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Differential Motor and Prefrontal Cerebello-Cortical Network Development: Evidence from Multimodal Neuroimaging

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Abstract

While our understanding of cerebellar structural development through adolescence and young adulthood has expanded, we still lack knowledge of the developmental patterns of cerebellar networks during this critical portion of the lifespan. Volume in lateral posterior cerebellar regions associated with cognition and the prefrontal cortex develops more slowly, reaching their peak volume in adulthood, particularly as compared to motor Lobule V. We predicted that resting state functional connectivity of the lateral posterior regions would show a similar pattern of development during adolescence and young adulthood. That is, we expected to see changes over time in Crus I and Crus II connectivity with the cortex, but no changes in Lobule V connectivity. Additionally, we were interested in how structural connectivity changes in cerebello-thalamo-cortical white matter are related to changes in functional connectivity. A sample of 23 individuals between 12 and 21 years old underwent neuroimaging scans at baseline and 12-months later. Functional networks of Crus I and Crus II showed significant connectivity decreases over 12-months, though there were no differences in Lobule V. Furthermore, these functional connectivity changes were correlated with increases in white matter structural integrity in the corresponding cerebello-thalamo-cortical white matter tract. We suggest that these functional network changes are due to both later pruning in the prefrontal cortex as well as further development of the white matter tracts linking these brain regions.

Keywords

cerebellum; development; diffusion tensor imaging; functional connectivity MRI; longitudinal

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1. Introduction

The cerebellum, though classically thought of as a motor structure, is now known to contribute to cognitive and affective processes as well (Desmond et al., 2005; Schmahmann and Sherman, 1998; Stoodley and Schmahmann, 2009; Stoodley et al., 2012). Importantly, both nonhuman primate and human neuroimaging investigations suggest that there are distinct circuits connecting motor and prefrontal regions of the cortex to different sub-regions of the cerebellum (Dum and Strick 2003; Kelly and Strick 2003; Krienen and Buckner 2009; Strick et al. 2009; O'Reilly et al. 2010; Salmi et al. 2010; Bernard et al. 2012; Bernard, Peltier, et al. 2014). Functional neuroimaging and meta-analytic evidence also provides support for the dissociated motor and non-motor regions within the cerebellum, and suggests that the structure has a unique functional topography (Stoodley and Schmahmann, 2009; Stoodley et al., 2012). Anterior regions of the cerebellum, particularly Lobule V, are associated with motor behaviors and motor-cortical networks, while lateral posterior regions such as Crus I have been associated with cognitive behaviors and prefrontal-cortical networks (Kelly and Strick 2003; Salmi et al. 2010; Bernard et al. 2012; Stoodley et al. 2012). Interestingly, there is also evidence showing interactions between the cerebellum and cortex during non-motor task performance measured with effective connectivity (Kellermann et al., 2012; O'Reilly et al., 2008; Sokolov et al., 2012). Crus I and II show effective connectivity with cortical regions during biological motion processing, attentional tasks, and perceptual tasks. Given the diverse functional contributions of the cerebellum, understanding the development of this structure and its networks with the cortex (via the thalamus; Strick et al., 2009) may be particularly informative for our understanding of both cognitive and motor development. Indeed, the cerebellum has been implicated in a variety of developmental psychopathologies including but not limited to, autism (Fatemi et al., 2012; Mosconi et al., 2015; Mostofsky et al., 2009; Ziats and Rennert, 2013), developmental coordination disorder (Bo and Lee, 2013; O'Hare and Khalid, 2002; Piek and Dyck, 2004; Zwicker et al., 2009), and psychosis risk (Jessica A. Bernard et al., 2014; Dean et al., 2013). An understanding of cerebellar development may be especially informative for our understanding of developmental psychopathology, and healthy cognitive and motor development more broadly.

The period of adolescence is characterized by rapid physical changes, along with continued brain growth and development that correspond with changes in executive function, social interactions, and increases in risk taking behaviors (reviewed in Paus 2005; Casey et al. 2008; Steinberg 2010; Blakemore 2012). Because of the wide range of functional contributions of the cerebellum, understanding the development of the networks connecting this important brain region to the rest of the brain, and particularly the prefrontal cortex may therefore be especially important. With respect to cerebellar structure, development has been relatively well studied. Prior research investigating whole brain development indicates that volume develops more slowly in higher order association cortices as compared to primary sensory and motor cortices (Gogtay et al., 2004). With respect to cerebellar development, it had also been suggested that regions associated with the prefrontal cortex would follow a similar more protracted developmental trajectory, and would be associated with cognitive progression (Diamond, 2000). That is, development would occur more slowly in these

cerebellar regions, and continue through late adolescence and into young adulthood. Indeed, investigations of regional cerebellar gray matter support this notion (Bernard et al., 2015; Tiemeier et al., 2010a). Not only do the relationships between age and cerebellar gray matter volume differ across cerebellar sub-regions, but those regions that are typically associated with cognitive functions and have know connections with the prefrontal cortex (eg. Crus I, Crus II; Krienen and Buckner, 2009; O'Reilly et al., 2010; Bernard et al., 2012) show non-linear relationships with age (Bernard et al., 2015; Tiemeier et al., 2010a). Peak volume in these regions is seen during later adolescence and into early adulthood. Finally, recent work specifically investigating non-linear gray matter associations with age revealed a network of regions that followed a quadratic "inverted-u" pattern (Douaud et al., 2014). Most critically, this network includes higher order association cortices (prefrontal and parietal), but also lateral posterior regions of the cerebellum that are associated with the prefrontal cortex and cognitive function (Douaud et al., 2014). However, though studies of brain volume indicate that cerebellar regions associated with the prefrontal cortex and cognitive function show a more protracted pattern of development, investigations of functional networks between the cerebellum and cortex have not been investigated during development, particularly during adolescence and into young adulthood when these regions reach maturity.

While investigations into regional cerebellar morphology have provided important insights into our understanding of brain development, we still lack an understanding of how *networks* between the cerebellum and cortex develop during adolescence through young adulthood. Interactions between regions are necessary for behavioral performance, and taking a network perspective to cerebellar development, particularly how it relates to the prefrontal cortex, is especially informative. Using a longitudinal study design, we investigated cerebellar functional networks in a sample of healthy adolescents and young adults. Individuals underwent brain imaging at baseline and at follow-up one year later. Our objective was to investigate differential development of cerebello-cortical motor and prefrontal networks. We hypothesized that functional networks of the posterior lateral aspects of the cerebellum (eg. Crus I and Crus II) which have known connections with the prefrontal cortex (Kelly and Strick 2003; Krienen and Buckner 2009; O'Reilly et al. 2010; Salmi et al. 2010; Bernard et al. 2012) will show protracted patterns of development, particularly when compared with anterior Lobule V which is associated with motor cortex and motor function. Specifically, we expected to see changes in resting state connectivity over time when using Crus I and Crus II seeds, but we did not expect to see any changes when using lobule V given its motor nature. Though cerebello-cortical networks have not been investigated from a developmental perspective, cross-sectional work comparing prefrontal connectivity between children and adults has demonstrated decreased connectivity with development (Kelly et al., 2009), and given the prefrontal regions associated with Crus I and Crus II, we expected to see a similar pattern here. Correlations between Crus I and II and the prefrontal cortex would then be lower at follow-up. Furthermore, we were interested in whether these changes in resting state connectivity were associated with changes in structural connectivity of white matter circuits connecting the cerebellum to the cortex (via the thalamus). Thus, we also mapped cerebello-thalamo-cortical structural connectivity to assess whether it was correlated with functional connectivity, quantified using fractional anisotropy (FA), and associated with developmental changes in these resting state networks.

