The Effects of a Single High School Football Season on the Brain: A Multimodal Neuroimaging Study

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The Effects of a Single High School Football Season on the Brain: A Multimodal Neuroimaging Study

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Abstract

Objective: To assess brain function and structure in adolescent athletes after a single season of high school football.

Methods: 15 high school football players completed a brief clinical interview that included concussion history, neuroimaging, and neuropsychological testing at three time points: pre-season (T1), post-season (T2), and at three-months post-season (T3). Neuroimaging techniques included: pseudo-continuous arterial spin labeling (pCASL) MRI to measure cerebral blood flow (CBF), functional connectivity MRI to measure synchrony of affected brain region, and diffuse tensor imaging (DTI) MRI to assess gray matter structural integrity of the brain region.

Results: The results suggest underlying functional and structural changes to the right insula with minimal increase on some neurocognitive test scores. The right insula showed a significant decrease in CBF post-season and at the three-month follow up, p<0.05 [FWE-Corrected]. Consequently, the insula’s network (i.e. salience network) connectivity increased to potentially compensate for the decrease in CBF of the right insula, ΔT₁₂: p=0.014. Moreover, an increase in mean diffusivity of the right insula suggests structural changes at post-season and three-months post season: ΔT₁₂: p=0.007, ΔT₁₃: p=0.001. There were no significant changes on any neuropsychological tests from pre- to post-season. However, there was an increase on test scores from pre-season to three-months post-season on letter fluency (p=0.006), digit span (p=0.006), and coding (p=0.02) likely due to practice effects.

Conclusion: The current study, to our knowledge is the only study to demonstrate functional and structural brain changes across one athletic season in non-concussed athletes, with continued effects three-months post-season.
What are the findings?

- Results demonstrated a significant decrease in cerebral blood flow in the right insula at post-season and three-month follow up, p<0.05 [FWE Corrected].
- The insula’s network connectivity became hyperactive at post-season, $\Delta T_{12}$: p=0.014
- The right insula’s structure, as measured by mean diffusivity, showed lower integrity at post-season and three-month follow up compared to pre-season; $\Delta T_{12}$: p=0.007, $\Delta T_{13}$: p=0.001.

How might it impact on clinical practice in the future?

- Brain changes occur in adolescent athletes in the absence of a concussion and can either represent a change as a result of subconcussive impacts or normal development
- More research is warranted to tease out the reason for brain changes
INTRODUCTION

High school football athletes experience hundreds of impacts per season\(^1\). The potential effects of repeated impacts on the structure and function of the brain has become a popular area of study, as some have suggested that a gradual accrual of “subconcussive” hits may negatively influence neurocognitive and neurophysiological function \(^1,2\). Even with the use of helmet sensors in some investigations, subconcussive impacts are difficult to define and quantify. Thus, some investigators have studied the effects of potential head impacts on athletes’ brains by comparing pre- and post-season data via neurocognitive assessments and/or neuroimaging techniques \(^3,4\). Recent improvements in neuroimaging techniques have made it possible to detect subtle functional and structural changes of the brain even in the absence of clinical symptoms \(^5,6\). Comparison of pre-season and post-season performance on measures of neurocognitive and neural function can demonstrate whether head impacts throughout an athletic season may influence brain functioning in the absence of a concussion.

