Functional Neuroimaging to Characterize Visual System Development in Children with Retinoblastoma

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PURPOSE. To use functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI) to investigate visual system development in children being treated for retinoblastoma.

METHODS. Informed consent was obtained for all participants ($N = 42$) in this institutional review board-approved study. Participants were imaged with a 1.5-T scanner while under propofol sedation. Diagnostic brain and orbital imaging was followed by investigational functional neuroimaging, which included fMRI during photic stimulation through closed eyelids, to measure functional activation in the visual cortex, and DTI, to evaluate diffusion parameters of white matter tracts in the corpus callosum and the periventricular optic radiations. Analysis included 115 examinations of 39 patients with a median age of 16.4 months and age range from 1.5 to 101.5 months at first evaluation.

RESULTS. The blood oxygen level-dependent signal was predominantly negative and located in the anterior visual cortex. Activation was affected by tumor lateralization (unilateral or bilateral), macular involvement, and retinal detachment. Patients who had undergone unilateral enucleation showed cortical dominance corresponding to the projection from the nasal hemiretina in the unaffected eye. Diffusion parameters followed a normal developmental trajectory in the optic radiations and corpus callosum, but variability was greater in the splenium than in the genu of the corpus callosum.

CONCLUSIONS. Longitudinal functional neuroimaging demonstrated important effects of disease and treatment. Therefore, fMRI and DTI may be useful for characterizing the impact of retinoblastoma on the developing visual system and improving

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Retinoblastoma, a childhood cancer of the eye, typically affects children during the first 3 years of life, a time of rapid development in the central nervous system.^{1,2} Overall survival at 5 years after diagnosis of retinoblastoma exceeds 90% with recent treatment protocols^{3,4} and there is increased interest in factors such as visual outcome that influence quality of life in this population. Because it tends to arise during a period of rapid and crucial development of the eye and brain, retinoblastoma has a profound effect on long-term visual function in survivors. Visual outcome is influenced by tumor characteristics (i.e., tumor location, macular involvement, retinal detachment, and vitreous seeding),^{5,6} but it remains largely unpredictable on the basis of initial presentation of disease within the eye.^{7,8} This fact suggests that visual outcome also depends on how retinoblastoma and its treatment affect the more distal elements of the visual system, such as the visual cortex and associated white matter pathways.

Sensory processing and signaling downstream from the retina during early development play an important part in the patterning of the visual cortex.^{9–11} Monocular deprivation of normal visual input during early development disrupts the normal formation of visual fields and ocular dominance columns in the primary visual cortex and leads to visual deficit.^{12–15} Other diseases of the visual system that occur early in life affect development in portions of the brain associated with vision.^{16–18} Therefore, altered visual input caused by the presence of tumor or enucleation in patients with retinoblastoma most likely disrupts visual system development. However, little is known about the direct effects of retinoblastoma on this process. Functional magnetic resonance imaging (fMRI) and diffusion tensor imaging (DTI) have been used successfully to investigate the visual system in healthy and diseased brains.^{19–25} We used fMRI during photic stimulation and DTI to investigate basic elements of the developing visual system in patients being treated for retinoblastoma.

METHODS

Subjects

Participants were enrolled in an ongoing prospective clinical protocol designed to improve the treatment and understanding of retinoblastoma. Participation in the investigational functional neuroimaging examination was optional, and written informed consent was obtained for all subjects as approved by the St. Jude Children's Research Hospital Institutional Review Board in compliance with the guidelines in the Declaration of Helsinki. Ophthalmologists performed primary enucleation (after first performing an MRI) in all patients with either advanced unilateral disease or bilateral disease and unsalvageable eye(s). Treatment for early unilateral or advanced bilateral disease

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consisted of different chemotherapy regimens, focal treatments, and, in some cases, radiation therapy. Laterality of disease, date of enucleation, macular involvement, and retinal detachment were determined by chart review.

