The Cerebellum (2022) 21:380–390
https://doi.org/10.1007/s12311-021-01307-x

ORIGINAL PAPER

White Matter Microstructural Properties of the Cerebellar Peduncles Predict Change in Symptoms of Psychopathology in Adolescent Girls

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Accepted: 14 July 2021 / Published online: 26 July 2021

Abstract

Internalizing symptoms typically emerge in adolescence and are more prevalent in females than in males; in contrast, externalizing symptoms typically emerge in childhood and are more commonly observed in males. Previous research has implicated aspects of white matter organization, including fractional anisotropy (FA), of cerebral tracts in both internalizing and externalizing symptoms. Although the cerebellum has been posited to integrate limbic and cortical regions, its role in psychopathology is not well understood. In this longitudinal study, we investigated whether FA of the superior (SCP), middle (MCP), and inferior cerebellar peduncles (ICP) predict change in symptoms and whether sex moderates this association. One hundred eleven adolescents completed the Youth Self-Report, assessing symptoms at baseline (ages 9–13 years) and again 2 years later. Participants also underwent diffusion-weighted imaging at baseline. We used deterministic tractography to segment and compute mean FA of the cerebellar peduncles. Lower FA of the right SCP at baseline predicted increases in internalizing symptoms in females only. Lower FA in the right SCP and ICP also predicted increases in externalizing symptoms in females, but these associations did not survive multiple comparison correction. There was no association between FA of the cerebellar peduncles and change in symptoms in males or between MCP FA and symptom changes in males or females. Organizational properties of the SCP may be a sex-specific marker of internalizing symptom changes in adolescence. The cerebellar peduncles should be explored further in future studies to elucidate sex differences in symptoms.

Keywords Cerebellum · Diffusion MRI tractography · White matter · Psychopathology · Sex differences · Adolescence

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Introduction

Researchers have documented sex differences in internalizing and externalizing symptoms in males and females [18, 43, 51]. Specifically, whereas internalizing symptoms typically emerge in adolescence and are twice as prevalent in females as in males, externalizing symptoms commonly emerge in childhood and are three times more prevalent in males than in females [32].

Adolescence marks a critical period during which individuals undergo significant pubertal, behavioral, and neural development [14, 46]. In addition to sex differences in the prevalence of a number of forms of psychopathology [28], males and females also differ in neural factors associated with the emergence of symptoms in adolescence [3]. In this context, widely distributed cortical and subcortical regions of the brain and their white matter connections have been associated with internalizing and externalizing symptoms in adolescents [9–11, 20, 37]. In contrast, structural properties of the cerebellum have been relatively neglected in relation

to affective functioning. Importantly, much of the existing work has been conducted with adult patient and clinical samples. For example, Schmahmann and Shermann [44] documented dysregulated emotion in patients with cerebellar trauma or lesions (e.g., from a stroke, tumor, or neurogenerative disorder), referred to as the "cerebellar cognitive affective syndrome." Further, among patients with degenerative cerebellar diseases, over 75% had comorbid psychiatric disorders [25]. These foundational studies with patients led to subsequent research implicating altered cerebellar volume in depression [39], bipolar disorder [4], and schizophrenia [34].

Fewer studies have assessed the relation between structural properties of the cerebellum and affect in adolescents and adults. One recent study of over 1,400 adolescents aged 8–23 years reported that reduced gray matter volume of the cerebellum was a stronger predictor of psychopathology than were structural metrics of other cortical and subcortical regions [35],similarly, Romer et al. [41] found that gray matter volume of the cerebellum was negatively associated with psychopathology in young adults. Only two studies to date have assessed *white matter* of the cerebellum in relation to affect in unselected samples of adults. The major white matter pathways of the cerebellum are the middle (MCP), inferior (ICPs), and superior cerebellar peduncles (SCPs). The MCP contains afferent fibers that connect the cerebellum to the contralateral cerebral cortex via the pontine nuclei [7, 8, 36]. The ICPs contain afferent fibers that send signals from the spinal cord and olivary nucleus into the cerebellum and efferent fibers from the cerebellum toward the vestibular nuclei situated at the junction between the pons and medulla oblongata [7, 8, 36]. Finally, the SCPs contain mainly efferent fibers that emerge from the deep cerebellar nuclei, travel through the dorsal pons, decussate at the inferior colliculi, and continue via the contralateral thalamus to the cerebral cortex [7, 8, 36]. Romer et al. [41] found that lower FA of the left SCP was related to higher psychopathology in young adults. In a subsequent investigation in an independent sample of older adults, Romer et al. [42] found that lower microstructural properties of cortico-cerebellar tracts were also associated with greater risk for psychopathology. This is the first study, however, to examine the role of white matter of the cerebellar peduncles longitudinally in adolescents.

