.[1] In adolescents and young adults with epilepsy, executive dysfunction is common,[3-7] and likely contributes to poor outcomes in social, academic, behavioral and quality of life domains.[8-12] Executive dysfunction also results in poor adherence to medical treatment through deficits in planning and working memory.[13-15]

Executive dysfunction is observed in adolescents with localization-related and generalized epilepsy, raising the possibility of a common neural basis regardless of epilepsy type. Identification of neuroimaging biomarkers of brain network connectivity would provide insight into the neural mechanisms of executive dysfunction in epilepsy and facilitate more objective assessment of disruptions in EF-related brain networks. These neuroimaging biomarkers could potentially also be useful in identifying children who are likely to benefit from EF-related interventions [16] and characterizing treatment-related changes at a neural level.

Working memory is a specific component of executive function that involves short-term retention and manipulation of information. One of the primary components of the working memory system is the central executive component which is involved regulation of information flow within the working memory system, retrieval of information from other memory systems (such as long term memory), and the processing of information storage.[17] The resources used by the central executive component are capacity limited, and may lead to decreased efficiency when there are greater simultaneous demands placed on the working memory system.[18] Functional MRI (fMRI) studies have demonstrated activation of specific brain regions and networks during performance of working memory tasks.[19-21] One of the more
widely used fMRI paradigms to target working memory regions in the brain is the n-back task. The n-back task requires subjects to monitor the identity or location of a series of presented stimuli, and to respond (typically with button-clicks) when the currently presented stimulus is the same as the one presented n trials previously. This is compared to a baseline condition 0-back, where the participant responds whenever a target stimulus is presented, which does not require the manipulation of information within working memory.

Typical regions of the brain activated during the n-back paradigm include the lateral premotor cortex, dorsal cingulate and medial premotor cortex, dorsolateral and ventrolateral prefrontal cortex, frontal poles and medial and lateral posterior parietal cortex[19]. Additionally, using functional connectivity analysis of fMRI, two discrete brain networks have been identified that play a role in executive function, namely the fronto-parietal network, consisting of the dorsolateral prefrontal cortex and the superior parietal lobule and adjacent intraparietal sulcus, and the cingulo-opercular network, consisting of the dorsal anterior cingulate and medial superior frontal cortex, the anterior insula and adjacent frontal operculum, and the anterior prefrontal cortex.[20, 21]

Since the n-back task requires on-line monitoring, updating, and manipulation of recently presented information, it is assumed to place great demands on many key processes in the working memory network.[19] N-back tasks are very demanding and require participants to continually adjust the information held in working memory to incorporate the most recently presented stimulus while simultaneously rejecting or ignoring more temporally distant stimuli. [19] As adolescents with epilepsy are at a greater risk of working memory disability, we were interested in identifying underlying brain activation and functional network connectivity with fMRI and the n-back task to potentially explain neuronal differences related to poor working memory in this group. We hypothesized that fMRI activation in brain regions involved in working memory would be significantly different in children with epilepsy relative to healthy controls and that
activation patterns would correlate with neuropsychological measures of working memory ability.

METHODS

Following IRB approval, a prospective case-control study design was used.

Participants: Only participants with English as the primary language and a body mass index between the 5th and 99th percentile for age and sex were recruited.

Adolescents with epilepsy: All adolescents with epilepsy were recruited via Neurology clinics. To be included in the study, these adolescents had to have a confirmed diagnosis of epilepsy, be on antiepileptic medication and had at least one seizure within the past 12 months. Any adolescent with a significant developmental delay (e.g. autism), previous history of head trauma, pregnancy in female participants on verbal screening, or prescribed psychoactive medications other than antiepileptic medications and stimulants for ADHD was excluded from the study. Additionally, exclusion from participation occurred when patients had an epilepsy diagnosis of symptomatic etiology, such as a brain lesion clinically determined to underlie the epilepsy diagnosis, any brain lesion on prior MRI that caused anatomical distortion of brain structures or brain surgery. There were 55 eligible participants with epilepsy, of whom 40 agreed to participate in the study (73%). However, 9 participants did not complete neuropsychological testing or imaging at their second study visit (n=5 withdrew, n=4 lost to follow-up; retention=78%). One participant did not have MRI due to braces and another participant did not complete fMRI due to claustrophobia in the MRI environment. Thus, our final sample included 29 adolescents with epilepsy.

