Cerebello–thalamo–cerebral connections in pediatric brain tumor patients: Impact on working memory

Nicole Law a,b,c, Eric Bouffet d, Suzanne Laughlin e, Normand Laperriere f, Marie-Eve Brière g, Douglas Strother h, Dina McConnell i, Juliette Hukin j, Christopher Fryer k, Conrad Rockel a, Jolynn Dickson a, Donald Mabbott a,b,d,⁎

a Program in Neuroscience and Mental Health, Hospital for Sick Children, Toronto, Ontario, Canada
b Department of Psychology, University of Toronto, Toronto, Ontario, Canada
c Department of Oncology, British Columbia Children’s Hospital, Vancouver, BC, Canada
d Department of Psychology, University of Toronto, Toronto, Ontario, Canada
e Program in Neuroscience, University of Toronto, Toronto, Ontario, Canada
f Department of Haematology/Oncology, Hospital for Sick Children, Toronto, Ontario, Canada
g Radiation Oncology, Princess Margaret Hospital, Toronto, Ontario, Canada
h Diagnostic Imaging, Hospital for Sick Children, Toronto, Ontario, Canada
i Southern Alberta Cancer Program, Alberta Children’s Hospital, Calgary Alberta, Canada
j Department of Psychology, British Columbia Children’s Hospital, Vancouver, BC, Canada
k Department of Oncology, British Columbia Children’s Hospital, Vancouver, BC, Canada

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A B S T R A C T
Brain tumors are the leading cause of death and disability from childhood disease in developed countries. Pediatric posterior fossa tumors are often effectively controlled with a combination of surgery, radiation, and chemotherapy, depending on tumor type. White matter injury following resection of tumor and radiation treatment is associated with cognitive declines, including working memory deficits. We investigated how brain injury following treatment for posterior fossa tumors results in deficits in working memory. We used diffusion tensor imaging and probabilistic tractography to examine the structural integrity of cerebello–thalamo–cerebral tracts in patients and healthy children. We also compared working memory outcome in patients versus controls, and related this function to integrity of cerebello–thalamo–cerebral tracts. Bilateral cerebello–thalamo–cerebral tracts were delineated in all participants. Patients treated with a combination of surgery and radiation had lower mean anisotropy and higher mean radial diffusivity within the cerebellar regions of the cerebello–thalamo–cerebral tract compared to patients treated with surgery only and healthy controls. Poorer working memory scores were observed for the cranial radiation group relative to controls. Reduced anisotropy and higher radial diffusivity within the entire cerebello–thalamo–cerebral pathway predicted lower working memory. Our finding that working memory function is related to the integrity of cerebello–thalamo–cerebral connections is a novel contribution to the understanding of cerebral–cerebellar communication. Identifying differences in the structural integrity of white matter for specific pathways is an essential step in attempting to localize the effects of posterior fossa tumors and their treatment methods.

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Introduction
With advances in medical treatment in the last 20 years, most children now survive childhood brain tumors, with five year survival rates approaching 70% (Strother et al., 2002). The majority of pediatric brain tumors arise in the cerebellar hemispheres or fourth ventricle of the posterior fossa (PF), including low grade astrocytomas, ependymomas, and medulloblastomas (Strother et al., 2002). Low grade astrocytomas are treated effectively with surgery only, whereas ependymomas are typically treated with surgery and focal radiation. Medulloblastomas are treated with surgery, chemotherapy, and cranial-spinal radiation (CSR) with a boost to the PF (Strother et al., 2002). Unfortunately, significant impairment often accompanies treatment. The long term adverse effects of treatment for PF tumors on thinking and learning are well documented (Beebe, 2005; Dennis et al., 1998; Mabbott et al., 2008; Mulhern et al., 2004; Reddick et al., 2003; Ris et al., 2001; Spiegler et al., 2004).

Surgery for PF tumors can result in impairments of speech and communication, executive function, and behavioral regulation (Aarsen et al., 2004; Beebe, 2005; Ersahin et al., 1996; Huber et al., 2006; Levisohn et al., 2002; Spiegler et al., 2004).
et al., 2000; Riva and Giorgi, 2000; Steinlin et al., 2003). CSR is associated with significant neuro-toxicity, including white matter damage and cognitive morbidity (Edwards-Brown and Jakacki, 1999; Khong et al., 2004). However, the underlying neurobiological mechanisms that result in the late effects are poorly understood.