Evidence suggests that neurophysiological changes such as reductions in cerebral blood flow (CBF) occur prior to neurostructural changes post head injury \(^7\), which makes it an ideal marker of subtle change in non-concussed athletes. Meier et al. (2015) assessed change in CBF as an early marker of neurophysiological change in American football players who suffered concussion \(^8\). They found lower CBF in the right insular cortex compared to athletes without concussion where the insular CBF gradually recovered in the concussed athletes after four weeks. They argued that regional measures of CBF may be more reliable than global measures of CBF or functional connectivity MRI (fcMRI), which relies on changes in cerebral blood volume. However, there is limited research utilizing pseudo-continuous arterial spin labeling (pCASL) MRI to assess CBF because most studies utilize fcMRI and diffusion tensor imaging (DTI).
Abbas and colleagues used resting state fcMRI to show hyperconnectivity of the default mode network after a single athletic season in high school football athletes without concussion.\textsuperscript{9} Connectivity alterations were demonstrated in comparison to control non-contact athletes, as well as to individual athletes’ own pre-season baselines\textsuperscript{1,9}. Several other DTI studies have demonstrated changes in white matter structural integrity in youth football athletes over the course of 1-2 seasons with little investigation of the impact on gray matter microstructural changes\textsuperscript{10 11 12}. Gong et. al., (2018) evaluated deep gray matter structures over a single football season. They found an increase in MD in deep gray matter structures when comparing athletes’ post-season measures to their baseline\textsuperscript{13}, suggesting gray matter as a potential biomarker of microstructural change following a concussion.

The key purpose of this study was to examine functional and structural brain changes in high school football athletes without concussion after a single football season. We examined functional and structural brain changes using multimodal neuroimaging with a focus on CBF as the initial marker of subtle change across three time points: pre-season (T1), post-season (T2) and three-months post-season (T3). This study investigated CBF changes by pCASL MRI, synchrony of brain regions by fcMRI, and gray matter structural integrity of the brain by DTI MRI. We hypothesized that following a single football season; non-concussed athletes would show a reduction in CBF, an increase in the salience network’s functional connectivity and an increase in MD, a marker of structural integrity. We also examined neurocognitive change across the three time points and hypothesized that the non-concussed athletes would show no significant changes in neurocognitive performance.
METHODS

Participants
We recruited 15 high school football athletes, ages 15 to 18 ($M=16.7$; $SD=0.9$) from a local public high school. The criteria for inclusion included native English speakers participating in high school football. Exclusionary criteria included MR scanning contraindications, history of neurological or psychiatric conditions, elevated endorsement of depressive symptoms (Beck Depression Inventory-2nd edition; BDI-II>14), and recent history of concussion (during the course of the study).

Patient and Public Involvement
This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

Procedures
Approval for the study was obtained from the Institutional Review Board at the University of Texas at Dallas and the University of Texas Southwestern Medical Center. Informed consent was obtained from participants’ ≥18 years old. Participants <18 years old provided written assent with signed consent from their legal guardians. The study consisted of a brief clinical interview that included concussion history, neuroimaging, and neuropsychological testing at three time points: pre-season (T1), post-season (T2), and at 3-months post-season (T3). Pre-season data was collected within two weeks prior to the first contact practice, post-season was collected within two weeks following the last game, and the post-season follow up occurred
3 months after T2 (within a two and half week timeframe). The athletes played 12 games (10 regular season and 2 playoff games).

**MRI Acquisition**

MRI scans were performed on a 3 Tesla MR system (Philips Medical System, Best, Netherlands). A body coil was used for radiofrequency transmission and an 8-channel head coil with parallel imaging capability was used for signal reception. We used four different MRI techniques to investigate functional and structural changes: a pCASL sequence was used to measure CBF\(^{14}\), fcMRI was used to assess functional connectivity of the brain regions\(^{15}\), DTI MRI to provide an assessment of brain structure integrity\(^{16}\), and a high-resolution T1-weighted image as a structural reference. The details of the imaging parameters are available in the supplemental section.