Information about clinical, electroencephalographic, psychological, academic and social history for children with epilepsy was obtained from their electronic medical record and interviews with their parents, and included age of seizure onset in months, seizure type (generalized, localization related, unclassified), seizure etiology (idiopathic, cryptogenic),
presence of seizures in the past year, time since diagnosis in months, and psychological 
comorbidities (e.g. learning disorders, ADHD).

**Controls:** Healthy controls matched on age, gender, race, and Duncan score [22] (+/- 5.00), a 
measure of socioeconomic status, were recruited from the database maintained by the clinical 
trials office at Cincinnati Children’s Hospital Medical Center, the healthy control registry 
maintained by the Pediatric Neuroimaging Research Consortium, and by advertising in the 
community. For controls, history of neurologic or psychiatric disease in the participant or first 
degree relative, personal history of ADHD, history of head trauma, gestational age of less than 
37 weeks, birth weight less than the 10th percentile, personal history of chronic illnesses (e.g. 
diabetes, asthma) or prescribed psychoactive medications excluded them from the study. All 
potential healthy control participants were screened with the Behavior Rating Inventory of 
Executive Function (BRIEF)-Parent report [23, 24], a screening instrument for executive function 
via phone. If any subscale on the BRIEF questionnaire was in the clinically elevated range, they 
were excluded. This resulted in exclusion of three participants in the control group. Of the 
remaining 23 healthy controls that met eligibility and agreed to participate in the study, 3 did not 
attend their study visit. Therefore, 20 healthy controls participated in the study.

Informed consent was obtained from all participants and their parents. All participants 
received monetary compensation for time and travel.

**Neuropsychological testing**

Cognitive testing was conducted by a trained psychometrist. All but one participant had both 
fMRI and neuropsychological testing completed in the same day (one participant completed 
neuropsychological testing within 2 weeks after fMRI). The battery of tests included standardized 
measures of executive function and parent surveys to rate executive function and behavior. 
Adolescents completed the (BRIEF®)[23, 24] self-report. Working memory was assessed via 
the Wechsler Intelligence Scale for Children (WISC-V) working memory subtests [25] (including 
Digit Span and Picture Span) or the Wechsler Adult Intelligence Scale (WAIS-IV) working
memory subtests (Digit Span and Arithmetic), depending on age (adolescents 17 years or older received the WAIS). The Working Memory Composite score was obtained from either the WISC-V or WAIS-IV. T-tests were used to identify neuropsychological scores that were significantly different between adolescents with epilepsy and healthy controls in the parent- and child- report BRIEF and the WISC/WAIS Working Memory scores. Correlations between neuropsychological working memory scores and n-back fMRI brain activation were assessed.

**Neuroimaging**

**N-back task design:** All participants performed an N-back fMRI task (block design 2-back versus 0-back condition with single-letter stimuli). The scan time was 5 min 20 seconds and included five cycles of 0-back and 2-back stimulus presentation and a 12 second fixation. Each of the 0-back and 2-back blocks lasted 26 seconds and had 2 seconds of instruction followed by 16 trials of stimuli in 24 seconds. For the 0-back trial, the participant was instructed to press a button if the current stimulus matched the initial instruction at the beginning of the block. For the 2-back trial, the participant pressed a button if the stimulus presented two trials previously matched the initial instruction. Participants made responses via button-press. Information regarding task performance, time lag and accuracy of button-press were recorded. BOLD imaging using echo-planar sequences was performed on a 3 Tesla-MRI scanner (Philips Achieva, Best, Netherlands) using a 32 channel head coil with the following parameters: TR/TE 2000/35 ms, FOV 24 x24 cm, matrix 64x64, voxel size 3.75 x 3.75 x 5 mm, 38 axial interleaved slices without gap. High resolution 3D T1 weighted 1 mm isotropic anatomical images were acquired for coregistration and alignment.