Higher FA values are thought to be indicative of better white matter integrity. A review across multiple studies combining structural and functional connectivity indicates that resting state functional connectivity strength is typically positively associated with structural connectivity (Damoiseaux and Greicius, 2009). With this in mind, we predicted that changes in FA would be correlated with developmental differences in resting state cerebello-cortical connectivity.

2. Methods

2.1 Participants

Healthy participants between the ages of 12 and 21 were recruited to participate as controls in a broader longitudinal study of psychosis risk. All participants were healthy and absent of any psychopathology and substance dependence as assessed by the Structured Clinical Interview for Axis-I DSM-IV Disorders (First et al., 1995). Furthermore, all participants were free from prior head injury, and screened to ensure eligibility for the magnetic resonance imaging environment. The current analyses include data from those individuals who have completed neuroimaging at two time points, baseline and 12-months later. A total of 78 people have been recruited to the study thus far and 42 were eligible for 12-month follow-up assessments at the time of data processing and analysis. Of the 42 individuals eligible for follow-up, 3 declined to complete the baseline scan, and 5 additional individuals declined to complete the scan at 12-month follow-up. 2 individuals were no longer interested in participating, 1 individual moved, and we were unable to get in touch with 5 of our participants from baseline. Thus, 26 individuals completed baseline and 12-month follow-up including scans at both time points. Finally, 3 additional participants were excluded due to issues with data quality and collection at the scanner. 23 individuals were ultimately included in our analyses (12–21 years old; mean age 17.78 ± 2.73 years; 13 females). The study procedures were approved by the University of Colorado Boulder Institutional Review Board, and all participants completed an IRB approved consent form. For individuals under the age of 18, parents provided consent, and the participant provided their assent to participate.

2.2 Brain Imaging Procedure

All individuals completed a brain imaging session that included structural, DTI, and fcMRI scans. All of the scans were acquired using a 3-Tesla Siemens Tim Trio MRI scanner (Siemens AG, Munich, Germany) using a standard 12-channel head coil. Structural images were acquired with a T1-weighted 3D magnetization prepared rapid gradient multi-echo sequence (MPRAGE; sagittal plane; repetition time [TR] = 2,530 ms; echo times [TE] = 1.64 ms, 3.5 ms, 5.36 ms, 7.22 ms, 9.08 ms; GRAPPA parallel imaging factor of 2; 1 mm^3 isomorphic voxels, 192 interleaved slices; FOV = 256 mm; flip angle = 7°; time=6:03 min). A five minute 34 second resting state blood-oxygen-level dependent (BOLD) scan was acquired with a T2-weighted echo-planar functional protocol (number of volumes = 165; TR = 2,000ms; TE = 29 ms; matrix size = 64 × 64 × 33; FA = 75°; 3.8 × 3.8 × 3.5 mm^3 voxels; 33 slices; FOV = 240 mm). Participants were instructed to relax with their eyes closed during this time. A turbo spin echo proton density (PD)/T2-weighted acquisition (TSE; axial oblique aligned with anterior commissure-posterior commissure line; TR = 3,720 ms; TE =

89 ms; GRAPPA parallel imaging factor of 2; FOV = 240 mm; flip angle: 120°; 0.9 × 0.9 mm^2 voxels; 77 interleaved 1.5 mm slices; time= 5:14 min) was generated to investigate incidental pathology. Studies indicate that the functional connectivity MRI (fcMRI) duration utilized in the present study provides equal power to longer scan times (Van Dijk et al., 2010). Furthermore, shorter scan durations may be optimal in developmental populations so as to minimize subject motion in the scanner. Structural connectivity was assessed with a diffusion-weighted scan (71 gradient directions; TR = 9600 ms; TE = 86 mm; GRAPPA parallel imaging factor 2; β-value = 1000 s/mm^2; FOV = 256 mm; 72 slices; 2 mm^3 isomorphic voxels; 7 β0 images; time= 11:13 min).

2.3 fcMRI Data Processing and Analysis

Data were first preprocessed in FSL (v.5; http://fsl.fmrib.ox.ac.uk/fsl), which involved motion correction, brain extraction, high-pass filtering (100 s), and spatial smoothing (6mm FWHM). Functional images were aligned to the MNI 2-mm brain template with a two-step procedure. First, the resting state scan was aligned to the high-resolution MPRAGE using a linear boundary-based registration method, which relies on white matter boundaries (Greve and Fischl, 2009; Jenkinson and Smith, 2001; Jenkinson et al., 2002). Second, the MPRAGE was nonlinearly aligned to the template (Andersson et al., 2010), and the two registrations were then combined to align the functional resting state scan to the template.

Recent papers have demonstrated the importance of properly correcting for motion by not only regressing out motion parameters, but also removing specific frames with motion outliers (motion scrubbing; Power et al. 2012). To accomplish this, we used the Artifact Rejection Toolbox (ART; http://www.nitrc.org/projects/artifact_detect/) to create confound regressors for motion parameters (3 translation and 3 rotation parameters) and to remove specific image frames with outliers based on brain activation and head movement. In order to identify outliers in brain activation, the mean global brain activity (i.e., the mean signal across all voxels) was calculated as a function of time, and was then Z normalized. Outliers were defined as any frames where the global mean signal exceeded 3 SD. Similarly, frame-wise measures of motion (composite measure of total motion across translation and rotation) were used to identify any motion outliers (i.e., motion spikes). Motion outliers were defined as any frame where the motion exceeded 1 mm.

All functional connectivity analysis was performed in the CONN toolbox version 14.p (Whitfield-Gabrieli and Nieto-Castanon, 2012), implemented with SPM8 (www.fil.ion.ucl.ac.uk/spm). Anatomical images were segmented into gray matter, white matter, and CSF with SPM8 in order to create masks for signal extraction. The Conn toolbox uses principal component analysis to extract 5 temporal components from the segmented CSF and white matter, which were entered as confound regressors in the subject-level GLM. This approach corrects for confounds of motion and physiological noise without regressing out global signal, which has been shown to introduce spurious anti-correlations (Chai et al., 2012; Murphy et al., 2009). Motion from the ART toolbox was also included as a confound regressor. In addition, ART identifies frames that are outlier in terms of framewise displacement, and creates regressors for each outlier in order to regress out those frames during analysis. These were also included as confound regressors to account for outliers.

The data also underwent linear detrending. Finally, all time series data were preprocess using a band-pass filter (0.008 to 0.09 Hz) to ensure analyses were completed within the frequency band of interest after the regression of the confounding variables. Lobule V, Crus I, and Crus II were used as seed regions. Masks of these regions were defined using the SUIT atlas (Diedrichsen, 2006; Diedrichsen et al., 2009), and these methods parallel those previously used in our work on cerebello-cortical resting state connectivity (Bernard et al. 2012, 2013; Bernard, Dean, et al. 2014). The mean time-series, averaged across all voxels within each seed region, was used as a predictor regressor, and correlated with all other voxels in the brain in seed-to-voxel connectivity analyses.