The present study was designed to address this gap in our knowledge. Given increasing evidence that the cerebellum is involved in affective functioning, we hypothesized that lower FA of the cerebellar peduncles, particularly the bilateral SCPs given their efferent connections to the contralateral cerebral cortex and findings reported by Romer et al. [41] in adults, will be associated with increasing internalizing and externalizing symptoms in adolescents over a 2-year period. Although we did not have strong hypotheses regarding the association between FA of the MCP and ICPs given the paucity of research on these regions and their structural organization, we included these pathways in our analyses to obtain a more complete understanding of how various cerebellar pathways are related to internalizing and externalizing symptoms. Further, given that males and females differ in white matter development [19], in the prevalence of internalizing and externalizing symptoms [32], and in the association of internalizing and externalizing symptoms with diffusivity of cerebral white matter pathways [3], we also examined whether sex moderated the association between FA of the cerebellar peduncles and changes in internalizing and externalizing symptoms.

Materials and Methods

Participants and Procedure

Two hundred fourteen adolescents from the San Francisco Bay Area participated in a longitudinal study investigating the effects of early life stress on psychobiological development and were recruited via media and online advertisements [9–11, 33]. At baseline, adolescents were 9–13 years of age, were proficient in English, and were sex-matched on pubertal status (see "Pubertal Status" in the Supplement). Exclusion criteria included a history of major illness or neurological disorder, contraindications for participating in a magnetic resonance imaging (MRI) scan (e.g., braces, metal implants), cognitive or physical challenges that interfered with their ability to comprehend or complete study procedures, and for females, the onset of menses prior to the baseline session. All participants and their legal guardian(s) assented and consented to the study, respectively, and were compensated for their participation. All study procedures were approved by the Stanford Institutional Review Board. Participants attended in-person laboratory sessions in which we obtained demographic information (baseline), neuroimaging data (baseline), and self-report measures of internalizing and externalizing symptoms (baseline and follow-up, 2 years later).

Participants who completed the Youth Self-Report (YSR; [1] at baseline and at a 2-year follow-up assessment (M interval = 2.04 years; SD = 0.38), and who had cerebellar coverage in a diffusion MRI (dMRI) scan at baseline, were included in the analyses. Of the 150 participants with cerebellar coverage, 74% completed the YSR at baseline and follow-up; thus, the final sample for the present study included 111 participants (62 female; baseline: ages 9–13 years, M = 11.42, SD = 1.01; follow-up: ages 11–15 years, M = 13.46, SD = 1.07; see Table 1).

Measures

Parental Income We assessed socioeconomic status by asking parents to report on their annual household income (binned from 1 (< $5,000) to 10 (> $150,000)).

Table. 1 Demographic and clinical characteristics of the sample

Variable	Male*M*(*SD*) or *N*(%)	Female*M*(*SD*) or *N*(%)	Statistic	*p* value
Sex	49	62		
Age at baseline	11.84 (0.88)	11.08 (0.99)	$t(109) = 4.23$	< .001
Age at follow-up	13.86 (0.96)	13.14 (1.06)	$t(109) = 3.74$	< .001
Interval	2.02 (0.34)	2.06 (0.40)	$t(109) = -0.52$	.606
FA of left SCP	0.50 (0.05)	0.49 (0.06)	$t(109) = 1.41$	.161
FA of right SCP	0.50 (0.06)	0.50 (0.05)	$t(108) = 0.37$	.712
FA of left ICP	0.44 (0.03)	0.45 (0.04)	$t(106) = -1.30$	.195
FA of right ICP	0.46 (0.04)	0.46 (0.04)	$t(102) = -0.55$	.587
FA of MCP	0.53 (0.06)	0.51 (0.06)	$t(107) = 1.74$	.085
Average relative motion	0.76 (0.51)	0.78 (0.35)	$t(109) = -0.16$	.873
Parent income				
< $5,000–$35,000	4 (8%)	7 (11%)	$\chi^2(3) = 0.60$	.897
$35,001–$150,000	23 (47%)	29 (47%)		
$150,000 +	19 (39%)	20 (32%)		
Don't know/missing data	3 (6%)	6 (10%)		
Income-to-needs ratio	1.36 (0.54)	1.28 (0.57)	$t(100) = 0.73$	.466
Child Race				
White	24 (49%)	31 (50%)	$\chi^2(5) = 1.17$	.948
Hispanic/Latinx	2 (4%)	4 (6%)		
Asian/Asian American	6 (12%)	7 (11%)		
Black/African American	5 (10%)	5 (8%)		
Two or more races	11 (22%)	12 (19%)		
Other races	1 (2%)	3 (5%)		
Internalizing at baseline	10.86 (7.71)	12.94 (9.73)	$t(109) = -1.22$	.224
Internalizing at follow-up	8.94 (7.57)	11.06 (9.37)	$t(109) = -1.29$	.200
Externalizing at baseline	10.04 (5.62)	8.66 (7.13)	$t(109) = 1.11$	.270
Externalizing at follow-up	9.69 (6.42)	8.13 (5.85)	$t(109) = 1.34$	.183