**MRI Analysis**

PCASL image series were realigned to the first volume for motion correction (SPM5’s realign function, University College London, UK). An in-house MATLAB (Mathworks, Natick, MA, USA) program calculated the difference between averaged control and label images. The difference image was corrected for imaging slice delay time to yield CBF-weight image, which was normalized to the Brain template from Montreal Neurological Institute (MNI). The absolute CBF was estimated similar to our previous publication in the units of mL blood/min/100 g of brain tissue\(^{14}\). Whole-brain blood flow values were calculated by averaging all the voxels in the brain. Finally, the voxel wise analyses were performed on the relative CBF (rCBF) maps, which included dividing the aCBF spatial maps, by the whole brain aCBF value. In a prior publication, we showed that this technique could improve the sensitivity of regional differences by reducing physiological variations\(^{17}\). In voxel-based analyses (VBA), the individual CBF maps were
spatially smoothed (with full-width half-maximum [FWHM] of 4 mm) to account for small
differences in sulci/gyri location across subjects. For cluster extent inference, we used
3dClustsim (with –acf option) in AFNI (NIMH Scientific and Statistical Computing Core,
Bethesda, MD, USA), which controlled false positive activation clusters over the set of all
activation clusters throughout the whole-brain volume. We refer to this procedure in Results as
family wise error correction (FWE corrected). For cluster inference, we tested the volume of
clusters that is conditional on two criteria: smoothness of the voxel map and cluster-defining
threshold. We set the cluster-defining threshold to a stringent p<0.0001. Then, the minimum
cluster size of 14 voxels (112 mm³) yielded an FWE-corrected significance level of 0.05.

Functional connectivity images were analyzed using AFNI (NIMH Scientific and
Statistical Computing Core, Bethesda, MD, USA). The dataset was preprocessed with slice
timing correction, motion correction, removal of the linear trend, transformation to standard MNI
space and smoothing by a Gaussian filter with a FWHM of 6 mm. The preprocessed images were
band-pass filtered (0.01–0.1 Hz) on a voxel-by-voxel basis to keep only the appropriate
frequency fluctuations. Next, the signals in white matter and cerebrospinal fluid were regressed
out using averaged signals from the white matter and the ventricles from each voxel time series.
A seed-based connectivity analysis was performed based on the right Insula region, which was
significant in the CBF analysis. The cross-correlation coefficient between the seed voxels and all
other voxels was calculated to generate a correlation map, which was transformed to a z-score
map using Fisher’s inverse hyperbolic tangent transformation. An ROI analysis was performed
based on the insula’s network: left insula, right insula and middle cingulate gyrus. The functional
ROIs were defined as follows: first, each region’s anatomical region was defined based on MNI
database. Then, a functional ROI was defined by choosing the top 500 voxels at each time point
(i.e., T1, T2, and T3). Last, the intersection (i.e. common voxels) of the masks from all three-time points was calculated to ensure the same voxels were being evaluated.

DTI data were analyzed by FSL (version 5.0.9), the FMRIB Software Library (http://fsl.fmrib.ox.ac.uk/fsl). First, diffusion-weighted data were corrected for motion and eddy current artifacts by using FMRIB’s diffusion toolbox. Then, a brain extraction tool was used to extract images for removing non-brain tissue and for each subject a binary brain mask was created. Next, a diffusion tensor model was fitted in each voxel and diagonal elements ($\lambda_1$, $\lambda_2$, $\lambda_3$), fractional anisotropy (FA) and mean diffusivity (MD) images were generated using FSL’s DTIFIT. All subjects’ maps were aligned to FMRIB58-FA template in Montreal Neurological Institute (MNI) 152 standard space and re-sampled to a spatial resolution $1\times1\times1$ mm$^3$ using FMRIB’s nonlinear registration tool FNIRT. In the same manner, the transformations were applied to the FA and MD maps for each subject. The right insula mask was resampled to $1\times1\times1$ mm$^3$ (from $2\times2\times2$ mm$^3$) and applied to FA and MD maps.

**Neurocognitive Measures**

Neurocognitive assessment included standard measures of verbal learning and memory [Hopkins Verbal Learning Test – Revised (HVLT-R)]$^{18}$; nonverbal learning and memory [Brief Visuospatial Memory Test – Revised (BVMT-R)]$^{19}$, language [Controlled Oral Word Association Test (COWAT)]$^{20}$; and Animal Fluency]$^{21}$, attention and executive functioning [Wechsler Adult Intelligence Scale (WAIS)]$^{22}$ and Trails A and B], processing speed (WAIS-IV Coding$^{22}$) and mood (BDI-II) were administered to participants at each time point of T1, T2 and T3. Alternate test forms were used, when possible, to minimize practice effects.