We conducted analyses to investigate the effect of development over 12-months on resting state connectivity. Connectivity between the seed ROI in the cerebellum was calculated with all other voxels in the brain. To investigate development over the 12-month period, differences between the two time points were assessed using a within-subjects t-test for all 3 cerebellar seed regions. Because we were also interested in the relationships between white matter development and functional connectivity development, we conducted additional regression analyses including FA from the corresponding white matter tracts (see below for DTI methodology and preprocessing details). These analyses investigated regions where functional connectivity strength was associated with FA (for more detail, see section 2.5, Analysis of the Relationships Between Structural and Functional Connectivity) All connectivity results were first thresholded at the voxel-level at $p_{uncorr} < .001$ and then corrected at the cluster-level to a false-discovery rate (FDR) of $p < .05$ (Chumbley and Friston, 2009). Finally, average z-scores from clusters that showed significant effects of development (connectivity differences over time from our within-subjects t-tests) were extracted and were used for additional correlation analyses with the DTI, implemented in SPSS Statistics version 22 (IBM Corporation).

2.4 DTI Data Processing and Analysis

Masks of right Lobule V and Crus I in the cerebellum were identical to those used as seed regions for the fcMRI analyses. We created additional masks of the left motor region of the thalamus and the left prefrontal region of the thalamus, defined using the tractography-based segmentation of Johansen-Berg and colleagues (Johansen-Berg et al., 2005) included in FSL. Finally, we included masks of the left primary motor cortex (M1) as defined by the Jülich histological atlas (Geyer et al., 1996), as well as the middle frontal gyrus (MFG) as defined by the Harvard-Oxford cortical atlas (Desikan et al., 2006), both of which are available in FSL. The masks were thresholded at 10% and then binarized for use in our analyses. The MFG was chosen based on the work of Salmi and colleagues (Salmi et al., 2010), which demonstrated that tractography originating in Crus I/II of the cerebellum was connected, via the thalamus, with large regions of the lateral prefrontal cortex. Furthermore, they demonstrated structural connectivity between Lobule V, also via the thalamus, with the primary motor cortex (Salmi et al., 2010). Because there is limited work investigating tractography between the cerebellum and cortex, we aimed to replicate the findings of Salmi and colleagues (2010) as closely as possible to ensure the tracts were being effectively mapped, and to aid in the interpretation of our findings.

Diffusion weighted images were processed using FSL's FDT toolbox. Images were first corrected for motion and eddy current distortions. Diffusion parameters were calculated at each voxel, accounting for crossing fibers in two directions using *BEDPOSTX* (Bayesian Estimation of Diffusion Parameters Obtained using Sampling Techniques) (Behrens et al., 2007). Separate probabilistic tractography analyses were performed between right Lobule V and the motor subregion of the thalamus and then from the motor subregion of the thalamus to M1. A similar two-step approach was taken from Crus I to the prefrontal subregion of the thalamus, and then on to the MFG. All tractography analyses were implemented in FSL using probtrackX using a step length of 0.5 mm, 5,000 streamlines from each voxel, a fiber threshold of 0.1, and modified Euler streamlining. Tracking was stopped when the streamline reached the edge of the brain mask, when tracking reached 2000 steps (equivalent to a distance of 1 m), or when the pathway exceeding ± 80 degrees from one step to the next.

All tractography was performed in individual subject space and then warped to standard MNI space, using nonlinear transformations between individual subject diffusion space and MNI standard space. In order to create a group average tract map, each individual subject tract map was divided by the total number of streamlines from the seed mask (i.e., the waytotal), thresholded at 10% to limit noise, and then binarized to create a mask. We summed together the individual masks, and divided by the total number of subjects to create the group probability map. This procedure was done for each seed mask. These maps were thresholded so that the tract passed through a given voxel in at least 50% of subjects. These maps were then visualized using FSLView and corrected to include only the tract connecting the thalamus to the motor or prefrontal cortex, respectively. Importantly, we investigated these tracts as two segments. The first segment was from the cerebellum to the relevant region of the thalamus, and the second segment was from the thalamus to the cortex. Mean FA was extracted from each tract segment for all subjects. Figure 1 shows the average Lobule V and Crus I loops across participants. Statistical analyses to investigate FA changes with development were completed using SPSS. Paired t-tests were used to investigate differences in each tract segment over the course of 12 months, and a repeated measures ANOVA was computed to investigate group by region interactions. That is, we were investigating whether or not patterns of development differed between the motor and prefrontal tract segments.

2.5 Analysis of the Relationships Between Structural and Functional Connectivity

We were particularly interested in whether and how FA is associated with changes in functional networks over time as assessed using resting state connectivity. Thus, two sets of analyses were computed. In both sets of analyses, we used FA difference scores, calculated by subtracting baseline FA from 12-month FA (time 2 – time 1) in all of the tracts of interest. Positive values indicate increases in FA over the 12-month period, while negative values indicate decreases.

First, we investigated whether or not FA is correlated with cerebellar resting state connectivity at baseline and 12-month follow up using regression models implemented in CONN to establish links between structural and functional connectivity in this sample. In these analyses, Crus I and Crus II connectivity was investigated only with Crus I-thalamic

and thalamo-prefrontal FA values, while Lobule V connectivity analyses were looked at only with the Lobule V-thalamic and thalamo-motor FA values. The Crus I/II connectivity patterns were investigated with tractography starting in Crus I given that the tracts mapped in this investigation parallel those mapped by Salmi and colleagues (Salmi et al., 2010). While resting state connectivity measures suggest that the functional networks of these regions are somewhat distinct (Bernard et al. 2012), we would expect partially overlapping white matter tracts would connect these regions, particularly via the cerebellar peduncles and through the anterior thalamic radiation.

Second, we were interested in whether FA change was correlated with functional connectivity specifically in regions that showed developmental change over 12-months. Thus, as described in section 2.3, average z-scores of regions showing significant changes in cerebello-cortical functional connectivity over time were extracted. We then investigated correlations between these z-scores and FA change in the corresponding white matter tracts using SPSS to understand the relationship between functional and structural connectivity development.

3. Results

3.1 Cerebello-Thalamo-Cortical Resting State Network Development

At both the baseline and 12-month scan session, average motion was very low, and did not differ between the two sessions (baseline: .32 ± .48 mm; follow-up: .21 ± .09 mm; $t_{(23)}$=. 991, p=.3). Likewise, very few outliers were scrubbed from the data at both time points. There were fewer outliers at time 2, though this relationship was only approaching trend-level (baseline: 8.0 ± 6.43 outliers; follow-up: 4.78 ± 5.62 outliers; $t_{(23)}$=1.73, p=.1). Most importantly, these analyses indicate that there was minimal motion at both time points, on average below .5 mm. Furthermore, the lack of significant differences in both average motion and the number of outlier frames does not differ across the two time points, suggesting that our findings are not confounded by the influence of motion, and decreases in motion with development.