Interval = years between baseline and follow-up; *FA* fractional anisotropy; *SCP* superior cerebellar peduncle; *ICP* inferior cerebellar peduncle; *MCP* middle cerebellar peduncle

Income-to-Needs Ratio We also calculated the income-to-needs ratio (INR) for each participant by dividing the midpoint of the selected income bin by the low-income value for Santa Clara county which takes into account the number of people in the home (https://www.huduser.gov/portal/datasets/il/il2017/2017summary.odn) [22].

Internalizing and Externalizing Symptoms The YSR [1] is a 112-item questionnaire developed for youth to assess symptoms experienced over the last 6 months. We examined the broadband YSR scales of internalizing (e.g., withdrawn/depressed) and externalizing (e.g., delinquent/aggressive behavior) symptoms. Each item is assessed on a 3-point scale from 0 (not true) to 2 (very true or often true), yielding total raw scores ranging from 0 to 60 (internalizing) and 64 (externalizing). The YSR has excellent psychometric properties, including high validity and reliability (baseline internalizing, $\alpha = 0.90$; baseline externalizing, $\alpha = 0.85$; follow-up internalizing. $\alpha = 0.91$; follow-up externalizing, $\alpha = 0.85$).

Diffusion MRI Preprocessing

At baseline, adolescents underwent diffusion MRI (dMRI) at the Center for Cognitive and Neurobiological Imaging (https://cni.stanford.edu). dMRI was obtained on a 3 T MRI scanner (Discovery MR750 scanner, General Electric Healthcare, Milwaukee, WI, USA) with a 32-channel head coil. T1-weighted images were obtained using a spoiled gradient echo pulse sequence (SPGR; repetition time (TR)/echo time (TE)/inversion time (TI) = 6.24/2.34/450 ms; flip angle = 12°; 186 sagittal slices; 0.9 mm isotropic voxels). Diffusion data were collected using an Echo-Planar Imaging sequence (TR/TE = 8500/93.5 ms; 64 axial slices;

0.938 × 0.939 × 2.00 mm voxel size; 60 b = 2000 diffusion-weighted directions, and 6 non-diffusion (b = 0) images at the beginning of the scan; anterior/posterior phase encoding direction; scan time: 9 min 30 s). Three participants had a different voxel size (2 × 2 × 2 mm) due to a scanner upgrade; therefore, we repeated analyses excluding these participants to examine the consistency of findings.

As described previously [16, 17], dMRI data were pre-processed using mrDiffusion (https://github.com/vistalab/vistasoft/tree/master/mrDiffusion) implemented in MATLAB R2018B (MathWorks, Natick, MA, USA). We used a rigid body alignment algorithm to correct participants' motion during the diffusion-weighted scan [40]. We registered all diffusion-weighted volumes to the average of the six b0 images and registered the average b0 image to the participant's ACPC-aligned T1-weighted image. The resulting transforms were combined and applied to the raw data (as well as the diffusion gradient directions [24]), which were then resampled to 2 × 2 × 2 mm isotropic voxels. We generated maps of FA using robust tensor fitting with outlier rejection [12].

Head Motion

In each participant, we quantified relative head motion by measuring the amount of motion correction (in voxels) in all three planes of a diffusion volume relative to the previous volume. We then counted the number of volumes that had 1 or more voxels (voxel size = 2 mm^3) of relative motion. The mean number of volumes with ≥ 1 voxel of relative motion was calculated based on all participants included in the final analysis ($M = 0.77$; $SD = 0.43$). Participants with scans with 30% of volumes affected by translational motion of 3 voxels or more relative to the prior volume were considered to have excessive head motion. No participant in the current sample met this criterion.

Tractography of the Cerebellar Peduncles

Automatic segmentation and quantification of the cerebellar peduncles in each participant's native space were performed using the Automated Fiber Quantification (AFQ; [50], https://github.com/yeatmanlab/AFQ) software package implemented in MATLAB R2018B. The methods and algorithms used for cerebellar segmentation in AFQ have been described in detail by Yeatman et al. [50] and Bruckert et al. [7, 8] and are summarized in the Supplement.