**Statistical Analysis**
A general statistical linear model (GLM) of repeated measures ANOVA was applied using SPSS (Version 22.0. Armonk, NY: IBM Corp.) to assess CBF, functional connectivity, diffusion, gray matter density and neurocognitive performance across three time points, (T1, T2, T3). We were primarily interested in how athletes’ brain measures changed across time; thus, we hypothesized changes in mean measures, either by T2 or T3, relative to T1. This hypothesis led to two contrasts: change from pre- to post-season (∆T₁₂) and change from pre-season to three months post-season (∆T₁₃). We were also interested in examining neurocognitive function across time and hypothesized that there would be no significant change in neurocognitive performance across the three time points.

RESULTS

Participant Characteristics

Table 1 summarizes demographic information of 15 African-American athletes. Five athletes reported one prior concussion (greater than one year) with only one with loss of consciousness. All 15 subjects completed a brief interview that included concussion history and a neuropsychological evaluation, with 12 subjects also completing the MRI scans. One athlete, however, was excluded from imaging analysis due to high motion of >3mm and >3 degrees across all MRI techniques. Thus, imaging analyses were performed on 11 athletes.

Neuroimaging Results

Global CBF was reduced from pre-season (67.9 mL/100g/min) to post-season (66.4 mL/100g/min) and three-months post-season follow up (60.8 mL/100g/min), ∆T₁₂: p=0.59, ∆T₁₃: p=0.01. To evaluate which brain regions may have contributed to the CBF decrease, we conducted a whole brain voxel-based analysis (VBA). Figure 1 shows the VBA results. We found a significant decrease in blood flow at three-months post-season in the right insula.
(Brodmann Area 13) compared with pre-season; MNI Coordinate [+42 -4 +10], T-Score=5.30. No other regions or contrasts were significant based on the threshold of p<0.05 [FWE-Corrected] and k≥112 mm³.

Following the observed CBF alterations in the right insula region, we characterized the functional connectivity by setting the seed region as the right insula. The components of this network consist of bilateral insula and middle cingulate gyrus, also known as the Salience Network. Figure 2A shows the average functional connectivity maps of the insula network for pre-season, post-season and three-months post-season. The functional connectivity of the right insula, the seed region, did not change from pre-season (T1) to post-season (T2) or three months post-season (T3), ΔT₁₂: p=0.41, ΔT₁₃: p=0.92. However, left insula showed an increase from T1 to T2 and T3, ΔT₁₂: p=0.04, ΔT₁₃: p=0.09. Additionally, middle cingulate gyrus connectivity showed a significant increase at the end of season, ΔT₁₂: p=0.001, but subsided at the three-month follow-up, ΔT₁₃: p=0.14. Interestingly, the average connectivity of the Salience Network (combined left insula, right insula and middle cingulate gyrus) showed a significant difference between T1 and T2 (ΔT₁₂: p=0.014) but not between T1 and T3 (ΔT₁₃: p=0.15), see Figure 2B.

We measured FA and MD of the right insula via DTI MRI and did not find a change in FA at T2 or T3 compared to T1, ΔT₁₂: p=0.18, ΔT₁₃: p=0.30 (Figure 3). However, the MD measure increased from pre- (T1) to post-season (T2) and remained elevated at the three-month follow-up (T3), ΔT₁₂: p=0.007, ΔT₁₃: p=0.001. Neuroimaging values are displayed in Table 2.

Neuropsychological Test Results

There were no significant changes on any neuropsychological tests from pre- (T1) to post-season (T2). There were, however, increased test scores from pre-season (T1) to three-months post-season (T3) on letter fluency (p=0.006), digit span (p=0.006), and coding (p=0.02). There
was no significant change on HVLT-R, BVMT-R, category fluency, Trails A and B, or BDI (Table 3).

