Tract profiles of the cerebellar peduncles in children who stutter

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Abstract
Cerebellar-cortical loops comprise critical neural circuitry that supports self-initiated movements and motor adjustments in response to perceived errors, functions that are affected in stuttering. It is unknown whether structural aspects of cerebellar circuitry are affected in stuttering, particularly in children close to symptom onset. Here we examined white matter diffusivity characteristics of the three cerebellar peduncles (CPs) based on diffusion MRI (dMRI) data collected from 41 children who stutter (CWS) and 42 controls in the 3–11 years range. We hypothesized that CWS would exhibit decreased fractional anisotropy (FA) in the right CPs given the contralateral connectivity of the cerebellar-cortical loops and past reports of structural differences in left cortical areas in stuttering speakers. Automatic Fiber Quantification (AFQ) was used to track and segment cerebellar white matter pathways and to extract diffusivity measures. We found significant group differences for FA in the right inferior CP (ICP) only: controls showed significantly higher FA in the right ventral ICP compared to CWS, controlling for age, sex, and verbal IQ. Furthermore, FA of right ICP was negatively correlated with stuttering frequency in CWS. These results suggest an early developmental difference in the right ICP for CWS compared to age-matched peers, which may indicate an alteration in error processing, a function previously linked to the ICP. Lower FA here may impact error monitoring and sensory input processing to guide motor corrections. Further longitudinal investigations in children may provide additional insights into how CP development links to stuttering persistence and recovery.

Keywords Stuttering · Cerebellum · Diffusion MRI · Fractional anisotropy · Error monitoring

Abbreviations
AFQ Automated fiber quantification
CP Cerebellar peduncle
CWS Children who stutter
dMRI Diffusion MRI
EVT Expressive vocabulary test
FA Fractional anisotropy
fMRI Functional magnetic resonance imaging
GFTA Goldman–Fristoe test of articulation
MD Mean diffusivity
ICP Inferior cerebellar peduncle
MCP Middle cerebellar peduncle
PPVT Peabody picture vocabulary test
ROI Region of interest
SCP Superior cerebellar peduncle
SLD Stuttering-like disfluency
STT Streamlines tracking tractography
WASI Wechsler abbreviated scale of intelligence
WPPSI Wechsler preschool and primary scale of intelligence

Introduction
Stuttering is a complex neurodevelopmental disorder characterized by frequent disruptions in the flow of speech. Neuroimaging investigations in the last two decades have revealed significant differences in speakers who stutter relative to typical speakers in functional neuroanatomy affecting auditory-motor integration, temporal processing of speech movements, and aberrant inter-hemispheric interactions (for a review, see Chang et al. 2018). Apart from some convergent findings (see discussion in Chang and Guenther 2020),

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there have been disparate reports of group differences that encompass many parts of the brain, reflecting the complex nature of stuttering and the probable involvement of multiple, network-level (versus region-specific) neural differences in stuttering. Examinations of the brain’s “hub” areas that support the integration of functions across multiple neural structures have thus been an area of significant interest in the context of stuttering. One such area is the basal ganglia, which plays a major role in coordinating and initiating movement sequences and has been discussed in recent reviews relevant to the neural bases of stuttering (for a discussion, see Chang and Guenther 2020; Chang et al. 2018; Craig-McQuaide et al. 2014). The cerebellum is another critical hub area of the brain (Akkal et al. 2007; Bostan et al. 2010, 2013; Brodal 1978; Glickstein et al. 1985; Hoover and Strick 1999; Hoshi et al. 2005; Kelly and Strick 2000, 2003; Leichnetz et al. 1984; Middleton and Strick 1994, 2001; Schmahmann and Pandya 1991, 1993, 1997; Snider and Maiti 1976; Strick et al. 2009; Tourville and Guenther 2011; Vilensky and Van Hoesen 1981; Zemanick et al. 1991). Arguably, the cerebellum has received much less exploration than other hub areas such as the basal ganglia, though a number of studies have reported cerebellar functional and structural differences in people who stutter (Beal et al. 2007; Brown et al. 2005; Budde et al. 2014; Chang et al. 2015; Chang et al. 2008, 2016; Chang and Zhu 2013; Chow and Chang 2017; Connally et al. 2014; De Nil et al. 2001; Garnett et al. 2018; Kell et al. 2018; Lu et al. 2009, 2010a, b, 2012; Sitek et al. 2016; Song et al. 2007; Watkins et al. 2007; Yang et al. 2016).

Several studies showed that people who stutter relative to controls exhibit greater activity in the right cerebellum during speech tasks (De Nil et al. 2001, 2003; Watkins et al. 2007; Lu et al. 2010c; Ingham et al. 2012). The cerebellar right hemisphere areas primarily inter-connect with left cerebral hemisphere regions, supporting functions including orofacial movements and speech motor control. In addition to motor functions, recent studies have provided strong evidence that the cerebellum is substantially involved in supporting higher-order cognitive processes. For example, working memory, semantic judgment, spatial awareness, procedural learning, decision making, and emotion processing all engage the cerebellum (for a review, see Stoodley and Schmahmann 2010). Given updated understanding of cerebellar function and cerebellar networks, further research into cerebellar functional neuroanatomy is expected to lead to a better understanding of the neural bases underlying the multifactorial nature of stuttering (for a discussion, see Smith and Weber 2017).