Diffuse white matter injury following radiation has been found to predict poor intellectual function in children with PF tumors (Khong et al., 2003; Mabbott et al., 2008; Mulhern et al., 2004; Ris et al., 2001; Schultheiss et al., 1995; Spiegler et al., 2004; Wong and Van der Kogel, 2004). However, the underlying neurobiological mechanisms that result in these late effects are poorly understood.

To examine a potential biological substrate of cognitive morbidity in PF tumors, we used Diffusion Tensor Imaging (DTI) to investigate whether treatment damages the primary white matter pathway connecting the cerebellum with frontal cortex, and if such damage contributes to the working memory deficits observed in this population. Notably, the cerebellum interacts with frontal cortex to support working memory function (Chen and Desmond, 2005; Salmi et al., 2010). Because PF tumors arise in or near the cerebellum, this structure and its connections with cerebral areas are particularly vulnerable to insult.

The role of the cerebellum in the coordination of motor function is well established (Evarts and Thach, 1969). Recent discoveries have also highlighted the role of the cerebellum in modulating cognitive function (Akshoomoff and Courchesne, 1992; Allen et al., 1997; Kim et al., 1994; Leiner et al., 1993; Timmann and Daum, 2007), likely as part of a distributed neural system involving reciprocal connectivity with the frontal lobes (Dum and Strick, 2003; Fiez, 1996; Kelly and Strick, 2003; Krienen and Buckner, 2009; Middleton and Strick, 1994, 2001; Ramnani et al., 2006; Schmahmann and Pandya, 1997a).

Specifically, the cerebellum is part of a distributed neural system that has reciprocal connectivity with the frontal lobes via cerebrocerebellar white matter circuitry (Akshoomoff and Courchesne, 1992; Desmond et al., 1997; Dum and Strick, 2003; Jissendi et al., 2008; Leiner et al., 1993; Middleton and Strick, 1994, 2001; Ramnani et al., 2006; Schmahmann and Pandya, 1995, 1997b; Strick et al., 2009; Timmann and Daum, 2007). The cerebello–thalamo–cerebral pathway is the primary feedback loop from the cerebellum to frontal cortex; it connects deep cerebellar nuclei to thalamic nuclei via the cerebellar peduncles and midbrain, terminating within the frontal cortex (Leiner et al., 1993; Middleton and Strick, 1991; Schmahmann, 1996). Identifying damage to this pathway as a result of treatment for PF tumors and how it may interfere with cognition is an important first step in discovering the neurobiological mechanisms that result in adverse neurocognitive outcome in these patients; this is the focus of our study.

There is recent DTI evidence that the Posterior Fossa Syndrome – a significant behavioral disturbance following PF surgery – is linked to compromised white matter regions within the cerebello–thalamo–cerebral pathway (Morris et al., 2009). What has not been examined is whether damage to this pathway contributes to the cognitive deficits seen in these patients, particularly working memory impairment (Mabbott et al., 2008). Working memory is a core cognitive process involving the concurrent storage, manipulation, and integration of information; crucial for knowledge acquisition in normal development (Baddeley, 2003; Case, 1995; Kail, 2000). Impaired working

The three main neural components of the cerebello–thalamo–cerebral pathway – the cerebellum, thalamus, and dorsolateral prefrontal cortex (DLPFC) – are all implicated in working memory function (Curtis and D’Esposito, 2003; Desmond et al., 1997; Funahashi et al., 1993; Kim et al., 1994; Peterburs et al.; Ravizza et al., 2006; Smith et al., 1996; Ziemus et al., 2007). Furthermore, human imaging studies have shown that: (a) the cerebellar, thalamic, and prefrontal areas activated during working memory performance are connected via cerebello–thalamo–cerebral white matter tracts (Salmi et al., 2010), (b) the cerebellum interacts with DLPFC in working memory function (Chen and Desmond, 2005), and (c) white matter within the cerebellar peduncles is related to working memory (Takahashi et al., 2010). Consequently, the cerebello–thalamo–cerebral pathway is a prime candidate as a possible neural substrate of working memory impairment seen following treatment for pediatric PF tumors. Thus, we examined whether the structural integrity of the feedback loop from the cerebellum to frontal cortex is related to working memory.