Magnetic Resonance Imaging

Imaging examinations were conducted at the time of diagnosis, after therapeutic enucleation if performed, and then at approximately 6-month intervals. Images were obtained with a 1.5-T MRI scanner (Symphony; Siemens, New York, NY), equipped with the standard receive-only head coil. All patients were sedated with propofol (250 $\mu\text{g/kg/min}$) during imaging and were carefully monitored by anesthesiologists. Each imaging examination included diagnostic brain and orbital MRIs for management of retinoblastoma. Investigational functional neuroimaging data were acquired after completion of the diagnostics scans, beginning approximately 40 minutes after induction of sedation.

fMRI Paradigm. Visual stimuli were projected onto a screen at the rear of the magnet and reflected onto the subjects' closed eyelids via a mirror attached to the head coil. The stimulus paradigm consisted of an alternating (8-Hz) black/white screen in block design with 20.6 seconds on/20.6 seconds off, 10 images per 20.6-second epoch. The stimulus was a square spanning a visual angle $30^\circ \times 30^\circ$, and the illumination intensity at the approximate location of the eye was 120 lux. The fMRI paradigm was implemented with experimental control software (Presentation; Neurobehavioral Systems, Davis, CA), and trigger pulses from the MRI scanner coordinated the stimulus presentation with image acquisition. Blood oxygenation level dependent (BOLD) functional images were acquired in an oblique axial plane, parallel to the plane containing the anterior-posterior commissure line. A 23-slice volume (slice thickness, 5 mm) provided full brain coverage and was obtained every 2.06 seconds. The T2*-weighted echo planar image pulse sequence used for the fMRI scans had the following parameters: field of view, 192×192 mm; image matrix, 64×64 ; echo time (TE), 50 ms; flip angle, 90° ; and readout bandwidth, 1954 Hz/pixel.²⁶

DTI Paradigm. Diffusion-weighted images were obtained with $b = 0$ and either 6 or 12 diffusion encoding gradient directions with $b = 1000$ seconds/ mm^2 . These images were acquired in four repetitions and were averaged after realignment to maximize the signal-to-noise ratio in the final product images. The double-spin-echo, echo planar image pulse sequence parameters were as follows: repetition time (TR), 7 seconds; echo time (TE), 101 ms; field of view 192×192 mm; and image matrix, 128×128 .

Data Analysis

fMRI Data Analysis. Functional images were preprocessed and analyzed by using Statistical Parametric Mapping software (SPM2, <http://www.fil.ion.ucl.ac.uk/spm/>) provided in the public domain by members and collaborators of the Wellcome Trust Centre for Neuroimaging, University College London, London, UK). Preprocessing included standard realignment, normalization, and smoothing.²⁷ Activation maps were generated from statistical parametric maps with threshold values of $P = 0.001$ (uncorrected at voxel level) and 5-voxel threshold. An occipital lobe mask²⁸ was applied to the activation map for each subject to evaluate both positive and negative BOLD signal changes during stimulation. The maximum t -statistic and total number of activated volume elements (voxels) were evaluated in the visual cortex. The activated volume was analyzed to determine the effects of age, laterality of disease, and enucleation. We assessed hemispheric asymmetries of activation volume in patients who had undergone unilateral enucleation to detect cortical dominance ipsilateral or contralateral to the remaining eye. Activation volume was analyzed in patients with bilateral disease to determine the impact of macular disease involvement and retinal detachment.

DTI Analysis. Diffusion tensor images were processed with the DTI Toolkit (<http://sourceforge.net/projects/spmtools>) for the Statistical Parametric Mapping software. Regions of interest were manually

drawn (ImageJ, <http://rsb.info.nih.gov/ij/>; developed by Wayne Rasband, National Institutes of Health, Bethesda MD) to evaluate fractional anisotropy (FA) and apparent diffusion coefficient (ADC) in the genu and splenium of the corpus callosum at the midline²⁹ and in the periventricular optic radiations (ORs).³⁰ The ADC is a measure of the magnitude of water diffusion and is approximately equal in gray matter and white matter. FA measures directional anisotropy of water diffusion; FA values are much higher in white matter than gray matter, because water diffuses more easily along than across white matter tracts.

Nonlinear regressions of FA and ADC versus age at examination were calculated for each brain region with the exponential models in equations 1 and 2, respectively, to characterize the developmental trends in these diffusion properties. Nonlinear mixed-effect analysis was used to account for the repeated measurements in the same individuals.

$$FA = a - b \cdot e^{-(\text{age}/c)} \quad (1)$$

$$ADC = a + b \cdot e^{-(\text{age}/c)} \quad (2)$$

The parameter a corresponds to the asymptotic value of the diffusion measurement at older age, the parameter b scales the developmental change in the measurement, and the parameter c corresponds to the time constant for the developmental change of the measurement. The units of a and b are the same as the modeled quantity and the unit of c is months. The regression models were fit to the data with commercial software (SAS, ver. 9.2; SAS Institute Inc., Cary, NC).