Major white matter tracts that interconnect the cerebellum and brainstem are bundled into cerebellar peduncles (CP). There are 3 pairs of CPs: the superior cerebellar peduncles (SCP), which contain almost all of the efferent projections from the cerebellum via the thalamus, such as those projecting to cortical motor areas; the middle cerebellar peduncles (MCP), which are the primary afferent pathway carrying input from the cortex via the pons; and the inferior cerebellar peduncles (ICP), which carry both afferent and efferent tracts. Afferent pathways that travel to the ICP originate in vestibular areas, the spinal cord, and the brainstem. Efferent channels from this peduncle also travel to vestibular regions and brainstem areas that mediate vital life functions, such as circadian rhythm and consciousness, as well as reflexes (e.g., Mangold and Das 2020). The ICP have also been associated with sensorimotor adaptation (Jossinger et al. 2020) and error detections during motor commands (for a review, see Shadmehr 2017). Relevant to speech-motor control theories, afferent information from the olivo-cerebellar fibers that enter the cerebellum through the ICP provide the cerebellum with sensory input from the periphery (Streng et al. 2018). This peripheral information could help guide the proper updating of motor commands through efferent copy mechanisms that are supported by the cerebellum (e.g., De Zeeuw et al. 1998; Miall et al. 1993; Rhodes and Bullock 2002; Wolpert et al. 1998).

Adults and children with persistent stuttering differ in microstructural characteristics of the cerebellar peduncles compared to controls and recovered children who stutter (CWS; Watkins et al. 2007; Connally et al. 2014; Chow and Chang 2017; Garnett et al. 2018). However, there is some disagreement on which peduncles may be affected in developmental stuttering. While some studies have suggested that all six peduncles have lower fractional anisotropy (FA) in adults who stutter (Connally et al. 2014), other studies have only found the right MCP to differ significantly in adults who stutter relative to controls (Watkins et al. 2007). Connally et al. (2014) also showed a significant age effect in the FA of the ICP: FA in ICP increased with age (14–42 years) in speakers who stutter, whereas there were no such age correlations in the controls. Another study found that while FA of the left ICP was associated with speech rate in adults who stutter, adults who stutter did not differ from controls in overall structural measures of any of the cerebellar peduncles (Jossinger et al. 2021). Both the ICP and MCP carry afferent signals from speech motor control substrates in the brainstem and the cerebral cortex, respectively. Given that there are some controversies surrounding whether speech motor learning differences exist in adults but not in children who stutter (Daliri et al. 2017; Kim et al. 2020), it is of interest to examine whether subtle differences reported in cerebellar peduncle structure in adults who stutter are also present in CWS. Examining CWS, who are less likely than adults to have acquired adaptive neuroplastic changes associated with reactions and compensations to one’s own stuttering, is critical to be able to differentiate any structural changes contributing to stuttering etiology versus adaptive
changes that may occur as the result of stuttering over many years.

In the above-mentioned studies, diffusion MRI (dMRI) was used to quantify water diffusion, reflecting brain tissue organization on a microstructural scale. The white matter structures inhabited by myelinated axons induce an anisotropic diffusion of water molecules, compared to gray matter structures or the cerebrospinal fluid, where water diffusion would be much more isotropic (i.e., mean diffusion probability occurring equally in all directions rather than in a preferential direction). FA is a summary measure derived from dMRI that provides a way to quantify how much of the water diffusion occurs in a principal direction compared to orthogonal directions. FA is influenced by various factors such as myelination, axonal diameter, presence of crossing fibers, and is commonly used in clinical studies. While FA can be examined using a voxel-based analysis (e.g., tract-based spatial statistics (TBSS); Smith et al. 2006) to compare between groups, there are some limitations in terms of ensuring that TBSS-based tracts correspond to tracts in individual brains (Tsang et al. 2010; Yeatman et al. 2012). Compared to voxel-wise analyses of FA, tractography involves probabilistic or deterministic algorithms to track the continuous trajectory of white matter tracts and is considered a more accurate method to identify white matter tracts in the human brain (Yeatman et al. 2012). However, most common methods for tractography investigations have relied on manual ROI definitions that were effortful and time-consuming. Furthermore, diffusion properties of tracts were often averaged along the length of the whole tract.

A new tractography method, Automated Fiber Quantification (AFQ), improves upon these limitations and provides a way to automatically identify major white matter tracts (Yeatman et al. 2012). AFQ also allows examining diffusion measurements at different locations on the tracts rather than just the mean across the entire tract. While this method has been applied to study adults who stutter (Jossinger et al. 2021; Kronfeld-Duenias et al. 2016a, b, 2018), it has not yet been applied to study white matter tracts in CWS who are relatively close to stuttering onset. Using this method, we examined five major cerebellar white matter tracts in CWS and their age-matched peers to compare diffusion properties at anatomically equivalent locations of each tract. These tracts included the bilateral SCPs, MCP, and bilateral ICPs.

Guided by previous reports showing decreased FA in cerebellar peduncles based on examining adults who stutter (Connally et al. 2014) and in general FA decreases across major white matter tracts when comparing stuttering and non-stuttering children (Chow and Chang 2017; Chang et al. 2015), we hypothesized that CWS would exhibit decreased FA in the cerebellar peduncles relative to controls. Furthermore, given the contralateral connectivity of the cerebellar cortical loops and previously reported convergent findings of differences focused on the left cerebral areas in people who stutter, we expected decreased FA in the right cerebellar peduncles for CWS relative to controls. Given the reported role of the ICP in error detection, and its role in carrying afferent fibers to the cerebellum with peripheral sensory information that may help support efference copy mechanisms during speech (e.g., Shadmehr 2017; Streng et al. 2018), we hypothesized that the right ICP would be associated with persistent stuttering and stuttering severity.