We compared FA, MD, AD, and RD within bilateral cerebello–thalamo–cerebral white matter pathways in three groups: PF tumor patients treated with cranial radiation (CR Group), PF tumor patients treated with surgery only (SO Group), and healthy controls. DTI can be used to generate quantitative indices (i.e. eigenvalues — $\lambda_1$, $\lambda_2$, $\lambda_3$) that reflect white matter tract microstructure and architecture, based on water molecule displacement and directionality. The first eigenvalue ($\lambda_1$) measures axial diffusivity (AD) — diffusion parallel to the axonal fibres (Basser, 1995). An increase in axial diffusivity may reflect axon degeneration (Song et al., 2002). Radial diffusivity (RD) is the average of the second ($\lambda_2$) and third ($\lambda_3$) eigenvalues and measures diffusion perpendicular to axonal fibres, reflecting the integrity of myelin sheath (Basser, 1995). The eigenvalues are combined to provide summary parameters. Directionality is indexed by fractional anisotropy (FA) and magnitude is indexed by mean diffusivity (MD). Reduced FA and increased MD reflect myelin breakdown and axonal degeneration (Beaulieu, 2002).

We compared FA, MD, AD, and RD within bilateral cerebello–thalamo–cerebral pathways between patients treated with cranial radiation, those treated with surgery only, and healthy controls, and also examined group differences in working memory. We expected that DTI indices reflecting compromised integrity of cerebello–thalamo–cerebral tracts would be evident in the patient groups and associated with lower working memory scores. The impact of medical and demographic variables on pathway integrity and working memory was also investigated. By examining the integrity of cerebello–thalamo–cerebral connections and relating DTI indices with working memory measures in patients with PF tumors, we strived to gain insight into a possible neural correlate of treatment-induced impairment of working memory function.

**Materials and methods**

**Participants**

Sixty-seven children were seen at Alberta Children’s Hospital in Calgary (ACH), British Columbia Children’s Hospital in Vancouver (BCCH), and The Hospital for Sick Children in Toronto (SickKids). Medical and demographic information is presented in Table 1. Twenty-nine patients were treated with surgery and cranial radiation for PF tumors (CR Group). Adjuvant chemotherapy was used for patients with medulloblastoma in this group. Depending on the treatment protocol employed, different combinations of cisplatin, vincristine, etoposide, ifosfamide, carboplatin, and cyclophosphamide were administered.

Twelve patients were treated with surgery only for PF tumors (SO Group). Considering their low grade pathology, the majority of patients had gross total resections and were not treated with chemotherapy. Twenty six healthy children also participated (Control Group). The protocol for this study was approved by the Research Ethics Boards of each participating site. All participants provided written informed consent or assent and parental consent was obtained where applicable. The groups did not differ in gender ($\chi^2(1) = 1.459$, $p = 0.227$), age at testing ($F(2,64) = 0.455$, $p = 0.637$), but did differ in average parental education ($F(2,62) = 20.589$, $p < 0.001$). Parental education was higher for the control group than the patient groups ($p < 0.003$). Patient groups did not differ in age at diagnosis ($F(1,39) = 0.635$, $p = 0.430$), time since diagnosis ($F(1,38) = 2.257$, $p = 0.141$), tumor size ($F(1,33) = 0.806$, $p = 0.376$), or extent of resection ($F(1,33) = 2.703$, $p = 0.110$). Incidence of cerebellar (i.e. ataxia, mutism, dysarthria) and other complications (i.e. hydrocephalus, nystagmus, hemiparesis, hearing impairment, diplopia, cranial nerve deficits) was also compared. The CR and SO groups did not differ in the frequency of either a single post-operative complication ($\chi^2(1) = 1.459$, $p = 0.227$), or multiple post-operative complications ($\chi^2(1) = 1.435$, $p = 0.231$).