**Image data processing:** First-level fMRI data were processed using FSL (FMRIB Software Library)[26, 27] and AFNI (Analysis of Functional Neuroimages).[28] Anatomical T1 data and functional data were first reoriented using FSL’s fslreorient2std. Next, the T1 data were bias corrected and brain extracted using FSL’s FAST[29] and BET respectively.[30] The brain
extracted T1 image was then normalized and resampled to the 2mm isotropic MNI ICBM 152 non-linear 6th generation template[31] using FSL’s FLIRT.[32, 33] For the functional data, typical pre-processing steps, such as slice timing correction and brain extraction, were carried out using FSL’s “slicetimer” and BET[30], respectively. Outlying functional volumes were detected using FSL’s “fsl_motion_outliers” using the default reference RMS metric. Motion correction of the BOLD time-series was carried out using MCFLIRT.[32] The motion related artifacts were then regressed from the data by setting up a general linear model design using 24 motion parameters[34] (6 motion parameters, the 6 motion parameters squared, a first order autoregressive model of the 6 motion parameters and a first order autoregressive model of the 6 motion parameters squared) and a 10 parameter CompCor model[35](5 white matter and 5 cerebrospinal fluid principle components) plus an additional parameter for each detected outlier. The residuals from the GLM were high-pass filtered in accordance with the task timing at 0.016 Hz and smoothed with a 6mm FWHM filter using AFNI’s 3dBandpass and the Z statistical map computed from the task design. The results were interpolated to a 2 mm isotropic voxel size and aligned to the Montreal Neurological Institute (MNI) template[31] by first co-registering it with the participant’s T1 using FSL’s FLIRT.[32, 33]

**Group activation and Region-of-interest (ROI) Analysis:** Using FSL, a general linear model (GLM) approach was used to create group activation maps contrasting 2-back vs 0-back for the epilepsy and control groups (z>2.3, corrected for multiple comparisons to p<.05) and both groups combined (z > 5, corrected for multiple comparisons to p<0.05). We assessed differences in activation maps between adolescents with epilepsy and healthy controls. After identifying neuropsychological working memory scores that were significantly different in the epilepsy and control groups, we used these scores as covariates in the GLM model, to assess for any correlation with the activation maps in the epilepsy and control groups. We also assessed parent and child BRIEF working memory sub-scores as covariates in the GLM model to assess for correlations. Finally, in the epilepsy group, we included age of onset of epilepsy,
time since epilepsy diagnosis and epilepsy type (generalized vs other) as covariates in the GLM model to for correlations with group activation. Adjustment for multiple testing was performed using FDR correction.

We identified functionally defined regions of interest (ROIs) based on clusters of combined group activation (Figure 1) in FSL. For each participant, mean Z-score values for fMRI activation were derived for each ROI. We then used Pearson correlation to identify relationship between Z-score values in each ROI with BRIEF working memory sub-scores and neuropsychological working memory scores and contrasted the epilepsy and control groups. In the epilepsy group, we correlated the Z-score values with epilepsy type (generalized vs. other), age of onset, and time since diagnosis, adjusted for multiple testing by using FDR correction.

**Functional Connectivity analysis (Region-of-interest (ROI) based):** These analyses were performed using CONN toolbox in SPM12 (http://www.nitrc.org/projects/conn), [36] Functional ROIs were selected based on N-back group composite z>5 and divided into cingulo-opercular and fronto-parietal Networks. Functional connectivity within three nodes in the cingulo-opercular network (anterior cingulate, bilateral frontal operculum including the middle and inferior frontal gyrus) and four nodes in the fronto-parietal network (bilateral dorsolateral prefrontal cortex, inferior parietal lobule) was computed as the temporal correlation of fMRI signal fluctuations between any two regions (connectivity index)[37] A group comparison was conducted using a general
linear model approach where connectivity index in a pair of ROIs were modeled as a function of group. We assessed correlations of BRIEF working memory scores and neuropsychological working memory scores with the connectivity indices in the epilepsy and control groups. In all analyses of the epilepsy group, we examined effects of epilepsy type (generalized vs. other) and time since diagnosis, adjusted for multiple comparison by FDR correction.

RESULTS

Demographics and Neuropsychological Testing: The groups did not differ in age or socioeconomic status (Table 1). Scores of neuropsychological tests related to working memory and child and parent BRIEF scores are presented in Tables 2 and 3.