**Methods**

**Participants**

A total of 83 children (41 stuttering, 26 boys; 42 controls, 21 boys) between 3 and 11 years of age participated. All were monolingual native North American English speakers without concomitant developmental disorders (e.g., dyslexia, attention-deficit/hyperactivity disorder, learning delay, psychiatric conditions). All children underwent careful screening to ensure typical speech and language developmental history except for the presence of stuttering in the experimental groups. The CWS and controls were matched in age, handedness (Oldfield 1971), and socioeconomic status (Holland et al. 2007). While most participants were strongly right-handed, 6 children were left-handed (3 CWS, 3 control) and 7 ambidextrous (5 CWS, 2 control). All participants were tested on a battery of standardized speech, language, and cognitive tests, audiometric hearing screening, oral-motor screening, and cognitive evaluations. The tests included the Peabody Picture Vocabulary Test (PPVT-3; Dunn and Dunn 2007), Expressive Vocabulary Test (EVT-2; Williams 2007), Goldman–Fristoe Test of Articulation (GFTA-2; Goldman 2000), Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III; for children 2: 6–7:3; Wechsler 2002), and Wechsler Abbreviated Scale of Intelligence (WASI; for children aged 7 and up; Wechsler 1999). All children scored within 2SD of the age normed average for all standardized assessments. The average test scores for each group are listed in Table 1.

Stuttering severity was assessed by collecting samples of spontaneous speech elicited through storytelling and conversational tasks with a parent and a certified speech-language pathologist. These samples were video recorded for further offline analyses. We calculated percent stuttered utterances per number of syllables based on narrative samples containing a conversation with the clinician and a monologue elicited with storytelling with a pictures-only book (‘Frog, where are you?’; Mayer 1969). In addition, the Stuttering Severity Instrument (SSI-4; Riley 2009) was used to examine the frequency and duration of disfluencies occurring in the speech sample, as well as any physical concomitants.
associated with stuttering; all of these measures were incorporated into a composite stuttering severity rating. To determine the measurement reliability of the SSI scores, an intraclass correlation coefficient was calculated based on the ratings from two independent judges on children’s speech samples.

While all CWS were diagnosed with stuttering at the initial study visit, they were categorized as recovered or persistent based on measurements acquired in subsequent visits that occurred up to 4 times for each child. A child was categorized as persistent with an SSI-4 score > 10 at two consecutive follow-up visits, and the onset of stuttering had been at least 36 months prior to his most recent visit. A child was considered recovered with an SSI-4 score ≤ 10 (corresponding to “very mild”) at two consecutive follow-up visits. Such determination also required the consideration of percent occurrence of stuttering-like disfluencies (%SLD) in the speech sample (≥ 3 for persistent) as well as clinician and parental reports. Similar criteria were used to determine recovery or persistency in previous studies (Yairi and Ambrose 1999). Using these criteria, we identified 13 recovered children (7 boys), hereafter “recovered” and 28 persistent children (18 boys), hereafter “persistent”. For controls, the inclusion criteria included never having been diagnosed with stuttering, no family history of stuttering, lack of parental concern for their child’s speech fluency, with %SLD < 3%.

Table 1 Demographic information and behavioral test scores of children who stutter (CWS) and control participants included in this study

|                      | Controls n = 42 (21 boys) | Children who stutter n = 41 (26 boys) |
|----------------------|---------------------------|--------------------------------------|
|                      | Mean (SD) | Range | Mean (SD) | Range |
| Age                  | 6.52 (2.03) | 3.25–10.75 | 6.28 (2.07) | 3.08–11 |
| SES (maternal education) | 6.36 (0.61) | 5–7 | 6.24 (0.76) | 4–7 |
| Full-scale IQ | 114.68 (14.33) | 84–144 | 105.90 (14.55) | 81–138 |
| Performance IQ | 111.58 (15.92) | 77–145 | 105.85 (13.92) | 79–135 |
| Verbal IQa | 117.04 (14.93) | 87–153 | 105.53 (14.20) | 77–137 |
| PPVT | 118.26 (13.61) | 95–151 | 110.07 (13.20) | 86–147 |
| EVTa | 115.65 (14.33) | 90—149 | 106.65 (12.25) | 87–137 |
| GFTA | 105.31 (7.95) | 81–123 | 103.61 (8.79) | 77–121 |
| %SLDb | 1.08 (0.87) | 0.0–3.23 | 6.16 (5.78) | 0.20–30.20 |
| %OD | 4.93 (2.67) | 0.0–13.73 | 5.29 (2.67) | 1.00–12.70 |
| SSI-4 at initial visit | N/A | N/A | 19.54 (8.01) | 6–48 |