![Fig. 1](image-url). a: the DLPCF defined on the surface of 3D-T1 FSPGR. b: the thalamus defined on the b0. c: the hemispheric cerebellar white matter defined.
MR Imaging and post-processing

MRI measurements were performed at SickKids using a GE LX 1.5T MRI scanner with 8 channel head coil (GE Healthcare, Milwaukee, Wis.) and at the BCCH and ACH using a Siemens 1.5T MRI scanner with 12 channel head coil (Siemens Canada Ltd., Mississauga, On.). The scanning protocol included a 3D-T1 FSPGR gradient echo, inversion recovery-prepared sequence (TR time = 400 ms; TE/TR = 4.2/10.056 ms; 116–124 contiguous axial slices; NEX = 1; 256 × 192 matrix, interpolated to 256 × 256; FOV = 24 × 24 cm; rbw = 162.734 kHz; slice thickness = 1.5 mm) and a diffusion-weighted sequence (single shot spin echo DTI sequence with EPI readout: 25–31 directions; b = 1000 s/mm²; TR/TE = 85.5/15000 ms; 45–50 contiguous axial slices; NEX = 1; 128 × 128 matrix, interpolated to 256 × 256; FOV = 24 × 24 cm; rbw = 1953.12 kHz; slice thickness = 3 mm). DTI data were corrected for inhomogeneity and eddy current and indices were calculated.

To evaluate inter-site consistency in MRI measurements, one individual (Research Coordinator) was scanned at all three hospitals. Signal-to-noise ratios for the zero diffusion images from SickKids, BCCH, and ACH were 24.7, 43.6, and 39.1, respectively, and signal-to-noise ratios for the echo-planar images were 14.0, 17.4, and 16.6, respectively. As ratios were higher for BCCH and ACH relative to SickKids, site of imaging (BCCH/ACH versus SickKids) was included as a covariate in subsequent analyses of imaging data.

DTI post processing, including seed/waypoint placement and probabilistic tractography were conducted using the FMRIB Software Library (Smith et al., 2004; Woolrich et al., 2009). First, MRI data were corrected for inhomogeneity and eddy current and DTI maps (FA, MD, \(\lambda_1\), \(\lambda_2\), and \(\lambda_3\)) were calculated. Second, the probability of connection between all voxels was calculated and served as a basis for fibre tracking analysis (Behrens et al., 2007). Seed, waypoint, and exclusion masks were placed on the zero diffusion image (b₀) for 10 healthy control children to create a standard template for tractography. The DLPFC was used as the seed point and the thalamus and cerebellum were used as waypoints for probabilistic tractography (see Fig. 1). Exclusion masks were placed on the corpus callosum and inferior medulla to constrain the tracts to the proposed pathway. Using Analyze (Biomedical Imaging Resource, Mayo Clinic, Rochester, Minn.) and FSLview (Smith et al., 2004; Woolrich et al., 2009), the DLPFC was defined on the surface of 3D-T1 FSPGR and included medial prefrontal cortex, inferior prefrontal gyrus, the superior and middle frontal gyri, and parts of the inferior frontal gyrus (i.e. pars opercularis and pars triangularis) (Fig. 1a). This region was registered to b₀ space and extended 2 mm into adjacent white matter. The thalamus was defined as the b₀ from the third ventricle superior to where the thalamus diminished and the lateral ventricles were evident inferiorly (Fig. 1b). Finally, the hemispheric cerebellar white matter was defined using a superior boundary of the cerebellar peduncles and an inferior boundary at the pons where the basal arteries are present (Fig. 1c). All masks were then registered to a single composite volume from the 10 healthy controls (Woods et al., 1998), and merged using Analyze. Masks were then converted back to the native space of all participants using affine transformation so that mask placement was standardized across the entire sample (Woods et al., 1998).

Probabilistic tractography (Behrens et al., 2007) using the seed and masks was used to delineate the cerebellum–thalamo–cerebral pathway bilaterally. Tracts were thresholded and edited to eliminate erroneous streamlines and, if necessary, manually edited to remove any erroneous tracts not comprising or included in the cerebellum–thalamo–cerebral pathway. Each tract was segmented into regions based on an anatomic template (Kabani et al., 2002; Mabott et al., 2009a). Anatomical regions produced from this segmentation included left and right frontal hemispheric white matter, left and right midbrain regions (i.e. internal capsule, thalamus), pons, and left and right cerebellar hemispheric white matter. Means and standard deviations for FA, MD, AD, and RD were calculated for the whole tract as well as each anatomic region.