Renderings of all segmented cerebellar peduncles were visually inspected; those which did not conform to known anatomical configurations were excluded from statistical analyses. We successfully tracked the bilateral SCPs, MCP, and bilateral ICPs in the majority of participants (see Fig. 1 for tractography in one representative participant). However, one participant did not track in the right SCP, three participants did not track in the left ICP, seven participants did not track in the right ICP, and two participants did not track in the MCP; all were therefore excluded from the respective analyses.

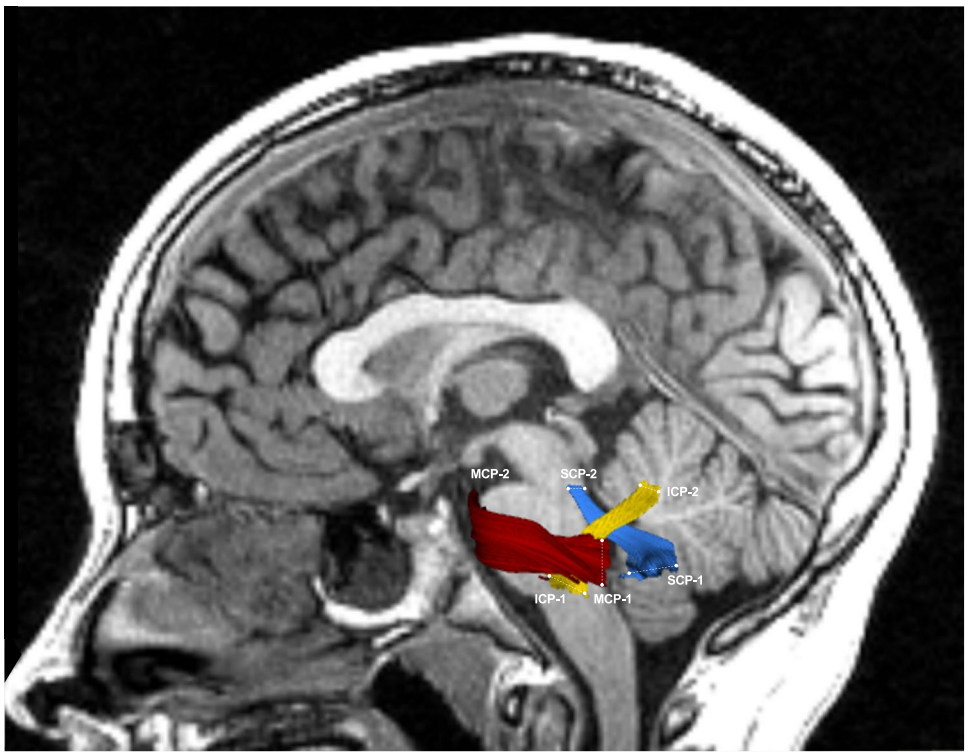

Fig. 1 Tractography of the cerebellar peduncles. Cerebellar peduncles are clipped at the Regions of Interest. Blue (superior cerebellar peduncle); red (middle cerebellar peduncle); yellow (inferior cerebellar peduncle)

Statistical Analyses

Statistical analyses were conducted using *R* v. 4.0.2 [48]. We first examined whether there were sex differences in demographic and clinical variables. Next, we tested whether the relation between FA of the cerebellar peduncles and change in symptoms from baseline to follow-up was moderated by sex using a series of linear regression models. Follow-up simple slopes were used to probe interactions. We controlled for age at baseline, the interval between baseline and follow-up, the respective YSR subscale at baseline (i.e., internalizing or externalizing), and average relative motion. We fit a total of ten linear regression models (four for the left and right SCPs and ICPs in predicting change in internalizing and externalizing scores and two models for the MCP). Sex was dummy coded; all continuous variables were z-scored. We used a false discovery rate (FDR) to correct for multiple comparisons.

Supplemental Analyses

We computed Pearson's correlations (FDR corrected) between FA of the cerebellar peduncles at baseline and internalizing and externalizing symptoms at baseline and follow-up separately in males and females (see Supplement; Figures S1 & S2). Further, we conducted additional linear models to examine the specificity of sex as the interaction term, compared to age and puberty, to ensure that findings were sex-related and were not driven by differences in age or pubertal stage. In the presence of significant main effects or interactions, we tested the association between FA of the respective cerebellar peduncles with baseline and follow-up symptoms to determine whether significant findings were driven by symptoms at baseline or follow-up. Further, in the presence of a significant interaction of sex and FA, we examined whether mean-tract RD, AD, or MD were also related to change in internalizing or externalizing symptoms. Finally, we excluded the three participants with a different dMRI acquisition to ensure the pattern of results held.