SD standard deviation, SES socioeconomic status, IQ intelligence quotient, PPVT Peabody Picture Vocabulary Test, EVT Expressive Vocabulary Test, GFTA Goldman–Fristoe Test of Articulation, SSI-4 Stuttering Severity Instrument Edition 4, %SLD stuttering-like disfluencies (e.g., sound-syllable repetitions word repetitions sound prolongations) occurring per 100 words during conversational speech, %OD other disfluencies (e.g., interjections, phrase repetitions) occurring per 100 words during conversational speech

a Controls exhibited significantly higher scores than the CWS group

b CWS exhibited significantly higher scores than in controls

MRI acquisition

MRI scans for this study were acquired on a GE 3 T SignaVR HDx MR scanner (GE Healthcare) with an 8-channel head coil. During each scanning session, 180 T1-weighted inversion recovery fast spoiled gradient-recalled images, with CSF suppressed, were collected to cover the entire brain with the following parameters: time of echo 3.8 ms, time of repetition of acquisition 8.6 ms, time of inversion 831 ms, repetition time of inversion 2,332 ms, flip angle 8 degrees, resolution 1-mm^3 isotropic, and receiver bandwidth ± 20.8 kHz. After collecting T1 data, high-order shimming procedures were performed to improve magnetic field homogeneity. The dMRI data were obtained with a dual spin-echo echo-planar imaging sequence for 12 min and 6 s with the following parameters: 48 contiguous 2.4-mm axial slices in an interleaved order, field of view 22 × 22 cm, matrix size 128 × 128, number of excitations = 2, echo time 77.5 ms, repetition time 13.7 s, 25 diffusion-weighted volumes (one per gradient direction) with b = 1000 s/mm^2, one
volume with \( b = 0 \) and parallel imaging acceleration factor = 2. A member of the research staff sat inside the scanner room next to the child being scanned for the duration of the scans to monitor participant comfort and to ensure the child was able to cooperate with scanning protocols. Children watched a movie to help them stay still during the acquisition of volumetric T1-weighted scans and dMRI scans. These scans were acquired as part of an on-going longitudinal study of childhood stuttering, where up to four scans were acquired from each child with an inter-scan interval of approximately 12 months. For the purposes of this investigation, only one dMRI scan collected from each child (i.e., typically the scan acquired during the child’s first year visit) was entered into the analyses. In cases where first year dMRI datasets could not be used (e.g., due to movement artifacts that are more common in younger years), the next available scan was used for that child. Of the 83 children, non-first year scans were used for 13 children: second year visit scans were used for 10 children (5 CWS and 5 controls), third year visit scans were used for two CWS, and a fourth year visit scan were used for one CWS. In these cases, the corresponding chronological year and linked behavioral scores are reported.

**Diffusion MRI data preprocessing**

Diffusion MRI data were preprocessed individually using the open-source software mrDiffusion (https://github.com/vistalab/vistasoft/tree/master/mrDiffusion) implemented in MATLAB R2017b. Eddy current distortions and subject motion in the diffusion-weighted images were removed by a 14-parameter constrained non-linear co-registration algorithm based on the expected pattern of eddy current distortions (Rohde et al. 2004). Each diffusion-weighted image was registered to the non-diffusion (b0) image; the b0 image was registered automatically to the T1 image, which had been aligned to the canonical MNI template. The combined transformation, incorporating both eddy-current correction and anatomical alignment, was applied to the raw diffusion data, and the transformed images were resampled at 2\( \times \)2\( \times \)2 mm\(^3\) isotropic voxels. Diffusion tensors were then fit using a robust least-squares algorithm. Eigenvectors and eigenvalues of the tensor were extracted. FA was calculated as the normalized standard deviation of the eigenvalues of the diffusion tensor, and mean diffusivity (MD) was calculated as the average of all three eigenvalues (Pierpaoli and Basser 1996). Head motion was quantified in each participant by calculating the degree of motion correction in each volume relative to prior volume (Bruckert et al. 2019). That is, relative head motion in each child was quantified by calculating the magnitude of motion correction in the x, y, and z planes of each volume relative to the prior volume. The average of the translational displacement was calculated for each child and compared between groups. Average displacement across participants 0.046 ± 0.030, and there was no group difference in the displacement (\( p > 0.05 \)).

**Tract identification and segmentation**

Five cerebellar peduncles (bilateral ICPs, bilateral SCPs, and MCP) were identified and quantified using the open-source software AFQ package (Yeatman et al. 2012). AFQ consists of three main processing steps: (1) whole-brain fiber tractography, (2) automatic tract segmentation based on template region of interest (ROIs) warped to native space, and (3) automatic tract quantification and cleaning. A whole-brain fiber group was tracked using a deterministic streamlines tracking tractography (STT) algorithm (Mori et al. 1999). Based on previous dMRI studies in children (Bruckert et al. 2019), the tracking algorithm was seeded with a white matter mask defined as all the voxels with FA value greater than 0.15. Tracking proceeded in both directions along the principal diffusion axes and stopped when FA estimated at the current position dropped below 0.10 or when the angle between the last path segment and next step direction was greater than 30°. Fiber tract segmentation was based on waypoint ROIs, which were first defined on the JHU MNI T1 template and then back-transformed to each participants’ native space (Bruckert et al. 2019; Jossinger et al. 2021). The core of the tract was calculated by defining 30 sample points along the tract and computing the robust mean position of the corresponding sample points. After tract segmentation, an automated cleaning algorithm was used to remove fiber longer than 1 standard deviation from the mean fiber length and spatially deviated more than 4 standard deviations from the core of the tract.