Table 1. Participant Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Control (n=20)</th>
<th>Epilepsy (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adolescent Age in Years</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>15.4 (1.5)</td>
<td>15.4 (1.5)</td>
</tr>
<tr>
<td><strong>Male gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 (40%)</td>
<td>7 (24.1%)</td>
</tr>
<tr>
<td><strong>Adolescent Race</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, Hispanic</td>
<td>0</td>
<td>2 (6.9%)</td>
</tr>
<tr>
<td>White, Non-Hispanic</td>
<td>15 (75%)</td>
<td>24 (82.8%)</td>
</tr>
<tr>
<td>Black, Non-Hispanic</td>
<td>3 (15%)</td>
<td>2 (6.9%)</td>
</tr>
<tr>
<td>More than One Race</td>
<td>2 (10%)</td>
<td>1 (3.4%)</td>
</tr>
<tr>
<td><strong>Body Mass Index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>21.6 (4.0)</td>
<td>23.3 (5.1)</td>
</tr>
<tr>
<td><strong>Family Duncan score</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>65.9 (15.6)b</td>
<td>59.01 (17.4)a</td>
</tr>
<tr>
<td><strong>Epilepsy Type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localization-related epilepsy</td>
<td></td>
<td>6 (20.7%)</td>
</tr>
<tr>
<td>Generalized epilepsy</td>
<td></td>
<td>17 (58.6%)</td>
</tr>
<tr>
<td>Unclassified epilepsy</td>
<td></td>
<td>6 (20.7%)</td>
</tr>
</tbody>
</table>

a Family Duncan scores of 59.01 represent occupations including legal assistants, transportation/ticket agents, and chief communications officers.
b Family Duncan scores of 65.9 represent occupations including insurance sales, special education teachers, and therapists.
Table 2. Working memory related neuropsychological tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Epilepsy Mean (SD)</th>
<th>Controls Mean (SD)</th>
<th>P-value (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WISC/WAIS Working memory composite</td>
<td>95.82 (14.74)</td>
<td>113.05 (15.46)</td>
<td>0.0007</td>
</tr>
<tr>
<td>WISC/WAIS Digit Span</td>
<td>8.7(2.4)</td>
<td>11.8(2.9)</td>
<td>0.0009</td>
</tr>
</tbody>
</table>