Statistical Analyses

Generalized estimating equations (GEEs) were used for comparisons among variables in this longitudinal dataset.³¹ For comparison between two measurements from a single scan (e.g., positive versus negative and contralateral versus ipsilateral), the difference between two measurements was first calculated and then was used as the response variable in the GEE. The intercept-only model was fit to test for differences. For comparison between two measurements from different scans (e.g., unilateral versus bilateral, nonenucleated versus enucleated, macular involvement of one eye versus two eyes, or retinal detachment in one eye versus two eyes), the measurement was used as the response variable, and the group indicator (e.g., unilateral versus bilateral) was fit as a covariate in the GEE, to investigate the difference of the two measurements. The GEE analysis was also used to determine the association between activation and age. The threshold for statistical inference was $P < 0.05$ for all tests.

RESULTS

Subjects

Clinical information about the subjects is summarized in Table 1. The first 42 patients enrolled in this ongoing study were analyzed for this report. This group included 24 patients with unilateral disease and 18 patients with bilateral disease. Three patients (two with unilateral disease and one with bilateral disease) had no fMRI or DTI scans. Thus, 106 fMRI scans ($n = 57$ unilateral, $n = 49$ bilateral) and 115 DTI scans ($n = 62$ unilateral, $n = 53$ bilateral) from 39 patients were included in our analyses. The mean age at the time of diagnosis of evaluable patients with unilateral retinoblastoma (31.9 months; range, 1.5–101.5 months) was significantly older than that of patients with bilateral disease (9.2 months; range, 2.6–22.2; $P = 0.0001$). The discrepancy between the total number of fMRI scans and DTI scans was due to technical failures of fMRI examinations.

TABLE 1. Clinical Information on Patients with Retinoblastoma Who Participated in Functional Neuroimaging Examination

Laterality of Disease	<i>n</i>	Age at Diagnosis (mo ± SD)	Patients with Enucleated Eyes (<i>n</i> Eyes)	Patients with Macular Involvement (<i>n</i> Eyes)	Patients with Retinal Detachment (<i>n</i> Eyes)
Unilateral	24	31.9 ± 24.8	23 (23)	22 (22)	13 (13)
Bilateral	18	9.2 ± 6.5	8 (9)	16 (22)	17 (22)

Functional MRI

Functional activation was detected in all but six completed scans (three scans of patients with unilateral and three with bilateral disease). The primary locus of activation was generally located in the anterior portion of the visual cortex (Fig. 1), corresponding to the peripheral visual field. The sign of the BOLD signal change during visual stimulation was predominately negative in 87 of 100 examinations (Fig. 2). As exemplified in Figure 1, the clusters of positive signal change were generally small and scattered in the striate and extrastriate visual areas. The median value of the maximum *t*-statistic was greater (GEE, $P < 0.001$) for the negative signal changes (8.5) than for the positive signal changes (4.5), and the number of activated voxels (volume) with a negative signal (1196 voxels) was 13.4 times greater ($P < 0.001$) than the number with a positive signal (89 voxels). The activated volume was associated with the peak signal change for both positive (GEE, $P < 0.001$) and negative (GEE, $P < 0.001$) changes during visual stimulation. The maximum *t*-statistic was associated with age (Fig. 3) for positive signal changes (GEE, $P = 0.03$), but not for negative responses. Visual inspection of Figure 3 shows that there was a similar increasing trend in the magnitude of both positive and negative signal changes with age for the low *t*-statistic values at the threshold of detection. There was no association between the volume of visual cortex activated and

age at examination. Subsequent analysis was based on the regions with negative BOLD signal.

The volume of activation was greater in patients with unilateral disease than in those with bilateral disease (1876 vs. 751 voxels/scan; GEE, $P = 0.04$). There was no difference between activation detected before enucleation and that detected afterward in patients with unilateral or bilateral disease or in all patients analyzed together. This comparison included all scans obtained before and after enucleation within each group. In addition, we analyzed scans from a group of nine patients with unilateral disease by focusing on the volume of activation in the last scan before enucleation and comparing the data with those from the first scan after enucleation in each patient. Again, there was no detectable effect of enucleation (data not shown).