A small number of CWS were excluded from each analysis because a tract could not be segmented or did not conform to anatomical norms. One child who stutters was excluded from the MCP, 1 from the right SCP, and 2 from the left SCP analyses. Diffusion properties (FA, MD) were calculated at 30 equidistant nodes along the core of each fiber tract bounded by the same two waypoint ROIs used for tract segmentation (Yeatman et al. 2012; Bruckert et al. 2019; Jossinger et al. 2021). Figure 1 shows the tracts of interest identified in six representative participants (three CWS and three controls); Fig. 2 shows visualization of FA profiles in each group and each CP.

**Statistical analysis**

Analyses were conducted on the core of each CP tract between the two waypoint-ROIs used for tractography.
Mean tract-diffusion indices (FA and MD) were calculated by averaging values of all 30 nodes for each participant and in each peduncle (hereafter referred to as “tract-FA and tract-MD”). Independent \( t \) tests were used to compare tract-FA and tract-MD between groups (CWS, controls) in the MCP, bilateral ICPs, and SCPs. For tracts showing significant group differences between CWS and controls, follow up post hoc \( t \)-tests were used to examine whether the group differences were driven by differences between persistent CWS and controls. In addition, we evaluated group differences of the local diffusivity measures along the 30 nodes in each tract, which may provide better sensitivity compared to the mean FA and MD for the whole tract. These along-track group comparison statistics were corrected using a non-parametric permutation test \( (p < 0.05) \) and controlling the family-wise error (FWE) corrected alpha at 0.05 (Nichols and Holmes 2002).

**Linear regression and correlations**

To account for the additional factors that could contribute to the group differences of diffusion properties in the CPs, a linear regression model was built to test group differences when age, sex, and verbal IQ were included as covariates.

**Results**

**Reduced FA in the right ICP in children who stutter**

Mean tract_FA and tract_MD for each CP tract are shown in Table 2 and Fig. 3. CWS showed reduced FA in right ICP \( (p = 0.013) \) and greater MD in MCP compared to controls \( (p = 0.051; \) Table 2). These group differences did not reach statistical significance when corrected for multiple comparisons \( (p < 0.01) \), though FA of the right ICP showed trend level significance. We further examined local diffusivity measures in the right ICP and found FA group differences between CWS and controls in the ventral nodes of the right ICP \( (p < 0.001, \) see Fig. 4 node 3–12). Post-hoc \( t \) test results show that there were group differences between persistent CWS and controls for both tract_FA \( (p = 0.003) \) and ventral nodes of the right ICP \( (p < 0.001) \); but no group differences were found between recovered CWS and controls, or persistent CWS and recovered CWS.
Fig. 2 FA tract profiles of the cerebellar peduncles, separately for controls (denoted by dark blue color; \(n=42\)) and CWS (denoted by orange color; \(n=41\)). Bilateral ICPs (top row), bilateral SCPs (middle row) show rendering of cerebellar tracts on sagittal T1 images, and MCP (bottom row) is shown overlaid on an axial T1 image in a representative subject. FA values are plotted for 30 equidistant locations/nodes in each tract. Locations of blue and yellow arrows in brain rendering correspond to blue and yellow arrows in nodes of the tract profiles. ICP inferior cerebellar peduncle, MCP middle cerebellar peduncle, SCP superior cerebellar peduncle.

Table 2 Fractional anisotropy (FA) and mean diffusivity (MD) profiles for controls and children who stutter (CWS)

|                        | Controls     | CWS         | Group difference | Age correlation in the full sample |
|------------------------|--------------|-------------|------------------|-----------------------------------|
|                        | Mean ± SD    | Mean        | \(t\) \(p\)      | \(r\) \(p\)                       |
| Fractional anisotropy  |              |             |                  |                                   |
| right ICP FA           | 0.475 ± 0.042| 0.451 ± 0.046| 2.544 0.013\*    | 0.283 0.009\*                     |
| left ICP FA            | 0.460 ± 0.049| 0.45 ± 0.049 | 0.925 0.358      | 0.32 0.003\*                     |
| right SCP FA           | 0.378 ± 0.054| 0.383 ± 0.045| −0.476 0.636     | 0.33 0.003\*                     |
| left SCP FA            | 0.368 ± 0.048| 0.372 ± 0.052| −0.317 0.752     | 0.255 0.021\*                    |
| MCP FA                 | 0.441 ± 0.057| 0.435 ± 0.06 | 0.518 ± 0.606    | 0.16 0.150                       |
| Mean diffusivity       |              |             |                  |                                   |
| right ICP MD           | 0.883 ± 0.182| 0.869 ± 0.074| 0.445 ± 0.657    | −0.116 0.298                      |
| left ICP MD            | 0.873 ± 0.107| 0.859 ± 0.06 | 0.706 ± 0.482    | −0.152 0.172                      |
| right SCP MD           | 1.245 ± 0.208| 1.242 ± 0.193| 0.072 ± 0.943    | −0.267 0.016\*                   |
| left SCP MD            | 1.186 ± 0.228| 1.176 ± 0.169| 0.218 ± 0.828    | −0.075 0.508                      |
| MCP MD                 | 1.022 ± 0.123| 1.088 ± 0.178| −1.985 ± 0.051\% | −0.165 0.140                      |