DISCUSSION

This study utilized multimodal imaging and neuropsychological measures in a sample of non-concussed high school athletes across three time points. Neuroimaging results in our sample showed a global decrease in brain perfusion predominantly in the right insula, which is consistent with findings from high school and college athletes following mild head injury. Wang et al. (2016) investigated high school and collegiate football athletes within 24 hours of a concussion and at eight days post-injury and found a significant decrease in CBF of several regions including the right insula at eight days post-injury compared to the first 24 hours after the concussion. Similarly, Meier et al. (2015) found that the right insula and superior temporal sulcus perfusion, as measured by pCASL MRI, decreased in college athletes post concussion and remained low for “slower-to-recover” athletes. This is an interesting finding since the insular cortex is a deep brain region that is known to be involved in sensorimotor processing (pain perception), cognitive processing (attention and salience processing), and emotional processing (depression and anxiety).

Neuroimaging Findings

The insula, as part of the salience network, is often associated with coordinating activation of the central executive network (CEN) and default mode network (DMN). This intricate communication system initiates appropriate responses to external stimuli. Previously, Abbas et al. showed hyperconnectivity of the default mode network after a single athletic season in high school football athletes without concussion. Similarly, we observed an increase in connectivity of the salience network in our non-concussed athletes. The increase in
connectivity of the left insula and middle cingulate may have occurred as a means to compensate for the reduced blood flow of the right insula.

Recently, Gong et al. (2018) reported an increase in mean diffusivity of gray matter following one season of high school football. Similarly, we evaluated diffusivity of the right insula and found that the right insula’s FA did not show any changes. However, MD, as measured by DTI, increased from pre- to post-season and remained elevated at three-months post-season. MD, an inverse measure of the membrane density, may be a more specific biomarker of gray matter microstructural change compared to FA, particularly after a TBI. These results suggest that a single season of playing football can influence microstructural changes of deep gray matter regions similar to our insula findings. Gong et al., (2018) suggested that during the recovery phase as the initial inflammatory response diminishes, the cell body and axons degenerate, which cause changes in the microstructure. It is important to note that in our sample, the MD remained elevated at three months post-season which warrants further longitudinal evaluations.

**Neurocognitive Findings**

Neurocognitive performance did not show meaningful changes from pre- to post-season or at three-months post season. Athletes showed some improvements on a few cognitive measures at three months post-season; however, this improvement may be attributable to practice effects as well as developmental maturation over the course of the season. It is worth noting that essentially all test scores improved from baseline. Only two scores (HVLT and Trails B) decreased slightly from pre- to post-season, though not significantly, and then improved by three months post season. In examining the literature in terms of neurocognitive performance across a season of a football, neurocognitive scores tend to remain stable. While our findings may suggest
neurophysiological and structural changes in these young football players, these differences were not associated with cognitive effects. As noted, others have found neurophysiological and neurostructural changes following one season in non-concussed athletes, including hyperconnectivity of the default mode network, reduced FA of the corpus callosum, and higher MD as measured by DTI. However, in the absence of consistent or meaningful behavioral changes in relation to these neurophysiological and neurostructural changes, the clinical implications, if any, remain unclear.

Limitations

The present findings need to be interpreted cautiously in light of several key limitations, including the small sample size and lack of a comparison group. Despite these limitations, we were able to replicate previous findings in multimodal MRI techniques across time. Future studies may benefit from a more expanded longitudinal investigation to examine neurocognitive and neurophysiological correlates over the course of multiple seasons and throughout various developmental periods (e.g., junior high, high school, collegiate, professional). Further, comparison of football players with other contact and non-contact sport athletes would improve our understanding of how brain structural and functional changes in relation to specific sport experiences.

Conclusion

The effects of repetitive head impact in the absence of a concussion are just beginning to be fully explored. The current findings demonstrated that playing a single season of football influenced functional and structural brain changes that continued at 3 months post-season. Specifically, the athletes showed a CBF decreased in the right insula, hyperconnectivity of salience network and structural changes across time. Overall, the research findings in these non-
concussed athletes resemble some of the findings from concussed athletes, which warrant more longitudinal studies.
ACKNOWLEDGEMENTS