Table 3. Child and Parent BRIEF scores

<table>
<thead>
<tr>
<th>CHILD BRIEF</th>
<th>Epilepsy</th>
<th>Control</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRIEF:Behavior Regulation Index</td>
<td>45.85(13.35)</td>
<td>45.35(10.29)</td>
<td>0.885</td>
</tr>
<tr>
<td></td>
<td>N=27</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF-Inhibit</td>
<td>44.37(12.12)</td>
<td>45.35(9.664)</td>
<td>0.759</td>
</tr>
<tr>
<td></td>
<td>N=27</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF-Shift</td>
<td>48.93 (11.56)</td>
<td>44(5.52)</td>
<td>0.117</td>
</tr>
<tr>
<td></td>
<td>N=27</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF-Emotional Control</td>
<td>47.67(12.44)</td>
<td>49.30(10.37)</td>
<td>0.627</td>
</tr>
<tr>
<td></td>
<td>N=27</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>BRIEF: Metacognition Index</td>
<td>48.85(11.97)</td>
<td>45.65(7.94)</td>
<td>0.277</td>
</tr>
<tr>
<td></td>
<td>N=27</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF- Working memory</td>
<td>53(14.87)</td>
<td>48.75(9.96)</td>
<td>0.247</td>
</tr>
<tr>
<td></td>
<td>N=27</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF – Plan/Organize</td>
<td>46.07(12.13)</td>
<td>44.30(7.09)</td>
<td>0.548</td>
</tr>
<tr>
<td></td>
<td>N=27</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF- Organization of Materials</td>
<td>44.70(7.86)</td>
<td>47.60(7.69)</td>
<td>0.213</td>
</tr>
<tr>
<td></td>
<td>N=27</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF- Monitor</td>
<td>44.89 (11.32)</td>
<td>45.90 (10.79)</td>
<td>0.757</td>
</tr>
<tr>
<td></td>
<td>N=27</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>BRIEF: Global Executive Composite</td>
<td>47.22(12.64)</td>
<td>45.25(8.87)</td>
<td>0.533</td>
</tr>
<tr>
<td></td>
<td>N=27</td>
<td>N=20</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>PARENT BRIEF</th>
<th>Epilepsy Mean (SD)</th>
<th>Control Mean (SD)</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRIEF:Behavior Regulation Index</td>
<td>46.24(9.97)</td>
<td>45.90(6.29)</td>
<td>0.893</td>
</tr>
<tr>
<td></td>
<td>N=29</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF-Inhibit</td>
<td>45.24 (6.84)</td>
<td>47.15 (5.234)</td>
<td>0.275</td>
</tr>
<tr>
<td></td>
<td>N=29</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF-Shift</td>
<td>46.59(9.87)</td>
<td>45.30(5.71)</td>
<td>0.603</td>
</tr>
<tr>
<td></td>
<td>N=29</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF-Emotional Control</td>
<td>48.34(11.39)</td>
<td>46.60(7.9)</td>
<td>0.530</td>
</tr>
<tr>
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<td>N=29</td>
<td>N=20</td>
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<tr>
<td>BRIEF: Metacognition Index</td>
<td>50.41(12.14)</td>
<td>45.65(5.14)</td>
<td>0.105</td>
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<tr>
<td></td>
<td>N=29</td>
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</tr>
<tr>
<td>• BRIEF- Initiate</td>
<td>51.31(10.83)</td>
<td>45.55(7.38)</td>
<td>0.032*</td>
</tr>
<tr>
<td></td>
<td>N=29</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF- Working memory</td>
<td>53.52(12.21)</td>
<td>46.10(5.47)</td>
<td>0.014*</td>
</tr>
<tr>
<td></td>
<td>N=29</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF – Plan/Organize</td>
<td>51.52 (12.34)</td>
<td>45.15(3.60)</td>
<td>0.030*</td>
</tr>
<tr>
<td></td>
<td>N=29</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF- Organization of Materials</td>
<td>45.28(12.49)</td>
<td>48.65(8.72)</td>
<td>0.271</td>
</tr>
<tr>
<td></td>
<td>N=29</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>• BRIEF- Monitor</td>
<td>47.38(9.68)</td>
<td>45.45(6.71)</td>
<td>0.414</td>
</tr>
<tr>
<td></td>
<td>N=29</td>
<td>N=20</td>
<td></td>
</tr>
<tr>
<td>BRIEF: Global Executive Composite</td>
<td>48.48(10.94)</td>
<td>45.20(4.88)</td>
<td>0.216</td>
</tr>
<tr>
<td></td>
<td>N=29</td>
<td>N=20</td>
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</table>

There was a significant difference in Parent BRIEF –Initiate, Working memory and Planning and organization sub-scores between the epilepsy and control groups. (Table 3)
**Task performance:** The mean percent of correct responses on the 0-back task was 99.1% in those with epilepsy and 99.5% in healthy controls. The mean percent of correct responses on the 2-back task was lower in those with epilepsy (88.75%) compared to healthy controls (92.37%); however, this was not significant (p=0.1). Epilepsy participants tended to have more “false alarms” on the 2-back task compared to controls.

**Group Activation:** For the 2-back greater than 0-back comparison, significant task related activation was observed in bilateral middle frontal and parietal regions, bilaterally, anterior cingulate cortex, and inferior parietal/occipital cortex and frontal operculum/insula bilaterally in both controls and epilepsy groups. When compared to the 2-back task, the 0-back task showed greater activation in the bilateral frontal pole, the bilateral frontal gyrus and the bilateral anterior temporal lobes, in the expected regions of the default mode network. (Figures 2 and 3)

There were no significant differences in group activation on fMRI n-back between the individuals with epilepsy and controls. In both, the epilepsy group and controls, group activation on during
the n-back task did not correlate with the neuropsychological working memory scores tested (Working Memory Composite, WISC/WAIS Digit Span scores) or BRIEF working memory subscores (parent and child). In adolescents with epilepsy, group activation did not correlate with, time since epilepsy diagnosis and did not differ by epilepsy type (generalized vs. not generalized).