CWS children who stutter, ICP Inferior cerebellar peduncle, MCP middle cerebellar peduncle, SCP superior cerebellar peduncle, SD standard deviation, L left, R right
\(\ast p < 0.05\); uncorrected; shown in bold
\(\# p < 0.10\); uncorrected; shown in italics
Fractional anisotropy of the right ICP scales with age and stuttering severity

Table 3 shows results from the linear regression models where group, age, sex, and IQ were included as covariates, and mean FA across the core of the right ICP tract (tract_FA), and mean FA for ventral nodes showing significant group difference (nodes 3 to 12) in the right ICP were...
dependent variables. Group differences were still significant for both tract_FA and mean FA for ventral nodes of the right ICP, when controlling for age, sex, and verbal IQ. In both models, age was positively associated with FA, that is, age was correlated with mean FA for ventral nodes (nodes 3–12) and across the core (nodes 1–30) of the right ICP (Fig. 5). Furthermore, we found that mean tract_FA of the right ICP in CWS was significantly correlated \( (r = -0.315, p = 0.045) \) with stuttering frequency (%SLD) (Fig. 6). The correlation was still significant when controlling for age \( (r = -0.314, p = 0.049) \). No significant correlation was found between mean FA for ventral nodes in the right ICP and stuttering frequency (%SLD) \( (r = -0.203, p = 0.203) \).

In addition to the right ICP FA, significant correlation of age and mean tract_FA were observed regardless of group in the left ICP, bilateral SCPs, and mean tract_MD of the right SCP (Table 2).

### Table 3
Regression analysis for the prediction of mean FA for ventral nodes and tract_FA in the right ICP

|                  | Mean FA for ventral nodes (nodes 3–12) |           |           |          | tract_FA (nodes 1–30) |           |           |          |
|------------------|----------------------------------------|-----------|-----------|----------|------------------------|-----------|-----------|----------|
|                  | Beta | t         | p         | Beta      | t         | p         | Beta      | t         | p         |
| Group            | -0.326 | -3.004 | 0.004*      | -0.240 | -2.127 | 0.037*      |
| Sex              | -0.021 | -0.206 | 0.837      | -0.089 | -0.830 | 0.409      |
| Age              | 0.303 | 2.942 | 0.004*      | 0.258 | 0.113 | 0.911      |
| IQ               | 0.026 | 0.237 | 0.813      | 0.013 | 2.413 | 0.018*      |

\( ^* p < 0.05; \) uncorrected; shown in bold

**Fig. 5** Both right ICP (a) ventral nodes (mean FA for nodes 3–12) and (b) tract_FA (mean FA across the core of the tract) showed a significant correlation with age regardless of group. CWS is denoted by orange squares and controls are denoted by dark blue circles. FA fractional anisotropy; ICP inferior cerebellar peduncle.

**Fig. 6** Stuttering frequency (%SLD) is correlated with the tract_FA of the right ICP in children who stutter (CWS). Greater stuttering frequency was associated with lower tract_FA in the right ICP. Persistent and recovered CWS are denoted by red and orange dots respectively; best linear fit lines are plotted for persistent (red), recovered (orange) and combined CWS (black).
Discussion

With its vast connections with cerebral structures associated with both motor and cognitive functions and its well-known critical role in supporting motor control, particularly in finely timed self-initiated movements such as speech, the cerebellum is an important yet understudied area of interest in the context of stuttering. To date, only a handful of studies have focused on examining cerebellar function and anatomy, all in adults and adolescents who stutter, with no studies reported to date in CWS. The current study examined white matter microstructural changes associated with childhood stuttering based on diffusion-weighted MRI data. The AFQ tractography method was used to overcome limitations of previous dMRI methods, to examine FA and MD derived from the core of major white matter tracts, both at the mean tract level as well as at specific nodes along each tract. The main results were that CWS have reduced FA in the right ICP of the cerebellum relative to controls. Across the right ICP, CWS had significantly lower FA, particularly in the ventral nodes of this CP. Right ICP FA was also negatively correlated with stuttering frequency in CWS. Overall, across both groups, FA in the right ICP was positively correlated with age. There were no significant group differences based on MD.

These results, derived from the novel application of AFQ methods to study CWS, provide additional insight into reports of CP microstructural differences described in previous studies of stuttering. Though one study examining persistent CWS compared to controls found that there was a significant age x group interaction of FA in the right ICP such that the growth rate was less in persistent CWS than controls (Chow and Chang 2017), other ICP findings based on examining adults who stutter have been inconsistent. In one study, lower FA was found in all three pairs of CPs for speakers who stutter (age range 14–42; N = 29) relative to controls (age range 14–45; N = 37), with the greatest group difference occurring in the left ICP (Connally et al. 2014). Another study reported lower FA in the right MCP in speakers who stutter (age range 14–27 years, N = 17; control group age range 14–27 years, N = 13) (Watkins et al. 2007), and in another, no differences were found between the stuttering and control groups, but the left ICP FA was negatively correlated with speech rate in adults who stutter (age range 19–52 years, N = 23; control group age range 19–53 years, N = 19) (Jossinger et al. 2021). The discrepancies reported by the studies mentioned above may be explained by differences in the age range of participants examined, varying statistical power to detect group differences, as well as differing scanning protocols and dMRI analysis methods applied in the analyses (for a discussion of the latter point, see Jossinger et al. 2021).