Tract-based spatial statistics (TBSS) were used for supplementary voxel-wise analysis based on the methods outlined in Smith et al. (2006). First, FA maps were nonlinearly registered to a representative image, which was then aligned using affine registration to standard space (MN152; Montreal Neurological Institute, McGill, Canada). Second, a cross-subject mean FA image was created and used to generate a skeleton FA map, thresholded at FA > .20, that included only the large fiber tracts. Finally, individual FA maps were aligned with the skeleton and only the maximum FA values along the width of each large fiber tract were considered in subsequent voxel-wise analyses.

Behavioral measures

The Verbal Comprehension Index (VCI) and the Perceptual Reasoning Index (PRI) of the Wechsler Intelligence Scale for Children — Fourth Edition, were used to measure intelligence and the Working Memory Index (WMI) measured working memory function (Wechsler, 2003). The VCI is a measure of audio-verbal intelligence and includes three core subtests; Similarities (abstract verbal reasoning), Vocabulary (the degree to which one has learned to comprehend and verbally express vocabulary), and Comprehension (the ability to deal with abstract social rules and conventions). The PRI measures visuo-spatial abilities such as visual perception, organization, and planning and is comprised of three core subtests; Block Design (visual abstract processing and problem solving), Matrix Reasoning (nonverbal abstract problem solving and spatial reasoning), and Picture Completion (perceiving visual details quickly). The WMI is comprised of the Digit Span and Letter–Number Sequencing subtests. For Digit Span, children repeated series of orally presented digits, in forward and reverse order. For Letter–Number Sequencing, children repeated series of orally presented letters and numbers, sorting the series into numerical order and alphabetical order. Raw scores were converted into scaled scores, and subsequently combined into standardized index scores (mean = 100, SD = 15).¹

Statistics

First, for each DTI index, a 3 (group — CR, SO, Control) × 2 (hemisphere — left, right) × 4 (anatomic region— frontal, mid, pons, cerebellum) analysis of covariance was conducted with repeated measures on the latter two variables (controlling for site). Tests of simple effects were used to examine whether patients would show decreased white matter integrity relative to controls. Second, voxel-wise analyses were used to examine group differences in white matter integrity across the entire brain. Such analyses are important for interpreting any differences in the cerebello–thalamo–cerebral pathway within the broader context of whole brain white matter. Specifically, TBSS was used to test for local differences between the CR, SO, and Control groups in FA across whole brain white matter. TBSS includes a multiple-comparison correction by creating a “skeleton” FA map and thus reducing the number of tests. Additionally, TBSS controls for family-wise errors using a permutation methodology. Only those voxels common to all subjects were included in considering group differences, with clusters defined by t > 3. The null distribution of the cluster-size statistic was built up over 5000 random permutations of group membership. Cluster size was thresholded at p < .05, which is fully corrected for multiple comparisons across space.

Third, group differences in VCI, PRI, and WMI were examined using analysis of covariance controlling for parental education. Fourth, ¹ The Wechsler Adult Intelligence Scale — Third Edition was used for one participant and the Fourth Edition was used for another patient as they were both 17 years old. For both versions, the WMI is composed of the Digit Span and Arithmetic subtests. For the Arithmetic task, participants were asked to manipulate mental mathematical problems.
before making inferences regarding the relation between damage to the cerebellum–thalamocortical pathway and working memory in children with brain tumors, it is important to establish the nature of such brain–behavior relations in typical development. Hence, we evaluated the specificity of any relations between the cerebellum–thalamocortical pathway and cognitive function in the healthy control children. Specifically, stepwise regression analyses were conducted using DTI indices to predict WMI. These analyses were also conducted on the VCI and PRI scores to test whether associations were specific to working memory, or whether the cerebellum–thalamocortical pathway was also related to broader cognitive functioning. Because FA, MD, AD, and RD are all derived from the same signal source, regressions were conducted for the set of DTI indices from each of the bilateral pathways in a single model in order to limit the number of comparisons and identify the best predictor. Fifth, correlation and regression analyses were used to determine the relative contribution of treatment for brain tumors versus DTI indices in predicting WMI, controlling for parental education. For all regression analyses, mean DTI values across the entire cerebellum–thalamocortical pathway were used. Finally, correlations between age at diagnosis, time since diagnosis, tumor size, DTI indices, and WMI were calculated separately for the CR and SO groups.