Results

Participants

Demographic and clinical characteristics of the sample are presented in Table 1. Our sample was representative of the San Francisco Bay Area population with regards to race and income (https://www.census.gov/quickfacts/sanfranciscocountycalifornia). Further, raw internalizing and externalizing scores were relatively low and did not differ by sex (Table 1). In recruiting participants for the parent study, males and females were matched on pubertal status; thus, by design, female participants were younger than male participants at both baseline and follow-up (Table 1). Adolescents included in the sample did not differ from excluded adolescents with respect to sex ($\chi^2(1) = 0.01$, $p = 0.942$), race ($\chi^2(5) = 1.17$, $p = 0.948$), parental income ($\chi^2(3) = 0.45$, $p = 0.929$), or age at baseline ($t(212) = -0.51$, $p = 0.611$); they also did not differ in baseline internalizing ($t(186) = -0.54$, $p = 0.588$) or externalizing scores ($t(186) = 0.27$, $p = 0.788$). Further, males and females did not differ significantly in parental income, race, income-to-needs ratio, baseline or follow-up YSR scores, average relative motion, or FA of the cerebellar peduncles (all $p > 0.050$; Table 1). 26.13% of the sample had an INR below 1, indicating they were low-income based on county-specific thresholds. Further, 8.11% of the sample had an INR of 0.29 or lower, indicating that they were living below the county-specific poverty line (https://www.census.gov/quickfacts/fact/table/santaclaracountycalifornia,US/PST045219).

Internalizing and externalizing symptoms were strongly positively correlated at baseline ($r(109) = 0.66$, $p < 0.001$) and follow-up ($r(109) = 0.68$, $p < 0.001$), reflecting the comorbidity of these symptoms. A correlation matrix of the cerebellar peduncles and baseline and follow-up internalizing and externalizing symptoms is presented, separately by sex, in the Supplement (Figures S1 & S2). At the 0-order level, FA of the left and right SCPs were negatively associated with internalizing and externalizing symptoms at follow-up in females only; FDR corrected (Figure S2).

Interaction Effects of Sex and Cerebellar FA on Change in Symptoms

Sex, data collection interval, and relative motion did not contribute significant variance to any model (Tables 2–4); however, age at baseline contributed variance to change in internalizing models (Table 2 models 3–4; Table 3 model 3). There was a significant interaction of sex and FA of the right SCP in predicting change in both internalizing ($t(102) = -3.22$, $p = 0.002$) (Fig. 2) and externalizing symptoms, $t(102) = -2.31$, $p = 0.023$ (Figure S3). Simple slope analyses revealed that in females only, lower FA of the right SCP predicted increasing internalizing (Fig. 2) and externalizing symptoms (Figure S3). The overall models accounted for 44.2% and 38.0% of the variance in internalizing and externalizing symptom change scores, respectively. Further, there was an interaction of sex and FA of the right ICP in predicting change in externalizing symptoms, $t(96) = -2.50$, $p = 0.014$ (Figure S4): similar to the right SCP, lower FA of the right ICP predicted increasing externalizing symptoms in females only. The overall model explained 42.9% of the variance in the change score. The interaction of sex and the right SCP in predicting change in internalizing symptoms survived FDR correction (adjusted $p = 0.017$),

Table. 2 Fractional anisotropy of the superior cerebellar peduncles in relation to change in symptoms of psychopathology

Effect	Model 1 Externalizing	Model 2 Externalizing	Model 3 Internalizing	Model 4 Internalizing
Sex	−0.14 (0.16)	−0.12 (0.16)	0.25 (0.16)	0.30 (0.16)
Age at baseline	0.06 (0.08)	0.08 (0.09)	0.19 (0.08)*	0.22 (0.08)**
Interval	0.03 (0.08)	0.04 (0.08)	0.09 (0.07)	0.12 (0.07)
YSR subscale baseline	−0.61 (0.08)***	−0.62 (0.08)***	−0.65 (0.08)***	−0.68 (0.07)***
Relative motion	−0.09 (0.08)	−0.05 (0.08)	0.01 (0.08)	0.06 (0.07)
Left SCP FA	0.22 (0.28)	-	0.16 (0.28)	-
Left SCP FA x Sex	−0.24 (0.16)	-	−0.21 (0.16)	-
Right SCP FA	-	0.43 (0.25)	-	0.62 (0.24)*
Right SCP FA x Sex	-	−0.36 (0.16)***	-	−0.48 (0.15)**
Adjusted R^2	37.8%***	38.0%***	41.1%***	44.2%***

Interval = years between baseline and follow-up; *YSR* Youth Self-Report; *FA* fractional anisotropy; *SCP* superior cerebellar peduncle
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, uncorrected