Concerning age, Jossinger et al. (2021) point out that group differences reported by Connally et al. (2014) for FA of the ICP decreased dramatically with age. This result may suggest that white matter development for people who stutter in ICP may be delayed but eventually catch up to levels seen in controls during adulthood. Jossinger et al. also did not find significant group differences in the ICP in their participants who had a higher mean age than in the other two studies. The current study is the first to examine CP white matter microstructure in children ranging from 3 to 11 years of age, with the largest sample size reported of the studies published to date. We found significant group differences in FA of the right ICP only and age-related increases in this same tract for both groups, which offers some clarifications on the early development of the CPs that differentiate people who stutter from age-matched peers. Like Jossinger et al. (2021) that used identical methods as the current study employing AFQ, we did not find significant group differences in any of the diffusivity measures in the MCP (only trend level increase in MD found for CWS relative to controls), bilateral SCPs, or left ICP. Unlike Jossinger et al. (2021), we found group differences in the right ICP where they did not find a significant group difference; they only reported a relationship between FA in the left ICP with speech rate in people who stutter. Findings from the two studies collectively point to the ICP as potentially most relevant among the cerebellar peduncles in relation to stuttering: while speculative, weaker white matter development in the right ICP during childhood may lead to possible maladaptive changes in the homologous left ICP that is linked to slower speech rate that may be a compensatory process associated with years of stuttering.

The results of the current study, which shows reduced FA in the ICP that conveys error feedback via the mossy fibers, indicate that the error monitoring function of the cerebellum may be affected in people who stutter, which has implications for updating internal models for adaptive motor control. The afferent fibers from the olivary nucleus relay error signals from mismatches between motor commands and peripheral input (Streng et al. 2018). Other studies have also suggested the olivo-cerebellar fibers are associated with sensorimotor adaptation (Jossinger et al. 2020) and detecting motor command errors (for a review, see Shadmehr 2017). More specifically, the ICP carry afferent fibers from the olivary nucleus, which is one of the two main afferent inputs to the cerebellum. These olivary inputs are carried through the climbing fibers, which convey bottom-up sensory feedback-related signals. The cerebellum also receives afferent input from the pons via the mossy fibers, which relay information from cortical areas. These inputs are thought to carry efference copy information, relaying an estimate of the sensory feedback predicted from the execution of the movement. When there is a mismatch between the predicted and actual sensory feedback from the movement, the cerebellum
can signal this discrepancy back to the cerebrum, resulting in adaptation of the movement so that any discrepancies between predicted and actual sensory feedback can be minimized in future movements. In a recent review paper on altered feedback studies in stuttering, the authors note that while people who stutter seem to show less compensation to sudden perturbation, they were particularly impaired in tasks that required adaptation of motor behavior that is induced by sustained perturbation of sensory feedback (Bradshaw et al. 2021). Of the two types of responses induced by perturbation tasks—automatic compensation to sudden/unexpected perturbation vs. adaptation in response to sustained perturbation—it is the latter in which cerebellar activity is most reliably evoked (Johnson et al. 2019). Namely, the cerebellum plays a critical role in motor adaptation that enables adjusting predictions for subsequent behavior and enabling fine-tuning of behavior based on feedback. Collectively, the findings reviewed here suggest that altered performance on adaptation studies reported in people who stutter may be linked to deficits in cerebellar error monitoring and/or signaling to the cerebrum for adaptive changes in response to sensory feedback perturbation.

Developmental stuttering is often connected to aberrant error monitoring (Postma and Kolk 1992; Max et al. 2004). Some influential theoretical perspectives have suggested that people who stutter have aberrant internal models, which integrate prediction of the state of the speech system as a result of motor plans, and sensory feedback that results from speech (Max et al. 2004). The cerebellum is hypothesized to build associations between sensory states of speech and the motor cortex during development (for a review, see Tourville and Guenther 2011) and support learning associated with inverse models (e.g., Kawato 1999). Structural differences in this pathway in CWS suggest that the development of internal models may be atypical. Auditory perturbation studies have found that adults who stutter are unable to update their motor commands in response to errors in their feedback (Loucks et al. 2012; Cai et al. 2012, 2014; Daliri et al. 2017; Daliri and Max 2018; Sares et al. 2018; Kim et al. 2020). Previous behavioral studies of auditory perturbation have variable results in CWS (Daliri et al. 2017; Kim et al. 2020). The current AFQ analyses suggest that cerebellar pathways associated with error monitoring are reduced in FA in CWS and that the higher the stuttering severity, the lower the FA in the same tracts. Structural differences in the ICP that carry olivo-cerebellar tracts may lead to variable error detection signals during speech, resulting in atypical updating of motor plans and less compensatory behaviors during perturbation trials. Additionally, associations between the frequency of stuttering and FA of the right ICP in CWS further suggest that structural differences in this pathway may contribute to greater frequency of disfluencies in speech. Because this analysis combined both persistent and recovered CWS, and stuttering severity scores were skewed toward milder ranges with only a few participants falling into moderate-severe stuttering severity in our sample, further investigations with larger sample sizes are warranted to replicate and expand on possible relationships between stuttering severity and structural changes in the ICP.