We then evaluated the difference in the volumes of activation between hemispheres in patients with unilateral disease who underwent enucleation. Activation was significantly greater in the hemisphere contralateral to the remaining eye (1221 vs. 657 voxels/scan; GEE, $P < 0.002$). To demonstrate that there was no left or right hemispheric bias in this finding, we compared left and right hemisphere activation in this same group of patients and found no significant difference ($P = 0.113$). In patients with bilateral disease who had not undergone enucleation, the volume of activation in those with mac-

FIGURE 1. Representative fMRIs of a pediatric patient with retinoblastoma. Orthogonal views show activation with a negative BOLD signal (blue) in the anterior portion of the visual cortex and a small cluster of activation with a positive signal (red) at the occipital pole. The coronal slices in the lower right quadrant demonstrate the localization of the activation to the gray matter along the calcarine sulcus. This examination was after enucleation in a 40-month-old patient with unilateral disease. The activation parameters for this patient were approximately equal to the cohort median values for both positive (maximum *t*, 5.1; activated voxels, 117) and negative (maximum *t*, -7.5; activated voxels, 1072) signal changes (see also Figs. 2 and 3).

FIGURE 2. Characteristics of visual cortex activation in patients with retinoblastoma. The main graph shows the relationship between the number of activated voxels and the maximum *t*-statistic for both positive (*white*) and negative (*gray*) BOLD signal changes. The positive and negative activation for each patient are connected by lines. *Inset*: an expanded view of a smaller number of activated voxels. The box-and-whisker plots at the top and right of the figure summarize the percentile distribution of the activation data (*circles*: 5th and 95th, *whiskers*: 10 and 90th, *box*: 25th and 75th, line within box: 50th). Activation was predominately negative in 87 of 100 examinations.

ular involvement in one eye was significantly greater than that in patients with macular involvement in both eyes (1672 vs. 426 voxels/scan; GEE, $P = 0.003$). Finally, the volume of activation in patients with bilateral disease was greater in those with retinal detachment in one eye than in those with retinal detachment in both eyes (990 vs. 199 voxels/scan; GEE, $P = 0.03$). Images of retinoblastoma involving the macula, retino-

FIGURE 3. Maximum *t*-statistic for BOLD signal changes versus age at the time of the fMRI examination. There was a significant association for the positive signal changes (*white*) and the age, but not for the negative signal changes (*gray*). Note that there appears to be a similar increasing trend in the magnitude of the signal for both positive and negative responses near the detection threshold.

FIGURE 4. Funduscopy images showing retinoblastoma tumors involving (A) the macula, (B) the peripheral retina, and (C) retinal detachment. (D) Image of a normal retina.

blastoma involving the retinal periphery, retinoblastoma with retinal detachment, and normal retina, are shown in Figure 4.

Diffusion Tensor Imaging

There was no difference between the mean FA ($P = 0.20$) and ADC ($P = 0.83$) values measured with 6 (90 scans) gradient directions compared with those measured with 12 (25 scans); therefore, we analyzed all the DTI data together. The color map images demonstrated qualitative developmental changes in the brain structure of children with retinoblastoma (Fig. 5). FA in the genu and splenium increased to comparable values with age (Fig. 6A). However, the variability of FA was greater in the splenium than in the genu. Similarly, ADC in the genu and splenium decreased to comparable values (7.6×10^{-4} and $7.8 \times 10^{-4} \text{ mm}^2/\text{s}$, respectively) with age (Fig. 6A) and was also more variable in the splenium than in the genu. The FA in the left and right hemisphere ORs increased to comparable values and had similar variability (Fig. 6B). Similarly, ADC in the left and right hemisphere ORs decreased to the same value ($8.8 \times 10^{-4} \text{ mm}^2/\text{s}$) and had similar variability. After appropriate adjustments of the diffusion parameters for age according to the regression equations from all the regions of interest (Table 2), FA was greater in patients with unilateral disease than in those with bilateral disease ($+0.01$; $P = 0.013$), and ADC was lower in patients with unilateral disease than in those with bilateral disease ($-1.8 \times 10^{-5} \text{ mm}^2/\text{s}$; $P = 0.008$).

DISCUSSION

Our results demonstrate the feasibility of fMRI and DTI analyses in pediatric patients with retinoblastoma. Our functional neuroimaging findings in this patient population were generally consistent with previous reports describing specific developmental features of both the BOLD signal in the visual cortex^{22,32} and the diffusion characteristics of major white matter tracts^{21,33} in infants and young children. However, the patterns of activation in the visual cortex during photic stimulation and diffusion parameters in the associated white matter pathways reflected important effects of disease and treatment. Therefore, fMRI and DTI may be useful for characterizing the impact of retinoblastoma on the developing visual system and improving the prediction of visual outcome in survivors.