**ROI Analysis:** There were significant correlations between the WISC/WAIS Digit Span scores and mean level of activation (z-score) in the left frontal pole (r = 0.53, p = 0.013), left frontal operculum/insula (r = 0.53, p = 0.015) and left parieto-occipital area (r = 0.48, p = 0.034) in controls, which was not seen in epilepsy patients. There was no correlation between Z-scores and Working Memory Composite scores in either group. In adolescents with epilepsy, there was significant correlation between activation level in the left temporal lobe and parent BRIEF working memory scores (r = 0.49, p = 0.009). The Child BRIEF working memory scores did not correlate with ROI activation in either group. There was no relationship between ROI Z-score and time since epilepsy diagnosis and did not differ by epilepsy type (generalized vs. not generalized) in adolescents with epilepsy.

**Functional connectivity analysis:** There was significantly reduced functional connectivity (p < 0.05) between the left frontal operculum to the anterior cingulate gyrus (components of the cingulo-opercular network) in adolescents with epilepsy when compared to controls. There were no differences in fronto-parietal network connectivity between the two groups. There were no significant correlations between either functional network and time since epilepsy diagnosis and connectivity did not differ by epilepsy type (generalized vs. not generalized) in the epilepsy group. There was no relationship between functional connectivity and WISC/WAIS Working Memory Index, or parent and child BRIEF - working memory scores.
DISCUSSION

In this study we demonstrate evidence of functional reorganization during performance of working memory task and changes in functional connectivity between adolescents with epilepsy and healthy controls. Although the n-back fMRI task design has been used extensively to study working memory function, our study is the first to use this tool to evaluate working memory in pediatric epilepsy. We identified significantly decreased functional connectivity between the left inferior frontal gyrus and the cingulate gyrus in adolescents with epilepsy compared to controls. This connection forms a part of the cingulo-opercular network, a component of the executive function network, which is important for attention, alertness, initiation, maintenance, feedback and adjustment in working memory. [20, 21, 38, 39] Our results are in keeping with similar findings of decreased functional connections to the left frontal lobe on fMRI working memory tasks in children with autism and working memory deficits. [40] The importance of the cingulo-opercular network in working memory function is underscored by these observations. Specifically, the left frontal lobe connections are shown to be involved in verbal working memory related tasks, such as the n-back fMRI design used in our study.[41, 42, 43]

Our cohort of adolescents with epilepsy had significantly worse working memory function compared to healthy controls identified by the behavioral questionnaire (Parent BRIEF) and neuropsychological tests (Working Memory Composite, WISC/WAIS Digit Span scores). We identified neuroimaging network changes that were associated with these neuropsychological outcomes. On ROI measures of brain activation, we found correlations between the working memory network regions in the left frontal pole, left frontal operculum and left parieto-occipital region and the WISC/WAIS Digit Span scores in healthy controls, but not in the epilepsy group, further evidence for disrupted working memory network or presence of an alternate working memory network in the latter group. The Digit Span scores are shown to be an effective measure of working memory function with specific activation pattern in the ventrolateral frontal
Similar findings of decreased left frontal activation with the n-back task have been seen with working memory deficits in children with autism [40, 42] and traumatic brain injury, [45] especially during tasks that require greater working memory capacity.[41, 42, 43] Decreased activation in the working memory related frontal and parietal regions was also demonstrated in a study of adolescents with epilepsy and ADHD, when stimulant medication was withheld.[5]