Disruptions to error detection systems of the cerebellum may result in atypical speech motor control development. Reduced compensation and adaptation responses to auditory perturbation have been consistently reported in adults who stutter (Loucks et al. 2012; Cai et al. 2012, 2014; Daliri et al. 2017; Daliri and Max 2018; Sares et al. 2018; Kim et al. 2020), but not as consistently for CWS (Daliri et al. 2017; Kim et al. 2020) may at least be partially be explained by changes in the speech motor control systems over a lifetime of stuttering in people who stutter (Loucks et al. 2012; Cai et al. 2012, 2014; Daliri et al. 2017; Daliri and Max 2018; Sares et al. 2018; Kim et al. 2020). Over time, an inability for the cerebellum to efficiently detect errors used for error-based learning may lead to atypical internal model development that may be especially evident in adults who stutter. Alternatively, a recent study by Kim et al. (2020) suggests that when feedback alterations induce motor adjustments to aspects of vocal tract configuration (requiring intrinsic movement control), decreases in adaptation were pronounced in young CWS relative to age-matched controls. This result contrasts with those reported by Daliri et al. (2017), which showed that while significantly reduced adaptation was observed in adults who stutter compared to controls, CWS did not differ from controls in adaptation extent. However, the latter study used feedback perturbations that altered perception of the phoneme (vowel target), which may have led to conscious adjustments to one’s speech. The difference between the more implicitly controlled speech perturbation paradigm in Kim et al. (2020) compared to Daliri et al. (2017), which may rely on explicit aspects of adaptation, could have led to the discrepancies between the two studies. Kim et al. (2020) argue that motor adaptation that involves implicit, rather than explicit, learning components might differ in people who stutter, regardless of age. Differences in implicit motor adaptation are present early in development rather than acquired later in life. Motor adaptation in response to feedback alterations that can be consciously monitored, however, may become more evident with age. Conscious motor adaptations rely on additional neural resources outside of the cerebellum, including attention networks. It may be that functional differences in these circuits differentiated adults who stutter from controls, but not between CWS and controls (Daliri et al. 2017). The implicit motor adaptation to self-initiated movement that occurs automatically and is mostly outside of conscious control, however, is supported by the cerebellum (Knolle et al. 2013). Therefore, the extent of data from...
published studies in stuttering suggests that motor adaptation deficits are present in both children and adults who stutter when implicit learning is involved, indicating that this may be a core characteristic in developmental stuttering. Namely, atypical internal model development reflected in altered cerebellar morphology could affect implicit motor adaptation starting in childhood rather than a process acquired later over a lifetime of stuttering. Because few studies have examined children to date on perturbation experiments, it is essential to conduct more studies that use perturbation paradigms that induce both implicit and explicit adaptation mechanisms to confirm whether children close to stuttering onset indeed primarily exhibit deficits in implicit motor learning. This finding would align with the current results that show that CWS have reduced FA white matter tracts associated with error monitoring function of the cerebellum.

In addition to significant group differences in the right ICP and its negative correlation with stuttering frequency, FA values in the right ICP showed significant age-related increases in both groups. This finding suggests that structural development of this largely afferent cerebellar pathway is ongoing at least during the age range of children we observed in this study (3–11 years). Age related FA increases were also observed in the left ICP as well as the bilateral SCPs (Table 2). White matter integrity increases during preschool and school-age developmental period thus seem to be on-going in the CPs that contain afferent fibers from the periphery to the cerebellum, as well as efferent fibers from the cerebellum to subcortical and cortical regions. It may be that during this period of dynamic growth, CWS are particularly vulnerable to aberrant growth in the ICP, which may either result in or be influenced by aberrant error-based motor learning and error monitoring in their speech. The directionality of this relationship is unclear and requires studying greater numbers of younger CWS and longitudinal studies.

Though AFQ presents an updated analysis method for diffusion metrics derived from dMRI, these results could be further extended and confirmed through tractography analyses using tools that have shown to be superior in resolving crossing fibers (Wedeen et al. 2008; Tournier et al. 2008; Yeatman et al. 2012). Future studies could also consider examining white matter microstructure in conjunction with functional connectivity analyses of resting state fMRI data or task-based fMRI that involve a motor learning paradigm. These approaches could further establish the links between the structure and function of cerebellar tracts that are linked to error monitoring and adaptive motor learning. In addition, future research on this topic could benefit from longitudinal analyses. Neural developmental trajectories differ between CWS and controls and between CWS who eventually recover from or persist in stuttering (e.g., Chang et al. 2018; Chow and Chang 2017). Future studies that examine structural development of the cerebellum, as well as functional and structural examinations of cerebello-cortical networks, would further provide much-needed information on whether and how specific cerebellar lobules critical to motor control and networks change over development and how that interacts with speech motor control development and stuttering severity.

In conclusion, results from this study demonstrate that FA measures are significantly reduced in the right ICP in CWS relative to age-matched peers, which may reflect attenuated structural integrity of this tract. Further, FA of the right ICP was found to be negatively correlated with the frequency of stuttering. The significant differences found in the white matter microstructure of the right ICP in developmental stuttering support theories that propose that error detection differences contribute to developmental stuttering.

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Availability of data and material The datasets analyzed for this research are available on reasonable written request and through data agreement.

Code availability Toolboxes used for AFQ analyses are freely available at GitHub (https://github.com/yeatmanlab/AFQ). Customized portion is available on request.

Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

Ethics approval All procedures were approved by the Michigan State University Institutional Review Board.

Consent to participate Written informed consent was obtained from one parent of the participants and assent from the participant prior to participation.

Consent for publication All authors approved this submission.

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