Resting state analysis of Lobule V, Crus I, and Crus II, averaged over the two time points revealed distinct motor and prefrontal cortical networks, comparable to prior analyses investigating resting connectivity in these regions (Habas et al. 2009; Krienen and Buckner 2009; O'Reilly et al. 2010; Bernard et al. 2012) (Supplementary Table 1). Consistent with our hypotheses, there was no effect of time in Lobule V resting state connectivity, evidenced by the lack of significant differences when baseline and 12-month connectivity were compared. However, there were significant differences between baseline and 12-month follow-up of connectivity for both Crus I and Crus II. Connectivity between Crus I and both the dorsolateral prefrontal cortex and the paracingulate cortex decreased with development, while connectivity between Crus II decreased with regions of the lateral frontal pole. Specific regions showing significant main effects of time can be seen in Figure 2, and statistical information and coordinates are presented in Table 1. Thus, it seems to be the case that developmental effects continue in cerebello-frontal networks later into adolescence and young adulthood, and this is in contrast to cerebello-motor networks, where no differences are seen.

Because the changes and development that occur over a 12-month period are likely to be quite dissimilar for different age ranges (e.g., 12 and 13 year olds as compared to 20 and 21 year olds), we completed an additional follow-up analysis of functional connectivity over time where we controlled for age. This additional analysis allowed us to investigate whether the effects of development are still present in this sample, while accounting for potential differences in the magnitude of the effects at the extreme ends of our age range. Using paired t-test controlling for age, we found that there are significant decreases in Crus I connectivity with prefrontal cortex over 12 months, though the regions are centered in slightly different areas of prefrontal cortex (please see Supplementary Table 2). Connectivity was lower at 12-month follow-up than at baseline. There were no significant findings for Crus II, and as in our initial analysis, these effects were not present for Lobule V connectivity. This suggests that at least with respect to Crus I, though the developmental differences at the extreme ends of our age group are likely different in magnitude, such changes in this wide age group are still present. This provides further support for our finding that development continues in cerebello-frontal networks into late adolescence.

3.2 FA and Associations With Resting State Networks

For all analyses including FA, one individual was excluded as an outlier because his or her mean values were greater than 3 standard deviations from the group mean. 23 individuals were therefore included in all of the analyses related to FA.

With respect to changes in FA over time, paired t-tests indicated that there were numerical increases in FA, though these were not statistically significant (for all tracts $p>.15$). Furthermore, our repeated measures ANOVA indicated that there were no significant region (motor versus prefrontal) by time point interactions (for both the cerebellar-thalamo tracts and cortico-cerebellar tracts, $p>.7$).

First, we completed regression analyses implemented in CONN to investigate links between cerebello-thalamo-cortical structural and functional connectivity in an adolescent population. FA values from the tracts corresponding to the cerebellar resting state seeds were correlated at both baseline and at 12-month follow-up. These results suggest that, consistent with prior work, structural and functional connectivity are correlated with one another (Damoiseaux and Greicius, 2009). However, the dynamics of these correlations differ in some cases. First, there were *negative* correlations between Crus I-thalamic FA and Crus I functional connectivity at baseline (trend-level), though the correlations between thalamo-prefrontal FA and Crus I functional connectivity were positive, which is consistent with the existing adult literature (Damoiseaux and Greicius, 2009). Furthermore, there were positive correlations between thalamo-motor FA and lobule V functional connectivity at baseline, though negative correlations were also observed. These correlations included premotor regions, somewhat consistent with what we would expect. Findings at 12-month follow-up were mixed but indicated some trend-level negative correlations. Statistical information and the coordinates for these correlations are presented in Table 2. Baseline correlations are illustrated in Figure 3.

While the above analysis provides insight into the associations between cerebello-thalamo-cortical FA and resting state connectivity in adolescents, this does not assess whether FA

changes are associated with the changes in Crus I and Crus II resting state connectivity over time. We were interested in understanding factors that contribute to functional connectivity development, and given the associations between structural and functional connectivity, structural connectivity was a key area of interest. To probe this, we first extracted the connectivity value for each of the significant clusters (averaged across all voxels in the cluster) that resulted from our comparison of baseline and 12-month networks (presented in Table 1). We then conducted correlation analysis with functional connectivity strength in significantly different clusters, and FA from both segments of the Crus I cerebellar-thalamo-cortical circuit. The FA difference in the Crus I-thalamic aspect of the tract was significantly correlated with the middle frontal gyrus (20, 34, 20) cluster ($r_{(22)}$=.44, p<.05) as well as the paracingulate (−6, 50, 6) gyrus cluster ($r_{(22)}$=.45, p<.05). Scatterplots illustrating these relationships are presented in Figure 4. However, there were no significant differences when we investigated the Crus II difference cluster. Thus, differences in white matter structural integrity are associated with functional connectivity development at least to some degree. Functional connectivity change between Crus I and the prefrontal cortex was associated with increases in FA over 12-months. Finally, to further explore how age and development may be impacting these associations, we investigated the correlation between age and FA in the Crus l-thalamic aspect of the tract. There was a trend-level positive correlation ($r_{(22)}$=.35, p=.1). It seems to be the case that in older adolescents FA increases, though there are decreases in younger adolescence.

4. Discussion

Using resting state functional connectivity, we investigated cerebello-thalamo-cortical network development. Consistent with our hypotheses, we found evidence for protracted development of the lateral-posterior cerebellar networks associated with the prefrontal cortex. Furthermore, our follow-up analysis incorporating white matter integrity data suggest that structural connectivity changes are associated with these developmental changes in functional connectivity. These important network results extend previous work demonstrating protracted structural development of the lateral-posterior regions of the cerebellum and prefrontal cortex (Bernard et al., 2015; Douaud et al., 2014; Tiemeier et al., 2010b) to include the functional networks associated with these regions, and are more broadly consistent with the finding that higher order cortices develop more slowly as compared to primary sensory cortices (Gogtay et al., 2004). Importantly, these findings extend this notion to suggest higher order *networks* also develop more slowly, and further our understanding of cerebellar development. They also have implications for our understanding of cognitive development as well as psychopathology.

4.1 Cerebellar Network Development

The cerebellum is associated with both motor and non-motor networks functional and structural networks (Dum and Strick 2003; Kelly and Strick 2003; Habas et al. 2009; Krienen and Buckner 2009; O'Reilly et al. 2010; Salmi et al. 2010; Bernard et al. 2012; Bernard, Peltier, et al. 2014), though to our knowledge, development of these networks during adolescence has not been previously investigated. Though there is a growing literature on structural development of the cerebellum and cerebellar subregions during this

period of development (Bernard et al., 2015; Tiemeier et al., 2010a), understanding functional network connectivity is particularly important, as it may also provide insight into cognitive development. Future work would benefit from investigating these associations.

Consistent with our hypotheses, the regions of the cerebellum that are associated with the prefrontal cortex and cognitive function (Salmi et al. 2010; Bernard et al. 2012), Crus I and Crus II, show slower and later development, as evidenced by connectivity changes over time in this older sample across adolescence. However, there were no changes in the motor Lobule V functional connectivity networks. Interestingly, these regions of the cerebellum showing later network development are also areas that are phylogenetically younger, and thought to have evolved with the prefrontal cortex (Balsters et al., 2010). Indeed, it has been suggested that these lateral regions of the cerebellum should track with the prefrontal cortex, in keeping with the evolution of these regions, with respect to structural development (Diamond, 2000), which is known to be slower than that of primary sensory and motor regions (Gogtay et al., 2004). Development of prefrontal cortical and cerebellar regions is thought to be slower and occur over a longer period of time through adolescence and into young adulthood (Blakemore and Choudhury, 2006; Blakemore, 2012; Gogtay et al., 2004). We have extended this finding, and suggest that there is also protracted functional network development in these regions as well, that continues through adolescence and into young adulthood.