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References


Table 1. Athlete Demographics

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<th>Range</th>
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<td>Education</td>
<td>10.5±0.7</td>
<td>9-11</td>
</tr>
<tr>
<td>Age</td>
<td>16.7±0.9</td>
<td>15-18</td>
</tr>
<tr>
<td>BMI</td>
<td>29.2±5.0</td>
<td>23.4-39.1</td>
</tr>
<tr>
<td>Total Concussions</td>
<td>0.33±0.49</td>
<td>0-1</td>
</tr>
<tr>
<td>Estimated FSIQ</td>
<td>92.7±8.1</td>
<td>75-104</td>
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Note: BMI – Body Mass Index; FSIQ – Full Scale Intelligence Quotient

Figure 1. Result of CBF voxel-based comparison superimposed on an average CBF map of all participants at p<0.05 [FWE Corrected] and k≥112 mm³. Right insula (in red) showed a linear reduction in blood flow from pre-season (T1) to three months post season (T3). Bar plot shows the normalized CBF of right insula at pre-, post- and three-months post-season.

Figure 2. (A) The average functional connectivity map of the football player group is overlaid on their average T1-weighted image. For illustration purposes, the z-score maps were arbitrarily thresholded (z-score ≥ 0.30, k ≥ 250) to qualitatively visualize the change in the intensity and cluster size. (B) Mean change in fcMRI z-scores are shown across time periods. The right insula (affected region per CBF analysis) does not show any change; however, left insula and middle cingulate gyrus show an increase in connectivity at post-season (T2), potentially a compensatory mechanism.
Figure 3. The right insula’s diffusivity as measured by FA and MD is shown. MD, a measurement of membrane density, showed a significant change at T2 and T3 compared to T1.

Table 2. Pre-season (T1), post-season (T2), and three-months post-season (T3) neuroimaging results per MR technique are shown (mean±SD).

<table>
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<tr>
<th>Time Points</th>
<th>Football</th>
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<tr>
<td></td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>CBF (Whole Brain)</td>
<td>67.9±7.6</td>
<td>66.4±9.7</td>
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<tr>
<td>fcMRI (R. Insula)</td>
<td>1.14±0.16</td>
<td>1.10±0.20</td>
</tr>
<tr>
<td>fcMRI (L. Insula)</td>
<td>0.60±0.13</td>
<td>0.74±0.14</td>
</tr>
<tr>
<td>fcMRI (Middle Cingulate)</td>
<td>0.43±0.09</td>
<td>0.58±0.14</td>
</tr>
<tr>
<td>fcMRI (All Nodes; Salience Network)</td>
<td>0.67±0.09</td>
<td>0.75±0.12</td>
</tr>
<tr>
<td>MD (L. Insula)</td>
<td>8.8E-04±7.0E-05</td>
<td>9.4E-04±5.1E-05</td>
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<tr>
<td>FA (L. Insula)</td>
<td>0.168±0.035</td>
<td>0.157±0.025</td>
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Notes: CBF, Cerebral Blood Flow; fcMRI, functional connectivity MRI; L/R, Left/Right; MD, Mean Diffusion; FA, Fractional Anisotropy.

Table 3. Neurocognitive Scores

<table>
<thead>
<tr>
<th>Time Points</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>∆T&lt;sub&gt;12&lt;/sub&gt;</th>
<th>∆T&lt;sub&gt;13&lt;/sub&gt;</th>
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<tr>
<td>HVLT-R Total (T)</td>
<td>29.73±11.43</td>
<td>27.33±9.80</td>
<td>34.47±12.56</td>
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<tr>
<td>BVMT-R Total (T)</td>
<td>40.13±11.82</td>
<td>46.73±10.19</td>
<td>45.47±11.97</td>
<td>0.115</td>
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<td>BVMT-R Delay (T)</td>
<td>42.27±12.76</td>
<td>44.33±9.91</td>
<td>41.27±10.24</td>
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<td>Category Fluency (T)</td>
<td>51.93±6.67</td>
<td>53.53±11.40</td>
<td>51.93±9.25</td>
<td>0.440</td>
<td>1.000</td>
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<tr>
<td>Letter Fluency (T)</td>
<td>52.73±11.19</td>
<td>57.67±11.11</td>
<td>59.33±9.71</td>
<td>0.269</td>
<td>0.006</td>
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<td>Digit Span (ss)</td>
<td>8.36±1.91</td>
<td>8.79±2.12</td>
<td>9.50±2.10</td>
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<tr>
<td>Trails A (T)</td>
<td>59.07±10.38</td>
<td>58.07±11.54</td>
<td>62.07±8.39</td>
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<td>Trails B (T)</td>
<td>54.73±10.41</td>
<td>58.53±10.60</td>
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<td>Coding (ss)</td>
<td>8.40±1.55</td>
<td>9.13±2.13</td>
<td>9.60±2.10</td>
<td>0.662</td>
<td>0.023</td>
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<tr>
<td>BDI-II (raw)</td>
<td>2.75±4.29</td>
<td>3.50±5.37</td>
<td>2.92±4.34</td>
<td>0.595</td>
<td>0.895</td>
</tr>
</tbody>
</table>