Table 3 Fractional anisotropy of the inferior cerebellar peduncles in relation to change in symptoms of psychopathology

Effect	Model 5 Externalizing	Model 6 Externalizing	Model 7 Internalizing	Model 8 Internalizing
Sex	−0.05 (0.17)	−0.06 (0.17)	0.34 (0.17)	0.36 (0.17)
Age at baseline	0.09 (0.09)	0.06 (0.09)	0.20 (0.08)*	0.22 (0.09)
Interval	0.01 (0.08)	−0.06 (0.08)	0.07 (0.08)	0.04 (0.08)
YSR subscale baseline	−0.63 (0.08)***	−0.69 (0.08)***	−0.64 (0.08)***	−0.67 (0.08)
Relative motion	−0.06 (0.08)	−0.03 (0.08)	0.04 (0.08)	0.05 (0.08)
Left ICP FA	0.07 (0.29)	-	-0.02 (0.29)	-
Left ICP FA x Sex	−0.10 (0.17)	-	-0.05 (0.17)	-
Right ICP FA	-	0.57 (0.26)*	-	0.28 (0.27)
Right ICP FA x Sex	-	−0.39 (0.16)*	-	−0.24 (0.16)
Adjusted R^2	35.4%***	42.9%***	37.7%***	39.2%***

Interval = years between baseline and follow-up; *YSR* Youth Self-Report; *FA* fractional anisotropy; *ICP* inferior cerebellar peduncle
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, uncorrected

Table 4 Fractional anisotropy of the middle cerebellar peduncle in relation to change in symptoms of psychopathology

Effect	Model 9 Externalizing	Model 10 Internalizing
Sex	−0.20 (0.17)	0.24 (0.17)
Age at baseline	0.04 (0.09)	0.17 (0.08)
Interval	0.00 (0.08)	0.07 (0.08)
YSR subscale baseline	−0.61 (0.08)***	−0.65 (0.08)***
Relative motion	−0.05 (0.08)	0.03 (0.28)
MCP FA	−0.01 (0.29)	0.04 (0.28)
MCP FA x Sex	−0.07 (0.17)	−0.09 (0.17)
Adjusted R^2	35.1%***	37.6%***

Interval = years between baseline and follow-up; *YSR* Youth Self-Report; *FA* fractional anisotropy; *MCP* middle cerebellar peduncle
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, uncorrected

whereas the interactions of sex and the right SCP and the right ICP in predicting change in externalizing symptoms did not (adjusted $p = 0.076$ and $p = 0.071$). There were no main effects (or interactions with sex) of FA of the left SCP, MCP, or left ICP in predicting change in internalizing or externalizing symptoms (all $p > 0.050$).

Supplemental Analyses

Specificity of Sex as a Moderator

The obtained interactions were specific to sex and were not driven by age or pubertal status. Specifically, the interaction of age and FA of the right SCP in predicting change in internalizing symptoms was not significant, $t(102) = 1.84$, $p = 0.069$. Further, the interaction of pubertal status and FA of the right SCP was not significant, $t(101) = -0.78$, $p = 0.437$.