More generally, this is consistent with cross-sectional work investigating development of anterior cingulate functional network development (Kelly et al., 2009). Functional connectivity in the prefrontal cortex was higher in children in comparison with young adults. The changes in connectivity between the cerebellum and prefrontal cortex are broadly consistent with this finding. However, developmental investigations of other functional networks have demonstrated increased connectivity from childhood and adolescence into adulthood in the default mode network (Supekar et al., 2010) and in striatal-cortical functional networks (Bo et al., 2014). Thus, though our findings are consistent with the literature on cerebellar structural development (Bernard et al., 2015; Tiemeier et al., 2010a) and development of prefrontal resting state networks (Kelly et al., 2009), patterns of functional network connectivity seems to differ based on the networks and seed regions in question.

Over a period of 12 months we found that there were *decreases* in cerebello-prefrontal functional connectivity. It seems to be the case that these resting state networks are being further refined to include key regions over this period of time. The areas of connectivity are getting smaller over time, and perhaps are limited to key areas or areas that are most consistently correlated with one another. One contributing factor may be synaptic pruning. During development, microglia prune synapses throughout the brain (Paolicelli et al., 2011). While this primarily occurs during early development, synaptic pruning of the prefrontal cortex seems to occur later on, and continue during adolescence (reviewed in Blakemore and Choudhury, 2006). Thus, this later pruning may be contributing to these more refined networks with smaller regions of prefrontal connectivity seen over 12-months in our sample, though this may be an indirect mechanism and direct investigations of this notion are difficult. However, it is also of note that these findings are somewhat consistent with the

notion of decreased local connectivity with development, and increased longer-range connectivity (Supekar et al., 2009; Uddin et al., 2010). The decreases in connectivity with the prefrontal cortex may be due to more refined local connectivity in the PFC, and as such we saw a decrease in the correlations with the cerebellum. Finally, these findings are consistent with the notion that cortico-subcortical connectivity is less prominent in young adults where cortico-cortical connectivity is a more dominant feature (Supekar et al., 2009). However, as our findings demonstrate, the development of these networks and changes in connectivity vary by region within the cerebellum, and further underscores the importance of investigating regional differences within the cerebellum.

Furthermore, our data also suggest that cerebello-thalamo-cortical structural integrity is also contributing to these changes. Though we only saw trend-level increases in FA of the Crus I-thalamo-prefrontal tract, there were significant associations with resting state connectivity. FA in the cerebello-thalamo-cortical tracts was correlated with functional connectivity of corresponding cerebellar seed regions, consistent with prior work and general trends in the literature (Damoiseaux and Greicius, 2009). We extend this work to show that structural connectivity is related to functional connectivity during development, and in cerebello-thalamo-coritcal networks. However, it is of note that not all of our associations were positive. At baseline, and at 12-months, we found negative correlations between FA, particularly when looking at the cerebellar-thalamic tract segments. While this is in contrast to the findings of Damoiseaux and Greicius (2009), there are several points to consider. First, this review only included 8 studies, and they looked primarily at adults, and in several cases older adults. Here, we are investigating adolescents, and the dynamics of these interactions may change over the course of development. Furthermore, Damoiseaux and Greicius (2009) reviewed cortical studies. Sub-cortical networks with the cortex, such as those with the cerebellum, may not follow the same pattern, and that seems to be the case here. Cerebellar-thalamic segments showed a negative association, though positive correlations were seen with the thalamo-cortical tracts. Additional work, particularly focused on cortico-subcortical connectivity and development is needed to better understand these associations. Finally, resting state connectivity may also be instantiated through other connections, while structural connectivity is a direct connection between regions. While this work further supports the relationships between structural and functional connectivity, it also further highlights the notion that other factors and circuits may contribute to functional connectivity.

However, it is of note that in our analyses investigating FA and connectivity values in areas showing developmental differences, these correlations are positive (Figure 4), consistent with the prior literature (Damoiseaux and Greicius, 2009). Within these regions where connectivity decreases over 12-months, those with the strongest connectivity with the cerebellum have the highest FA increases in the corresponding white matter tracts. While these correlation analyses do not allow us to investigate the directionality of this relationship, we would speculate that the structural development is impacting the functional changes that we are seeing. Furthermore, it is of note that the changes in FA were not significant over 12-months, but this suggests that even subtle changes in structural connectivity may impact brain function and functional networks. Thus, this work provides

important insight into the relationships between structural and functional network development of cerebello-cortical networks.

Though not a focus here, it is important to consider the impact of these network changes on cognitive development. Cognition continues to develop across domains throughout adolescence and into adulthood (Bernard et al., 2015; Hartshorne and Germine, 2015), and effective connectivity analyses of cerebello-cortical interactions during attentional and perceptual tasks highlight the potential for plasticity in these circuits (Kellermann et al., 2012; O'Reilly et al., 2008; Sokolov et al., 2012). Furthermore, cerebellar functional connectivity has been associated with executive function and working memory (Bernard et al., 2013; Reineberg et al., 2014). The plasticity in these circuits, along with their continued development in parallel with cognitive development suggest that these may be involved in development of cognition. Future work would benefit from investigating cerebellar resting state and structural networks with respect to cognitive development during adolescence.

Finally, the cerebellum has been implicated in developmental psychopathology, including autism (Fatemi et al., 2012, 2008; Kern, 2002), psychosis risk (Bernard and Mittal, 2014; Dean et al., 2013; Mittal et al., 2014), and attention deficit disorder (Berquin et al., 1998; Bledsoe et al., 2011). A better understanding of the development of the cerebellum, particularly with respect to its interactions with the rest of the brain thus provides an important first step to understanding potentially aberrant patterns across these disorders. Future work looking at cerebellar resting state and structural network development in these important clinical populations may provide new insights in disease etiology and symptomatology.

4.2 Limitations

There are several limitations to consider with respect to this study. First, the 12-month period in the present study may impact our ability to detect effects, particularly in white matter, and ultimately limits our perspective of this dynamic developmental period. For example, further changes may still occur through young adulthood beyond the upward age cut-off. Indeed, Lebel and Beaulieu have demonstrated that while changes occur into adulthood (Lebel and Beaulieu, 2011; Lebel et al., 2012, 2008), beyond the ages of the individuals assessed here, and we have previously demonstrated that cerebellar structure in Crus I and Crus II do not reach their peak volume until closer to 30 years old (Bernard et al., 2015). However, we would expect that with a longer follow-up period our effects would be more robust, and this does not discount the findings presented here. In a related point, the trajectory of change may be different for younger participants than older participants. However, when we examined this in the present sample (covarying for age), the pattern of findings did not change. Second, we did not see any significant differences in white matter structural integrity over time. Though there were numerical increases in FA in all of the tracts that we investigated, these were not statistically significant. This pattern of weak trends is generally consistent with prior longitudinal investigations of white matter development (Bava et al., 2010; Lebel and Beaulieu, 2011) showing maturation in white matter tracts into adolescence. While the longitudinal approach combining both structural and functional networks measures is a strength of this investigation, it may be the case that