**Results**

**Group differences in the integrity of cerebellum–thalamocortical tracts and whole brain white matter**

The cerebellum–thalamocortical pathway was produced bilaterally across all participants with DTI tractography (Fig. 2a and b). Close-up sections of the cerebellum–thalamocortical tract are shown in Fig. 3. Each pathway connected cerebellar hemispheric white matter proximal to the dentate nucleus with contralateral ventral thalamic nuclei, via superior cerebellar peduncles. From this point, the pathway extended anteriorly into prefrontal white matter. The other branch extended superiorly and medially within the DLPFC, terminating near the superior frontal gyrus. Mean regional DTI indices for each group are presented in Table 2. Significant main effects for region were evident across FA, MD, AD, and RD [Fs(1,61) ≥ 9.76, ps < 0.01]. Main effects for FA and RD were qualified by region X group interactions [Fs(1,61) ≥ 3.48, ps < 0.04]. Tests of simple effects revealed that cerebellar region FA was lower (Fig. 4a) and RD was higher (Fig. 4b) for the CR Group relative to the SO Group and healthy controls (p < 0.01).

Based on voxel-wise TBSS analyses, FA was significantly lower (p < 0.05) in patients treated with cranial radiation versus healthy controls in multiple areas of white matter across the brain. Notably, posterior regions showed the greatest difference between the CR Group and healthy controls, including cerebellar, occipital, parietal, and temporal white matter (please see Fig. 5). Relatively fewer clusters of difference were present in frontal white matter. The SO group did not differ from the CR group or the control group.

**Group differences in IQ and working memory outcome**

Significant differences existed between the groups for mean PRI and VCI [Fs(1,62) ≥ 5.96, ps < 0.001]. The CR Group had lower PRI scores than the SO Group and Control Group (p < 0.01) (see Table 1). For VCI, the CR Group performed worse than controls (p < 0.01), but were no different than the SO Group. A significant group effect was observed for WMI [F(2,59) = 4.012, p = 0.02]. The CR Group performed more poorly than the healthy control and SO groups (p < 0.04) (Fig. 4c). No differences were evident between the control and SO groups.

**Relations between white matter integrity and working memory in healthy children**

When all DTI indices for the tract connecting the right cerebellar hemisphere with the left DLPFC via the left thalamus (LRR cerebellum–thalamocortical pathway) were simultaneously regressed on WMI, the overall model was significant [F(1,24) = 5.88, p < 0.03]. Only RD uniquely predicted WMI (RD β = 0.44, p < 0.03 vs. FA β = −0.02, MD β = −0.54, AD β = −0.28; ps < 0.01). The model including all DTI indices for the LRR cerebellum–thalamocortical pathway did not predict VCI or PRI [Fs(1,24) ≤ 1.41, ps > 0.10]. For the tract linking the left cerebellar hemisphere to the right DLPFC via the right thalamus (RLL cerebellum–thalamocortical pathway), the model including all DTI indices did not predict WMI, VCI, or PRI [Fs(1,24) ≤ 0.14, ps > 0.10]. Hence, in our sample of healthy children, WMI appears to be specifically related to the integrity of the RLL cerebellum–thalamocortical pathway.

**Does compromised white matter integrity account for differences in working memory between patients treated with cranial radiation and healthy controls?**

Because we found that WMI was predicted only by the integrity of the RLL cerebellum–thalamocortical pathway in healthy children, we focused on this tract when investigating the relative contributions of treatment and pathway integrity to working memory function in patients. VCI and PRI were not considered in these analyses as these functions were not related to white matter integrity of the cerebellum–thalamocortical tracts in our sample of typically developing children. Further, as differences in WMI were evident between patients treated with cranial radiation and healthy children, only these groups were included in the analyses. FA was correlated with WMI in the sample of patients treated with cranial radiation and healthy controls (r = 0.28, p = 0.04). Subsequent regression analyses (Table 3) revealed that both treatment status (i.e. CR versus Control
Fig. 3. The RIL cerebello-thalamo-cerebral tract overlaid on the T1 image. Left: axial sections depicting the path of cerebello-thalamo-cerebral connections from caudal to rostral. Middle: coronal sections showing the cerebello-thalamo-cerebral connections from posterior to anterior. Right: sagittal sections depicting the cerebello-thalamo-cerebral pathway from lateral to medial to lateral. Adjacent sections depict the same part of the tract but in a different view. Starting in cerebellar hemispheric white matter proximal to the dentate nucleus (a) the tract proceeds into superior cerebellar peduncle and decussates at the level of the midbrain (b). The tract then continues to contralateral ventral thalamic nuclei (c), finally reaching ipsilateral DLPFC. One branch of which extends anteriorly into prefrontal white matter (d), while the other branch extends superiorly and medially and terminates near the superior frontal gyrus (e).
Table 2