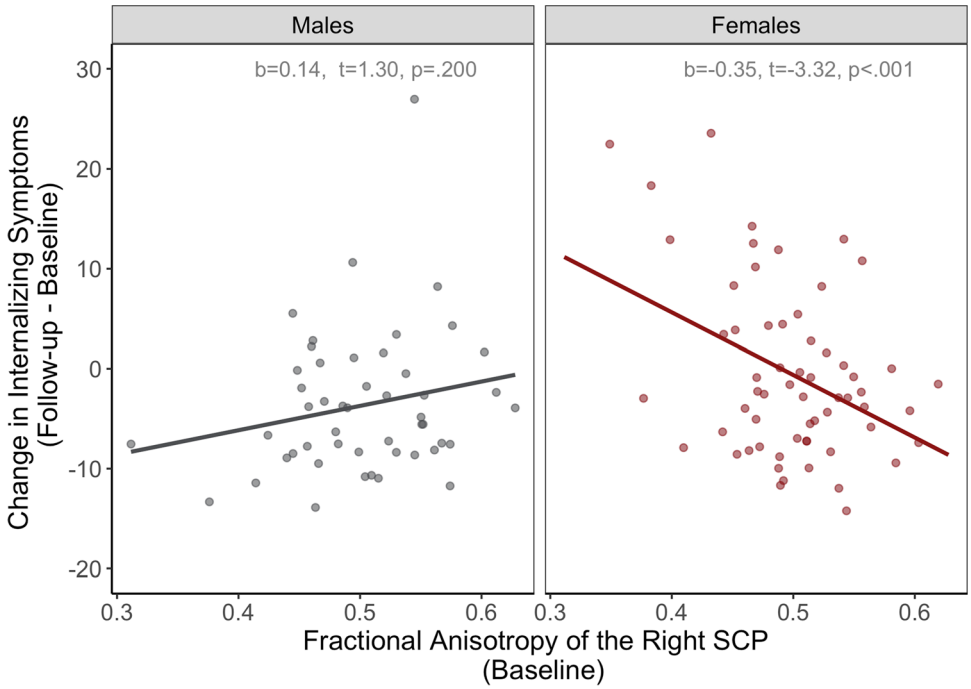

Fig. 2 Fractional anisotropy of the right superior cerebellar peduncle is related to change in internalizing symptoms in female adolescents. SCP = superior cerebellar peduncle. Covariates include: age at baseline, the interval between baseline and follow-up, internalizing symptoms at baseline, and average relative motion

Note. SCP=superior cerebellar peduncle. Covariates include: age at baseline, the interval between baseline and follow-up, internalizing symptoms at baseline, and average relative motion.

Interaction Effects of Sex and Cerebellar FA on Baseline and Follow-Up Symptoms

There was no interaction of sex and FA right SCP in explaining baseline internalizing scores, $t(104) = -1.48$, $p = 0.143$; however, the association between FA of the right SCP and follow-up internalizing symptoms was moderated by sex, $t(103) = -3.50$, $p < 0.001$. Specifically, female participants had a negative slope between FA of the right SCP and internalizing symptoms at follow-up (Figure S5).

Interaction Effects of Sex and Additional Cerebellar Diffusivity Metrics

There was a significant interaction of sex and RD of the right SCP in predicting change in internalizing symptoms $t(102) = 2.29$, $p = 0.024$): in females, higher RD was associated with increasing symptoms (Figure S6). AD and MD of the right SCP did not predict change in internalizing symptoms in males or females (all $p > 0.050$).

Sensitivity Analyses

The pattern of findings reported above was maintained after excluding participants with a different dMRI acquisition (FA of right SCP × sex interaction: $t(99) = -2.95$, $p = 0.004$).

Discussion

In a sample of adolescents, we examined whether fractional anisotropy (FA) of the cerebellar peduncles predicted change in internalizing and externalizing symptoms over 2 years. Further, we tested whether the association between FA of the cerebellar peduncles and symptoms of internalizing and externalizing was moderated by sex. Notably, in females only, FA of the right SCP predicted change in internalizing symptoms. Specifically, only in females, lower FA and higher RD of the right SCP predicted increasing internalizing symptoms. Finally, lower FA of the right SCP and right ICP predicted increasing externalizing symptoms in females but not in males, although these results did not survive FDR correction. Considered together, our findings highlight the role of the cerebellar peduncles as sex-specific biomarkers of the development of symptoms of psychopathology in female adolescents.

There is a growing body of research implicating the cerebellum in affective functioning [2], and our findings with adolescents are consistent with results reported by Romer et al. [41] with adults that lower FA of the left SCP is related to higher levels of psychopathology. The SCPs contain mostly efferent fibers, relaying information to the contralateral cerebral cortex. Therefore, increasing symptoms of psychopathology may be a result of altered connectivity and

integration between the cerebellum and the cerebral cortex. In fact, studies have shown aberrant functional connectivity between the cerebellum and limbic and cortical regions in patients with depression [29], and that functional connectivity between the cerebellum and cerebral cortex discriminates patients with major depressive disorder from control participants [30].

Importantly, our findings were not limited to changes in internalizing symptoms or to structural properties of the SCPs. Consistent with Romer et al. [41], it is possible that structural properties of the cerebellum predict psychopathology more generally. Indeed, we found negative associations between the right SCP and right ICP and change in externalizing symptoms in adolescent girls. The ICPs contain both afferent and efferent fibers and are posited to integrate sensorimotor information from the spinal cord and medulla oblongata. Structural properties of the ICPs may relate to increasing externalizing symptoms, such as hyperactivity, via motor functioning. In fact, Moberget and colleagues (2019) showed that features of the cerebellum were associated with externalizing symptoms such as psychosis and norm-violating behavior.

Finally, the MCP contains afferent fibers, relaying information to the cortex. The MCP has been implicated in clinical samples of children with attentional-deficit hyperactivity disorder [6] and adults with schizophrenia [38],however, we did not observe associations between the MCP and changes in internalizing or externalizing symptoms. This may be due to the age of our participants or to the severity of symptoms; it will be important for researchers to examine associations between alterations in the cerebellum and aspects of psychopathology more broadly.