we do not see significant white matter differences due to the time course of our investigation as noted above. The data from Bava and colleagues were collected at two time points 16-months apart (Bava et al., 2010), while that follow-up scan conducted by Lebel and Beaulieu occurred 4 years after the baseline scan (Lebel and Beaulieu, 2011). Thus, these changes may take place more slowly and as a result our data were not ideal for assessing this type of change. Additional follow-up data may therefore be necessary in this sample to see significant structural change. Third, the age range included in this investigation is quite large (12–21 years of age at baseline). This range was chosen to represent the large period of adolescence, though it is critical to note that development and brain structure and function differ greatly between those at the younger and older ends of our age range. Younger adolescence experience rapid changes socially, physically (eg. puberty), and neurally, and while some of these changes continue with further maturation later in adolescence (as demonstrated here) the effects of development may differ in magnitude at the two ends of our sample. While our supplementary analysis controlling for age suggests that developmental changes are present in this period when these potential differences in the magnitude of change are partially accounted for, the current study does not allow us to effectively account for these differences in developmental scale. Future work with larger samples that allows for both cross-sectional and longitudinal investigations of early and late adolescence is needed. Fourth, though we found evidence for protracted development of Crus I and Crus II networks in our functional connectivity data, this was not the case in the corresponding structural networks. However, as noted above, the time scale of our investigation may not be optimized for looking at white matter structural changes. Upon further follow-up, we would expect to see these patterns in our sample. Finally, it is important to note that we are combining structural and functional connectivity measures. While structural connectivity represents direct connections between regions (though not necessarily monosynaptic as is the case with cerebello-thalamo cortical networks), the connections underlying functional connectivity are not as clear. They may not be monosynaptic and indeed functional connectivity can also be present in cases where there are no structural connections (reviewed in Damoiseaux & Greicius, 2009). Thus, though we demonstrate relationships between structural and functional connectivity, other factors may also be influencing these functional methods, and this may in part, contribute to some of our mixed findings (negative correlations) at baseline and follow-up.

5. Conclusions

Here, we combined functional and structural connectivity to investigate cerebello-thalamo-cortical network development in adolescents and young adults. This work extends our understanding of cerebellar development to the network domain. We demonstrated protracted development in Crus I and Crus II networks associated with prefrontal cortex, but there were no changes in the Lobule V motor network. Furthermore, the functional network changes are associated with structural network changes in the corresponding white matter tracts. We suggest that decreases in functional connectivity represent a refining of these networks due to both synaptic pruning, as well as increases in structural connectivity. Future work including cognitive variables is warranted, and an extension of these findings into clinical populations may also be especially informative.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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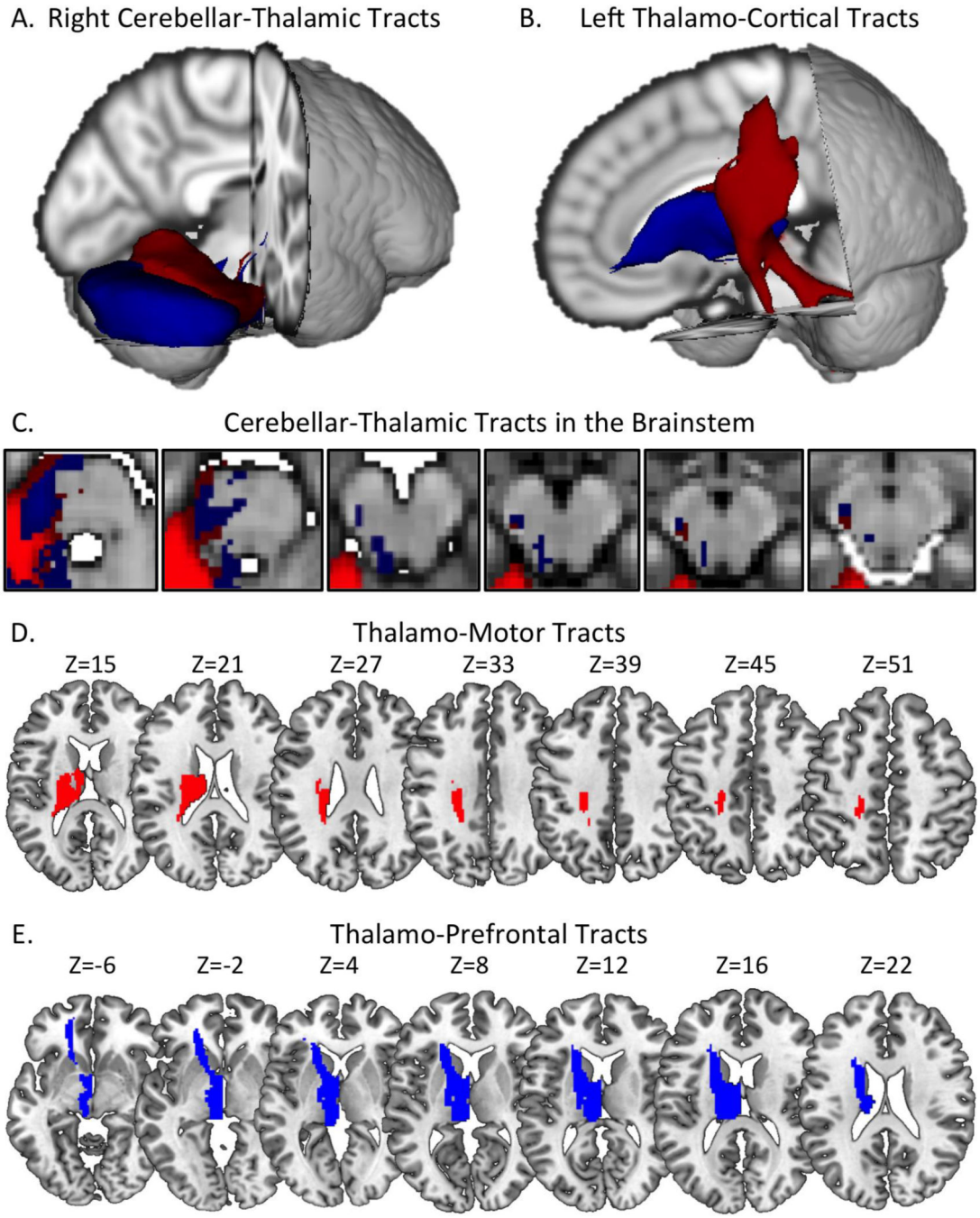

Figure 1.

A. Cerebellar-thalamic tracts from right cerebellar lobule V (red) and right Crus I(blue) to the thalamus. **B.** Cerebello-thalamo-cortical tracts as they continue from the thalamus to the primary motor cortex (red) and prefrontal cortex (blue) in the left hemisphere due to the cross-lateralization of the cerebellum. **C.** Lobule V and Crus I tracts as they pass through the brainstem in distinct but adjacent regions, comparable to what was found by Salmi and colleagues in young adults (Salmi et al., 2010). From left to right, inferior to posterior. Radiological orientation such that right is presented on the left **D.** Thalamo-motor segment

of the lobule V-thalamo-motor tract, from inferior to superior. **E.** Thalamo-prefrontal segment of the Crus I-thalamo-motor tract, from inferior to superior. Anatomical orientation such that right is presented on the right, and left on the left.