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<th>AD</th>
<th>RD</th>
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</table>

Different superscripts indicate significant mean difference across groups at p < 0.01.

Impact of medical and demographic variables

For the CR group, older age at diagnosis and treatment was related to lower AD for the LRR cerebello–thalamo–cerebral pathway (r = −0.397, p = 0.04). Longer time since diagnosis predicted lower FA (r = −0.558, p = 0.002), higher MD (r = 0.443, p = 0.02), and higher RD (r = 0.551, p = 0.003) for the LRR cerebello–thalamo–cerebral pathway. Finally, larger tumor size was associated with higher MD (r = 0.457, p = 0.03) and AD (r = 0.430, p = 0.046) for the LRR cerebello–thalamo–cerebral pathway, as well as lower WMI (r = −0.425, p = 0.04). For the SO group, older age at diagnosis and treatment was related to lower MD (r = −0.696, p = 0.02) and RD (r = −0.680, p = 0.02), and longer time since diagnosis predicted lower WMI (r = −0.716, p = 0.01).

Discussion

We used a model-driven paradigm of cerebellar–cerebral interaction (Brodal, 1978; Dum and Strick, 2003; Middleton and Strick, 2001; Schmahmann, 1996; Schmahmann and Pandya, 1995, 1997b) to investigate the role of the cerebello–thalamo–cerebral pathway in working memory deficits in children treated for PF tumors. We observed a number of novel findings.

For the first time in children, we delineated bilateral tracts connecting the cerebellum with DLPFC, replicating the cerebello–thalamo–cerebral pathway documented in animal and adult models (Dum and Strick, 2003; Jissendi et al., 2008; Krienen and Buckner, 2009; Middleton and Strick, 1994, 2001; Ramnani et al., 2006; Salmi et al., 2010; Schmahmann and Pandya, 1995). Regardless of group status, each pathway included the left/right DLPFC, the left/right thalamus, and the right/left cerebellar hemispheric white matter. Because neural activity in these areas is implicated in working memory (Curtis and D’Esposito, 2003; Funahashi et al., 1993; Kim et al., 1994; Smith et al., 1996; Ziemus et al., 2007), our findings that the cerebellum and DLPFC are structurally connected is further evidence that neural communication via the cerebello–thalamo–cerebral pathway is important in working memory.

Compromise has been previously documented in cerebral hemispheric white matter following cranial radiation (Khong et al., 2003, 2005, 2006; Mabbott et al., 2006; Reddick et al., 2005). We also found that treatment with cranial radiation for PF tumors damages white matter in multiple regions. Specifically, our TBSS analyses demonstrated damage throughout posterior areas of the brain. Aside from examining broad white matter changes, a unique contribution of our study is that we have documented the impact of cranial radiation on a discrete white matter pathway known to connect neural areas that may function together in mediating cognition. White matter damage was observed within the cerebellar region of the cerebello–thalamo–cerebral pathway in patients treated with cranial radiation. Although patients treated with surgery only did not show significant damage relative to controls, findings were in the expected direction of lower FA and higher RD. Decreased FA and higher RD may reflect breakdown of myelin (Basser, 1995; Beaulieu, 2002; Song et al., 2002). We also found that in patients treated with radiation, younger age at and longer time since diagnosis and treatment were associated with white matter compromise of the pathway. For patients treated with surgery only, younger age at diagnosis was associated with higher MD and RD. These findings are consistent with the vulnerability of the young brain to white matter damage. Because of the protracted period of
Fig. 4. a: average cerebellar white matter FA as a function of treatment. b: average cerebellar white matter RD as a function of treatment. Error bars show +/- two standard errors. c: mean WMI standard scores as a function of treatment. Error bars show +/- two standard errors.
pediatric brain tumors have not been associated with significant neurocognitive late effects, peripheral neuropathy resulting in motor slowing can be observed following treatment with vincristine (Gomber et al., 2010; Ramchandren et al., 2009), and auditory toxicity that may interfere with measurement of auditory-verbal functions can be associated with cisplatin (McHaney et al., 1983; Weatherly et al., 1991). There is a possibility that the chemotherapeutic agents used to treat pediatric brain tumors have an impact on cognitive function and thus requires continued examination.