Functional MRI produces distinctive patterns of activation in young subjects who are sedated with eyelids closed during

FIGURE 5. Developmental changes in DTI color maps of one pediatric patient with retinoblastoma. The transverse slice (*top row*) is approximately 1 cm above the AC-PC line, and the coronal slice passes through the splenium of the corpus callosum. The orientation of white matter fibers at each location is indicated by color: *red*, left-right; *green*, anterior-posterior; *blue*, superior-inferior (see also Fig. 6).

visual stimulation.^{22,34–36} These patterns differ from those seen in awake adult subjects^{37,38}; however, sedation, sleep, closed eyelids, and rapid brain development may contribute to these differences.^{35,39–44} The most notable differences are the sign of the BOLD signal change during stimulation and the location of the activation in the visual cortex. Sedated, asleep children have more negative signal during activation, and awake adults have more positive signal. Both positive and negative signal changes represent local changes in neural activity, but it is not fully understood why certain regions of the brain are more negatively activated. Our study confirmed the dominance of negative BOLD signal during visual stimulation through closed eyelids in sedated young patients with retinoblastoma.

Activation detected during photic stimulation was located in the anterior visual cortex corresponding to the peripheral visual field.^{45,46} The paucity of foveal visual cortex activation suggests that the stimulation on closed eyelids after approximately 40 minutes of anesthesia yielded dark adaptation of the eyes and scotopic stimulation conditions. Transmission through the eyelid is 5.6% for red light and 0.3% for green light, to which the rod receptors are most sensitive.⁴⁷ Thus, the illumination intensity through the eyelid was approximately 0.4 lux for our stimulus, which is at least an order of magnitude higher than the scotopic threshold reported for BOLD fMRI activation.⁴⁸ The visual response may also have been affected by the relatively low-frequency response of the rod receptors.⁴⁹ Much work is needed to determine the basic nature of BOLD signal changes in these developing young children, but despite these issues, the activation that we measured with fMRI was associated with specific features of retinoblastoma that affect vision.

Patients with unilateral retinoblastoma had more functional activation in the visual cortex than did those with bilateral disease. This difference in activation cannot be accounted for by the difference in the ages of the two patient groups or by the effect of enucleation, because neither of these variables had a significant effect on the volume of cortex activated. In fact, Figure 3 shows that the strength of activation was greatest in young patients. Therefore, it is likely that the difference was caused by greater tumor burden in the patients with bilateral disease, involving more of the retina and decreasing the volume of primary visual cortex activated. It is remarkable that there was little immediate effect of enucleation on the activa-

tion in the visual cortex. However, enucleated eyes most likely had little to no useful vision for a period before enucleation. These findings suggest that evaluation of visual cortical function with fMRI can help predict useful vision in affected eyes and help clinicians make informed decisions about administering conservative therapy.

Our fMRI results showed asymmetry of cortical activation in monocular children. It is well known that each eye contributes visual input to both hemispheres and that the nasal retina of each eye has a greater cortical representation than does the temporal retina.^{50,51} Previous fMRI studies have found that monocular eye stimulation of awake adults and sedated children yields greater activation of the brain hemisphere contralateral to the stimulated eye.^{50,52} We replicated this finding in young, sedated patients with unilateral retinoblastoma who had undergone enucleation. It will be interesting to see whether this difference in hemispheric activation persists into adulthood in subjects with early monocular vision. The plasticity of the early visual system may affect patterns of functional activation in enucleated subjects.^{53–57}

We found a significant effect of macular involvement and retinal detachment on the volume of functional activation in the visual cortex of patients with bilateral retinoblastoma. The macula mediates central vision in humans via its large density of cones and large representation in the cortical visual field relative to the rest of the retina.^{58,59} When the tumor involved the macula, it disrupted the synaptic network between the photoreceptors and the ganglion cells, thereby interfering with downstream visual signal processing.^{59,60} However, during retinal detachment, the synaptic activity of photoreceptors and ganglion cells often persists.^{61–63} Therefore, the potential for visual return is most likely greater in an eye in which the retina reattaches to the retinal pigment epithelium.