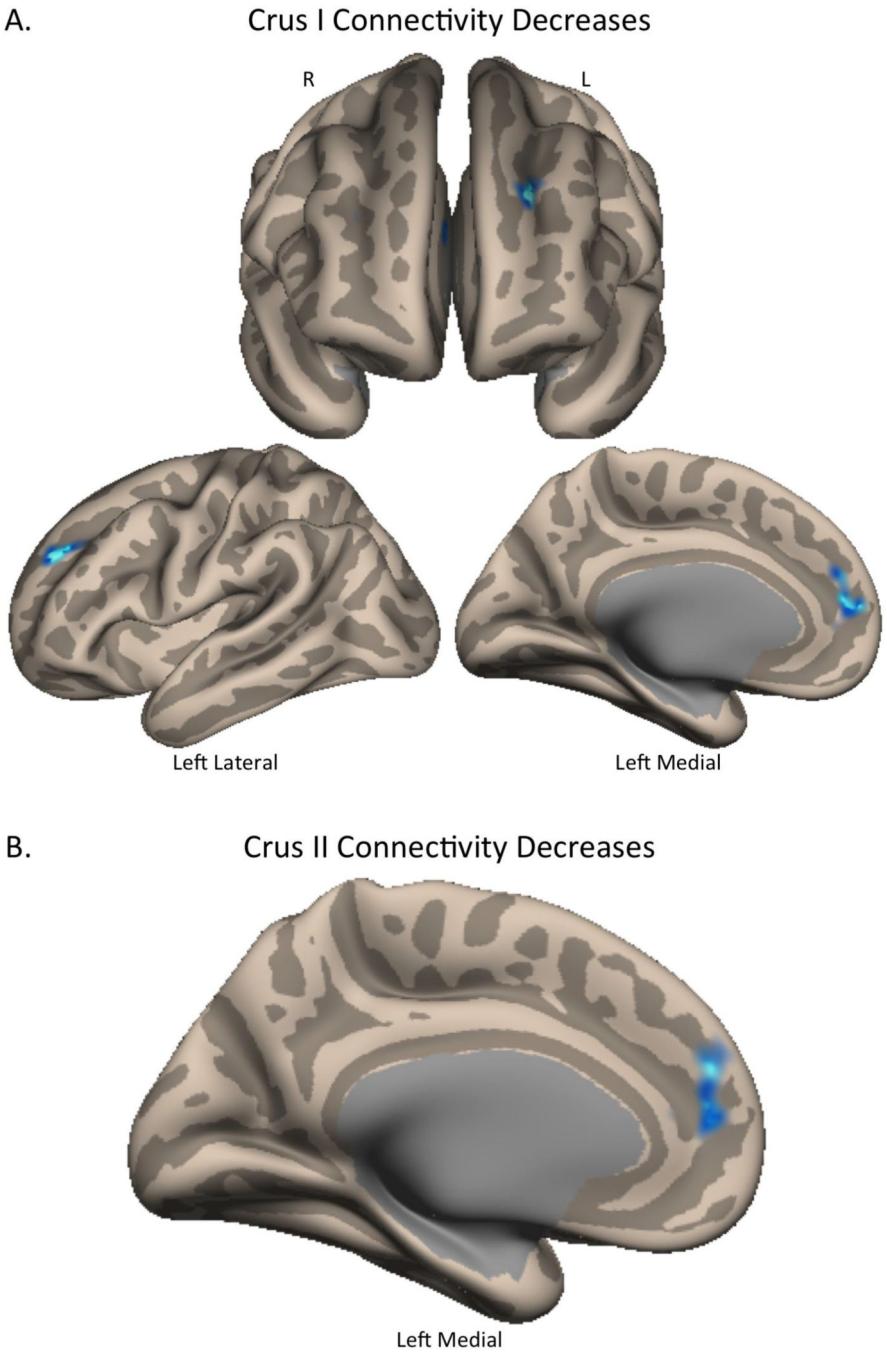

Figure 2.

Significant differences in cerebellar resting state connectivity in Crus I **(A)** and Crus II **(B)** over a period of 12-months. There were no significant differences in Lobule V connectivity over time. Table 1 provides the coordinates, t-values, and cluster sizes associated with these regions.

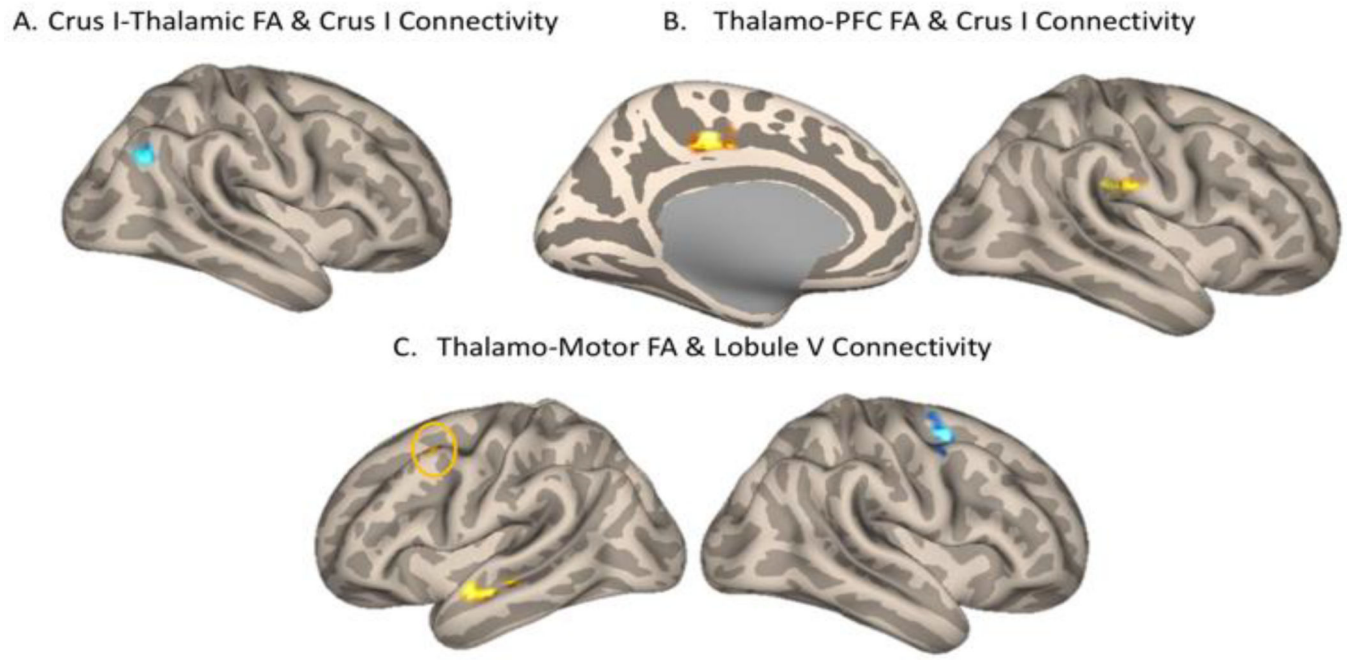

Figure 3.

Baseline correlations between structural and functional connectivity. **A.** Crus I – thalamus FA is negatively correlated (at trend-level) with Crus I connectivity in the right hemisphere. **B.** Thalamo-prefrontal FA is positively correlated with Crus I resting state connectivity in both left medial and right hemisphere regions. **C.** Mixed results were also seen with thalamo-motor connectivity and lobule V resting state networks. There were positive associations in the left hemisphere (left) while negative relationships were seen in the right hemisphere (right). There were no correlations between lobule V – thalamic tract FA and lobule V functional connectivity.