To our knowledge, this is the first study to demonstrate that cerebello–thalamo–cerebral pathway integrity is related to working memory outcome in children. Across all participants, we found that integrity of this pathway predicted working memory. The cerebellum is activated during tasks of verbal working memory and its role appears to involve evaluating the output of subvocal articulation (Desmond et al., 1997) and providing feedback to frontal control centers (Chen and Desmond, 2005). Our work shows that the structural integrity of the cerebello–thalamo–cerebral pathway is likely important in these feedback mechanisms. Further, based on regression analyses, we found that integrity of the pathway accounted for additional variance in working memory function even after treatment group was considered. This suggests that breakdown in myelin in this pathway following treatment for PF tumors may result in decreased efficiency of communication between the cerebellum and the frontal lobes leading to poor working memory. We acknowledge that many areas of white matter compromise were evident in our patients treated with cranial radiation and this diffuse damage may also contribute to the observed working memory deficits. Our intent is not to argue that all working memory impairments are solely related to damage within the cerebello–thalamo–cerebral pathway — rather to examine whether damage to this model-based pathway is one possible mechanism of working memory impairment.

Our findings must be considered in the context of the following limitations. First, we focused exclusively on the cerebello–thalamo–cerebral pathway and its role in working memory function. This pathway may be involved in other cognitive functions. Alternatively, other cerebellar inputs or outputs may contribute to working memory (Chen and Desmond, 2005). Furthermore, the cerebral portion of the cerebello–thalamo–cerebral pathway we identified included multiple fibre bundles and each of these may have a specific contribution to various components of working memory function. To test these specific contributions, a task that provides information on specific components of working memory is required and we did not employ such a task. Our findings do provide a solid foundation for future studies examining specific pathway contributions to working memory. Finally, heterogeneity in medical and treatment variables may limit the generalizability of our findings. Because of the relatively small number of patients in our sample treated with focal radiation, we combined these patients with those who had been treated with cranial spinal radiation. Group differences in chemotherapy and

**Table 3**

Regression model predicting working memory as a function of treatment and FA for the RLL cerebello–thalamo–cerebral pathway.

<table>
<thead>
<tr>
<th>Model</th>
<th>Model F ratio</th>
<th>p increment R²</th>
<th>Increment F ratio</th>
<th>p increment</th>
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<tr>
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<td>–</td>
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<td>Treatment status added</td>
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<td>.051</td>
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</tbody>
</table>

Fig. 5. Sagittal, coronal, and axial views (from left to right) of the TBSS difference map between the control and cranial radiation groups. Voxels have been filled/expanded to visually enhance the areas of difference between the two groups. The FA skeleton is depicted in green and the significant differences within the FA skeleton between the two groups are shown in red.
tumor pathology were also present. However, because these differences reflect current treatment protocols, it is not feasible to separate chemotherapy and radiation effects or to investigate pathology separate from treatment.

Knowledge about the role of the cerebellum—thalamo—cerebral pathway in mediating working memory is important for understanding how white matter connections in the brain work to support cognition. We have investigated this pathway as one possible mechanism by which treatment for PF tumors may contribute to working memory impairment. We have shown that the integrity of structural connections between cerebellum and frontal cortex is related to working memory. Thus, the ability to identify differences in the integrity of white matter for specific regions or pathways is an important step in attempting to localize the regional effects of PF tumors and their treatment methods in terms of structure—function relations. Such information is crucial in the ongoing modification of treatment protocols to reduce damage to healthy tissue and preserve neuro-cognitive function.

Acknowledgments

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References