Note: BDI-II-Beck Depression Inventory, 2nd edition; HVLT-R – Hopkins Verbal Learning Test, Revised; BVMT-R - Brief Visuospatial Memory Test, Revised; T - T-score; ss – scaled score.
Footnotes

Contributors. ND conceived the project and designed the study. ND, SA, MC, and JH contributed to the study protocol and key data interpretation. ND, JS, and LF were involved in patient assessments, visits, and study coordination. LF performed the statistical analysis on the neurocognitive testing. SA conducted all the neuroimaging analysis and statistical analysis of the neuroimaging data. ND and LF wrote the initial draft of the paper. ND, SA, LF, JS, MC, and JH critically edited and revised the paper.

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Competing Interests. None declared.

Patient Consent. Informed consent was obtained from participants’ ≥18 years old. Participants <18 years old provided written assent with signed consent from their legal guardians.

Ethics Approval. The institutional review boards at University of Texas at Dallas and the University of Texas Southwestern Medical Center approved the protocol.

Data Sharing Statement. These data have not been previously published and are original data.
MRI Acquisition

We used four different MRI techniques to investigate functional and structural changes: a pCASL sequence was used to measure CBF, fcMRI was used to assess functional connectivity of the brain regions, DTI MRI to provide an assessment of brain structure integrity, and a high-resolution T1-weighted image as a structural reference. The details of the MRI parameters are as follow:

The pCASL MRI sequence parameters were: single-shot gradient-echo EPI, field-of-view (FOV) = 240 × 240, matrix = 80 × 80, voxel size = 3 × 3 mm², 27 slices acquired in ascending order, slice thickness = 5 mm, no gap between slices, labeling duration = 1650 ms, time interval between consecutive slice acquisitions = 35.5 ms, TR/TE = 4020/14 ms, SENSE factor 2.5, number of controls/labels = 30 pairs, RF duration = 0.5 ms, pause between RF pulses = 0.5 ms, labeling pulse flip angle = 18°, bandwidth = 2.7 kHz, echo train length = 35, and scan duration 4.5 min. The fcMRI sequence parameters were FOV = 220 × 220, matrix = 64 × 64, slice thickness = 4 mm, no gap between slices, voxel size = 3.44 × 3.44 × 4 mm³, 36 axial slices, TR/TE = 2000/30 ms, flip angle = 70°, 120 image volumes, and scan duration = 4 min. The DTI sequence parameters were single-shot spin-echo EPI, FOV = 224 × 224 mm², matrix = 128 × 128, slice thickness = 3 mm (includes 1 mm slice gap), voxel size = 1.75 × 1.75 × 3 mm³, 50 slices, TR/TE = 4410/51 ms, SENSE factor 2.5, 30 gradient-encoding directions with a b value of 1000 s/mm², and scan duration = 3.7 min. The high-resolution T1-weighted image parameters were magnetization prepared rapid acquisition of gradient-echo (MPRAGE) sequence, TR/TE = 8.3/3.8 ms, shot interval = 2100 ms, inversion time = 1100 ms, flip angle = 12°,
160 sagittal slices, voxel size = 1 × 1 × 1 mm³, FOV = 256 × 256 × 160 mm³, and duration 4 min.