DTI provided a measure of development in important white matter tracts associated with vision. Ongoing functional and structural development of white matter fibers causes increased anisotropy and decreased magnitude of water diffusion in the brain.^{21,64,65} Therefore, the diffusion parameters FA and ADC are reliable indicators of normal or abnormal development in the white matter tracts. Color maps of white matter tracts identified in the developing brain of children with retinoblastoma appeared to be qualitatively comparable to similar images in healthy young subjects.^{66,67} However, quantitative evaluation of diffusion in the corpus callosum showed evidence of

disease- or treatment-related disruption of white matter maturation. FA was systematically lower and ADC was higher in patients with bilateral retinoblastoma compared with those measures in patients with unilateral disease. Diffusion parameters in both parts of the corpus callosum showed the dramatic developmental changes associated with myelination that were comparable, both qualitatively and quantitatively, to those observed in otherwise healthy children.^{21,68} However, the variability of the diffusion measurements with respect to the developmental trajectory was much greater in the splenium than in the genu. This variability in the splenium may reflect an interaction between disease and treatment and the complex patterning of the visual system that develops in young children.

FIGURE 6. Developmental changes in diffusion parameters for patients with bilateral (black) and unilateral (white) retinoblastoma. (A) FA and ADC showed similar age-related changes in the genu (left) and splenium (right) of the corpus callosum, but the variability of the diffusion parameters was greater in the splenium. (B) The same DTI parameters were assessed in the left and right ORs. The curves are fits of the regression models in equations 1 and 2; insets: the regression lines from the left and right panels together for comparison.

TABLE 2. Regression Parameters for Developmental Changes in Fractional Anisotropy and Apparent Diffusion Coefficient in the Genu and Splenium of the Corpus Callosum and in the Optic Radiations

	<i>a</i>	<i>b</i>	<i>c</i>
FA			
Genu	0.87 (0.01)	0.39 (0.02)	15.66 (1.71)
Splenium	0.87 (0.01)	0.33 (0.03)	10.75 (1.58)
OR left	0.58 (0.04)	0.18 (0.03)	32.71 (16.03)
OR right	0.57 (0.02)	0.19 (0.02)	19.53 (4.52)
ADC			
Genu	0.77 (0.01)	0.75 (0.04)	8.67 (0.81)
Splenium	0.78 (0.02)	0.71 (0.05)	10.10 (1.37)
OR left	0.88 (0.03)	0.29 (0.03)	25.31 (6.97)
OR right	0.92 (0.03)	0.25 (0.03)	21.10 (7.43)

Changes in fractional anisotropy were modeled by equation 1 and the apparent diffusion coefficient by equation 2. Regression parameters were determined by nonlinear mixed effect analysis to account for repeated measures within participants. The units of *a* and *b* are the same as the modeled quantity and the unit of *c* is months. Numbers in parentheses are the standard error of the parameter estimate. OR, optic radiation.

Despite the transient variability of diffusion parameters in the splenium, the FA and ADC converged toward normal values as the patients aged.

DTI measurements in the ORs showed no evidence of altered development in patients with retinoblastoma compared with similar measurements in the peripheral white matter of healthy children.^{21,69} Developmental trajectories of those values were comparable in the left and right brain hemispheres of our patients. However, no published studies to date have reported longitudinal FA and ADC values in the ORs of healthy children throughout development. Furthermore, the placement of a single planar region of interest in the ORs is difficult. Future analysis with fiber tracking may help improve the precision of the region of interest placement for parameter evaluation in the ORs and allow evaluation of other relevant white matter characteristics.^{70–72} For example, DTI with fiber tracking in patients with amblyopia has shown that, although FA and ADC were normal, the apparent length of the optic radiation fibers was abnormal.⁷³

There are two important issues related to the young age at which retinoblastoma develops that affect the interpretation of our functional neuroimaging results: Patients must be sedated for MRI examination, and the brains of these infants and young children are developing rapidly in structure and function. Sedation affects neural responsiveness and activity directly and may change the hemodynamic response to neural activity that is the basis of BOLD fMRI.^{74,75} Because of the requirement for sedation during MRI, there is limited normative functional imaging data of children during this period of rapid brain development. We note that we detected predominately positive sensorimotor cortex responses in young children evaluated for neurosurgical planning under the same anesthesia protocol that was used for the retinoblastoma patients reported herein.⁷⁶ Therefore, it is important to investigate the overlapping effects of disease traits, treatment, sedation, and normal development in the functional imaging findings of patients with retinoblastoma. Despite these challenges, we have shown that fMRI and DTI are promising methods to investigate visual system development in pediatric patients treated for retinoblastoma. Analysis of functional neuroimaging data in relation to ultimate visual outcome is necessary to determine the utility of these imaging methods in the clinical management of retinoblastoma.

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