Importantly, researchers have posited that sex differences in brain development contribute to differential risk for the emergence of internalizing and externalizing symptoms [19]. In fact, several studies have reported that patients with affective disorders also have more diffuse white matter (indexed by lower FA) in the frontal and temporal lobes [26, 45]. Further, Andre et al. [3] recently reported sex interactions with diffusion properties of the bilateral cingulum in 6- to 16-year-old participants, showing that more diffuse white matter (indexed by higher MD) was associated with more internalizing symptoms in females. In this study we provide evidence that lower FA is a risk factor for the development of symptoms in adolescent girls. Therefore, our work supports the formulation that more diffuse white matter properties confer risk for internalizing symptoms in females and extends our understanding of this association to the cerebellum.

In this context, Mankiw and colleagues [31] provided evidence that male participants had larger global and regional cerebellar volumes than did females. Relatedly, Herting et al. [15] reported that male participants aged 10–16 years had higher FA and lower RD in the left SCP than did females. We did not find sex differences in FA of the bilateral SCPs in our study. Because of the focus of the larger study on puberty [9–11], boys and girls were matched at entry into the study on pubertal status, not on age. Therefore, boys were older than girls at both assessments, which may have also contributed to the lack of sex differences in internalizing and externalizing symptoms in our sample.

Notably, externalizing disorders have an earlier age of onset than do internalizing disorders, which do not typically appear until age 15 [13]. In fact, Rutter et al. [43] note that sex differences in depression in childhood are minimal or non-existent and increase in later adolescence. Thus, because our sample was relatively young (*M* girls age at baseline = 11.08 years; *M* girls age at follow-up = 13.14; *M* boys age at baseline = 11.84; *M* boys age at follow-up = 13.86), it is possible that sex differences in symptoms will emerge later in the participants' development. Further, age-related decreases in psychopathology have been reported in several longitudinal studies [5, 9–11, 21].

Importantly, even subthreshold symptoms in adolescence have been found to predict poorer mental health outcomes [49] and worsening symptoms [27]; in the future; therefore, early identification and treatment of symptoms may improve long-term functioning, particularly for girls who have higher rates of depression and anxiety disorders than boys [27]. In this context, identifying robust biomarkers of internalizing symptoms will offer greater possibilities for intervening and altering the trajectory of these symptoms. While our findings should be replicated, they support the formulation that more diffuse white matter microstructure (as indicated by lower FA and higher RD of the right SCP) predicts increasing internalizing symptoms in females, indicating that there was sufficient variance in our symptom measures to document associations with other variables.

Limitations

We should note three limitations of our study. First, as is commonly observed with longitudinal measurements of internalizing and externalizing symptoms, we found trends consistent with regression to the mean for these measures in our sample. It is noteworthy, however, that adolescents have been found to exhibit decreasing internalizing and externalizing symptoms over time [23] and that trajectories of symptoms are heterogenous [47]. Second, although we had over 100 participants in this study, a larger sample may have yielded more significant associations between the cerebellar peduncles and internalizing and externalizing

symptoms. Finally, although common in the San Francisco Bay Area, our sample was from predominantly high-income backgrounds, potentially limiting the generalizability of our findings.

Conclusion

Despite these limitations, this is the first longitudinal study to examine the role of cerebellar white matter in relation to the development of internalizing and externalizing symptoms in adolescents. We assessed whether FA of the cerebellar peduncles, a set of tracts implicated in cognitive and affective integration that has been understudied in adolescents, predicted change in internalizing and externalizing symptoms over 2 years. We found that FA of the right SCP was related to change in internalizing symptoms in females, but not in males, thus potentially contributing to sex differences observed in psychopathology. These findings highlight the role of cerebellar white matter organization in the emergence of increases in internalizing symptoms during adolescence, particularly in females. Both we and Romer et al. [41] assessed adults and adolescents without clinically significant psychiatric symptoms. Given that FA of the SCPs is characterized by plasticity and continued maturation through adolescence [7, 8], it will be important for researchers to examine white matter of the SCPs in samples of youth who are experiencing more severe levels of psychopathology.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12311-021-01307-x.

Acknowledgements We thank Cat Camacho, Anna Cichocki, Monica Ellwood-Lowe, Megan Goyer, Amar Ojha, Holly Pham, Morgan Popolizio, Alexandra Price, Sophie Schouboe, and Lucinda Sisk for their assistance in data collection and management. We also thank all the adolescents and their families for participating in this research study.

Funding The National Institutes of Health supported this research (NIH; R37MH101495 to IHG, F32MH120975 to RC, K01MH117442 to TCH).

Declarations

Conflict of Interest The authors declare no competing interests.

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