Although we attribute lack of correlation between brain region Z-score values and neuropsychological scores in the epilepsy group in our study to disrupted normal working memory networks or presence of aberrant working memory networks, another potential explanation could be intrinsic brain maturational variation in the epilepsy group in spite of adequate age matching. [46-48] The prefrontal region matures later than other brain regions, and activities involving frontal lobe function such as working memory continue to mature through childhood and adolescence. [49] Synaptic density in the frontal regions continues to grow until mid to late adolescence, and other MRI-based structural measures such as gray matter thickness, myelination density and synaptic pruning also show protracted changes of development of prefrontal regions well into early adulthood. [50-56] We did not specifically test for structural MRI measures of maturation in this study, but we agree with other authors that in addition to structural changes, there is progressive maturation in working memory functional connectivity. [20, 46] This progressive maturation of working networks throughout childhood and adolescence has been demonstrated by resting state functional connectivity MRI. [20] Early in childhood, there is a unified working memory network in the brain, with development of two discrete and well segregated working memory networks, namely the cingulo-opercular and fronto-parietal networks in early adulthood. [20] [21] In adolescence, network connection pattern shows some overlap, with the anterior cingulate/medial frontal cortex (portions of the cingulo-opercular network) staying incorporated into the frontoparietal network. [20]
While there was a greater proportion of females in the epilepsy group compared to the
control group, this difference was not statistically significant. However, gender differences in
brain activation with working memory have been described, with females having lesser
hemodynamic response compared to males.[57] Spatial tasks of working memory are shown to
have greater gender difference in brain activation rather than the verbal working memory tasks,
such as the letter n-back task in adolescents.[58] Therefore, while the effect of gender cannot
completely be excluded, we feel that this effect on our results may be small.

Aside from the differences described above, the pattern of brain activation on n-back
fMRI in adolescents with epilepsy was similar to known working memory related brain regions in
children and adults.[19, 59] Multiple studies have tried to tease out the role of these different
brain regions in the various components of working memory. The frontal pole is suggested to be
engaged when problems involve more than one discrete cognitive process, [19] i.e., when the
application of one cognitive operation (such as a rule) on its own is not sufficient to solve the
problem and the integration of the results of two or more separate cognitive operations is
required to fulfill the higher behavioral goal. [60] The middle frontal regions are described as to
playing an essential role in increasing task performance by selecting appropriate high-level
organizational chunks that then serve to facilitate memory by reducing overall cognitive load.
[61] The frontal premotor cortex is considered to be responsible for maintaining visuospatial
and temporal attention, which is needed in the n-back task to recollect information received in
previous tasks.[61] Although the frontal operculum and insular activation has been implicated in
multiple cognitive processes, with respect to the n-back task, it is likely responsible for selection,
comparison and judgment of stimuli held in short-term and long-term memory[62], and the
implementation of an intended act or plan to remember or recall.[19] The precuneus and
bilateral inferior parietal lobule are also involved in a wide variety of cognitive tasks that overlap
with that of the prefrontal cortex. This region is frequently thought to be involved in the
implementation of stimulus response mapping [63-65] as well as in storage of working memory.
contents[66]. The anterior cingulate cortex, is described in relation to increased effort, complexity, or attention[67] and in error detection and response correction[68].

In addition to these areas of typical activation, we identified activation in bilateral anterior, medial and lateral temporal cortex in the 0-back as opposed to the 2-back task. We also observed correlation between the ROI brain activation in the left temporal lobe and parent BRIEF working memory scores in adolescents with epilepsy. Previous meta-analysis of brain working memory networks do not specifically describe the temporal lobe as a component of working memory, however smaller studies suggest that the temporal lobe is important for visual and verbal working memory function, as evidenced by poor verbal working memory function in adults with temporal lobe lesions.[69, 70] Activation in the anterior temporal lobes has been reported in a previous study in adults contrasting resting state with an n-back paradigm.[71] This may correspond to long-term memory or language related function or differences in working memory maturation in adolescents.

There were no differences in working memory related brain function based on epilepsy type (generalized vs non-generalized), which would suggest that either type of epilepsy may alter the working memory related brain networks. Larger scale studies may be able to tease out specific influences of different epilepsy types on working memory brain function. Overall, the results of our study showing differences in working memory related task brain function in adolescents with epilepsy, suggest a direction for future research of working memory dysfunction in adolescents with epilepsy.

**CONCLUSION**

Presence of identified decreased left cingulo-opercular network connectivity in adolescents with epilepsy when compared to normal individuals during the n-back task indicates altered pathway of working memory connectivity in adolescents with epilepsy compared to
healthy controls. Correlations between brain region activation and specific neuropsychological working memory scores that were significantly worse in adolescents with epilepsy suggests that the altered pattern of working memory processing in epilepsy patients may be inefficient.
BIBLIOGRAPHY