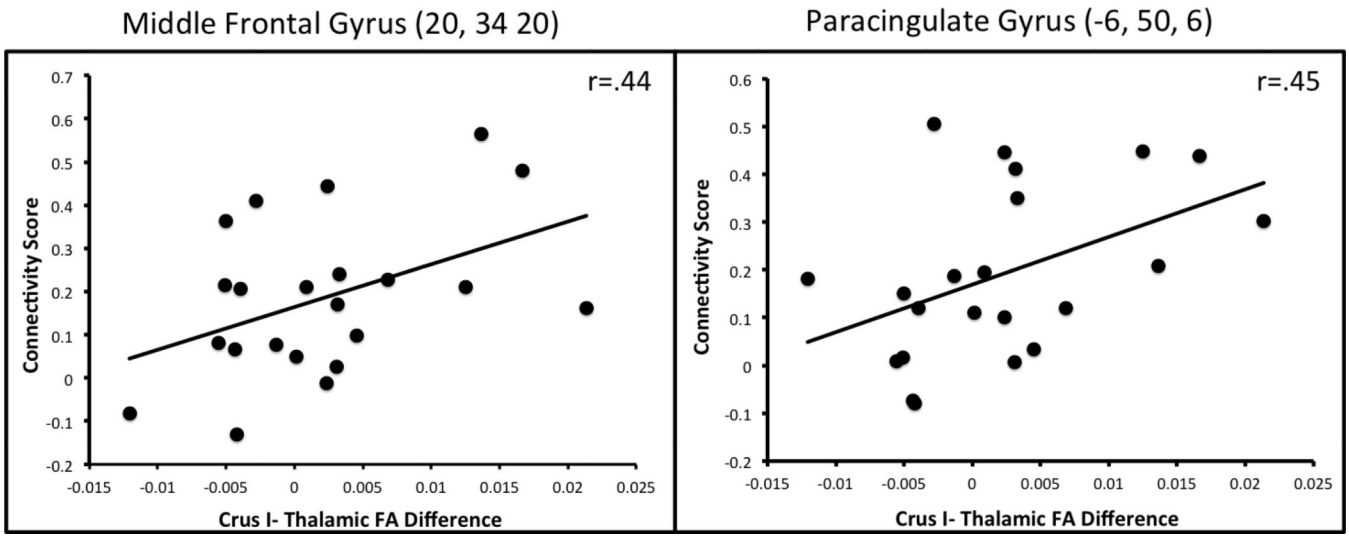

Figure 4.

Connectivity values extracted from regions in the Crus I network that show differences over 12-months are significantly correlated with FA changes in the corresponding cerebello-thalamo-cortical white matter tract. This provides further evidence for the notion that these resting state functional connectivity changes are associated with changes in structural connectivity.

Table 1

Significant main effect of time when comparing baseline to 12-month follow-up connectivity.

Seed	Region	BA	Cluster Size	MNI Coordinates			T-Value	$P_{(FDR)}$
				X	Y	Z		
Crus I	**Middle Frontal Gyrus**	**9**	**157**	**20**	**34**	**20**	**6.32**	**.017**
	Anterior Cingulate	24		8	22	15	5.26	
	Dorsolateral PFC	**9**	**160**	**−26**	**48**	**36**	**4.91**	**.017**
	Dorsolateral PFC	9		−26	38	38	3.76	
	Middle Frontal Gyrus	8		−22	32	30	3.70	
	Paracingulate Gyrus	**10**	**120**	**−6**	**50**	**6**	**4.41**	**.036**
	Paracingulate Gyrus	32		−6	48	22	4.31	
	Paracingulate Gyrus	32		−4	50	14	4.15	
	Paracingulate Gyrus	**32**	**151**	**−4**	**46**	**12**	**4.83**	**.030**
Crus II	Paracingulate Gyrus	32		−6	48	22	4.45	
	Medial Frontal Pole	10		−2	54	26	3.76	

There were no significant differences in Lobule V connectivity, though significant effects of time, indicative of further network development were seen for both Crus I and Crus II. Regions shown in bold indicate the cluster peak, and additional peaks with in the cluster are also reported. Anatomical regions are those defined by the Harvard-Oxford cortical structural atlas.

BA: Brodmann area

Table 2

Correlations between FA and cerebellar resting state connectivity at both baseline and 12-month follow up.

Seed	Region	BA	Cluster Size	MNI Coordinates X	Y	Z	T-Value	$P_{(FDR)}$
Baseline								
Crus I & Crus I – Thal FA: Negative Correlation	**Crus II**	--	**114**	**−44**	**−62**	**−46**	**5.42**	**.068**
	Crus I	--		−50	−56	−40	4.16	
	Crus II	--		−40	−72	−42	3.72	
	Lateral Occipital Cortex	**39**	**138**	**48**	**−68**	**30**	**5.15**	**.063**
	Lateral Occipital Cortex	39		46	−74	40	3.90	
Crus I & Thal – PFC FA: Positive Correlation	**Supramarginal Gyrus**	**40**	**116**	**46**	**−28**	**30**	**5.46**	**.003**
	Supramarginal Gyrus	40		56	−24	24	4.49	
	Post-Central Gyrus	2		62	−14	26	3.75	
	Posterior Cingulate Cortex	**31**	**151**	**−18**	**−28**	**38**	**5.10**	**.001**
	Posterior Cingulate Cortex	--		−10	−16	34	4.88	
	Cingulate Motor Area	--		−12	−20	42	4.56	
	Supplementary Motor Area	**31**	**115**	**4**	**−6**	**48**	**4.96**	**.003**
Lobule V & Thal – M1 FA: Negative Correlation	**Premotor Cortex**	**6**	**380**	**34**	**−4**	**54**	**5.36**	**.000**
	Premotor Cortex	6		26	−2	70	4.87	
	Premotor Cortex	6		22	2	54	4.85	
Lobule V & Thal – M1 FA: Positive Correlation	**Premotor Cortex**	**6**	**121**	**−38**	**12**	**60**	**6.20**	**.063**
	Premotor Cortex	6		−38	14	52	4.06	
	Superior Temporal Gyrus	**21**	**179**	**−52**	**−16**	**−18**	**5.47**	**.023**
	Middle Temporal Gyrus	--		−52	−6	−18	5.12	
	Middle Temporal Gyrus			−50	−24	−12	3.99	
12-Month Follow-Up								
Crus II & Crus I – Thal FA: Negative Correlation	**Middle Temporal Gyrus**	**21**	**121**	**62**	**−14**	**−16**	**4.47**	**.069**
	Middle Temporal Gyrus	21		68	−12	−10	4.34	

Seed	Region	BA	Cluster Size	MNI Coordinates X	Y	Z	T-Value	$P_{(FDR)}$
	Inferior Temporal Gyrus	20		60	−22	−29	3.97	

Negative relationships between Crus I and Crus I -- thalamic structural networks are reported, though they are only trend-level when cluster-level multiple comparisons corrections are applied. Follow-up relationships were minimal, but present at trend level. Positive and negative correlations are specified. Regions shown in bold indicate the cluster peak, and additional peaks with in the cluster are also reported. Anatomical regions are those defined by the Harvard-Oxford cortical structural atlas.

BA: Brodmann area.