Cancers of the brain and spinal cord are the most common solid tumors in children, accounting for almost 30% of all childhood cancers.1 With the advent of novel technologies and methods of treatments, survival rates are exceeding 70% in children and young adults.1 However, survivors experience profound impairments in neurocognitive and psychosocial domains,1,2 which lead to decreased quality of life following cranial radiation therapy (CRT). The most commonly affected brain domains incorporate overall cognitive ability and executive function, along with deficits in attention, processing speed, and inhibition control. These deficits appear months to years after CRT and impair quality of life.

Exercise has been shown to rejuvenate the brain and aid in recovery post-injury through its effects on neurogenesis and cognition. The purpose of this study was to test whether exercise ameliorates neurocognitive deficits following CRT.

Methods. We developed a juvenile rodent CRT model that reproduces neurocognitive deficits. Next, we utilized the model to test whether exercise ameliorates these deficits. Fischer rats (31 days old) were irradiated with a fractionated dose of 4 Gy × 5 days, trained and tested at 6, 9, and 12 months post-CRT using 5-choice serial reaction time task. After testing, fixed rat brains were imaged using diffusion tensor imaging and immunohistochemistry.

Results. CRT caused early and lasting impairments in task acquisition, accuracy, and latency to correct response, as well as causing stunting of growth and changes in brain volume and diffusion. Exercising after irradiation improved acquisition, behavioral control, and processing speed, mitigated the stunting of brain size, and increased brain fiber numbers compared with sedentary CRT values. Further, exercise partially restored global connectome organization, including assortativity and characteristic path length, and while it did not improve the specific regional connections that were lowered by CRT, it appeared to remodel these connections by increasing connectivity between alternate regional pairs.

Conclusions. Our data strongly suggest that exercise may be useful in combination with interventions aimed at improving cognitive outcome following pediatric CRT.
Importance of the study

Pediatric cranial radiation therapy, despite its effectiveness as a primary treatment for brain tumors, often results in late onset of complications and neurocognitive deficits in survivors. With these impairments, patients are often at a disadvantage compared with non-CRT counterparts and have a lower quality of life in that they often cannot achieve high educational levels or find and maintain gainful employment compared with their non-CRT siblings. As a result, there is a great need for the development of a translational model in which novel therapeutics and interventions can be tested. Our work presented here describes the successful development of such a model, investigates the use of exercise as a potential mitigator of neurocognitive side effects of CRT, and measures imaging changes and brain connectivity to gauge the effect of radiation and exercise.

Materials and Methods

Animals

Forty-five male Fischer (F344) rats (Harlan Laboratories), 24–27 days of age, were housed in a temperature-controlled (21°C) room on a reversed 12-hour light/dark cycle (lights on 8:00 pm–8:00 am). During training and testing on the 5-CSRTT, food was restricted to maintain the subjects at 85%–90% of their free-feeding weight in order to provide incentive to perform the task. Water was available ad libitum except in the testing chamber. A summary of the experimental design can be found in Supplementary Figure S1. All animal experiments were approved by the Institutional Animal Care and Use Committee at Baylor College of Medicine. The experiment incorporated 4 groups: sedentary sham (S-SHAM), sedentary irradiated (S-CRT), exercise sham (E-SHAM), and exercise irradiated (E-CRT). To investigate the role of exercise in brain recovery, a separate group of animals (12 in total) were subjected to the same regimen of radiation and exercise except that the exercise regimen was stopped after 14 days and animals euthanized for tissue and plasma collection. Animals were double-housed until one week before start of testing, then they were single-housed and food-restricted. The animals were handled for 5 minutes each for 3 days and fed a few reward pellets to acclimate them to the food reward offered in the 5-CSRTT chambers.

Irradiation

We have studied several fractionated CRT schemes, and the CRT scheme and age of animals used in this study were selected with the aim of mimicking pediatric clinical CRT paradigms. At 31 days of age, rats assigned to the radiation groups received fractionated whole brain irradiation of 20 Gy (4 Gy × 5 days) from behind the eyes and to the back of the ears with the body shielded, under isoflurane anesthesia using an RS 2000 biological X-ray irradiator 150 kVp, 25 mA (Rad Source Technologies). SHAM animals were anesthetized for the same amount of time but not irradiated.
Exercise

Exercise began 10 days after CRT. The regimen was composed of 5 days of voluntary wheel running and 2 days of rest for 7 consecutive weeks. Every rat was placed into a cage with an activity wheel (Techniplast) with food and water access ad libitum, for a period of 2 h within 2–6 h after the onset of the rats’ active cycle. Running wheel access was randomized daily to avoid any bias of running during a particular time of day. Exercise stopped one week before start of 5-CSRTT to allow adequate time for acclimation to single housing and food restriction. Activity wheels were equipped with counters that recorded the distance run.

5-Choice Serial Reaction Time Task

Executive function was assessed using 5-CSRTT (Med Associates). The 5-CSRTT box has dimensions of 25 × 25 × 25 cm, and software to run 5-CSRTT was obtained from Noldus Information Technology and Med Associates. At 9 weeks post-CRT, the training phase of the 5-CSRTT began. First, rats underwent daily 20 min habituation sessions, which consisted of placing 2 pellets in each of the 5 apertures and 10 in the food magazine, with all lights on. When the majority (>10) of pellets were consumed, the rats moved on from the habituation phase to training, which lasted 30–35 daily sessions. Target criteria per stage for the 5-CSRTT followed the protocol of Bari et al14; a summary of the protocol is found in the Supplementary material.

DTI and Connectome Analysis

Ex vivo DTI was carried out at 12 months post-CRT. Rat brains were prepared as described by Tyszka et al15 (and described in detail in the Supplementary material). Rat brains were DTI scanned using 20 distinct gradient directions on a Bruker BioSpec MRI scanner and tractography or connectome information generated. All details of computational processing using ROIEditor, DTI Studio,16 template maps from the Duke Center for In Vivo Microscopy,17 DiffeoMap,18 AIR algorithm,19,20 and LDDMM21 are in the Supplementary material. Connectomes were constructed individually for each rat brain in native space using the FMRIB Software Library,22 TrackVis,23,24 and Brain Connectivity Toolbox25 as detailed in the Supplementary material.

Immunohistochemistry

Once all DTI was completed, brains were removed from skulls and paraffin embedded. Sagittal sections of the brain were stained for glial fibrillary acidic protein (GFAP), myelin (Luxol fast blue), and ionized calcium binding adaptor molecule 1, and sections containing the corpus callosum were imaged using Leica Application Suite. Images were imported into ImageJ and width of the corpus callosum fibers located above the hippocampal formation was measured. The tissue of a smaller cohort of rats was stained for Ki67 and CD133 after CRT and 2 weeks of exercise to analyze the early effects of both CRT and exercise.

Brain-Derived Neurotrophic Factor Enzyme-Linked Immunosorbent Assay

Plasma was collected from a separate cohort of rats at 3 weeks post-CRT and after 2 weeks of running, and brain-derived neurotrophic factor (BDNF) levels were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) kit from Promega (BDNF Emax), according to the manufacturer’s instructions.

Statistical Analyses

Statistical analyses were conducted using SPSS (IBM) and GraphPad Prism 6. For 5-CSRTT training, number of trials to reach criterion per stage was analyzed by 3-way mixed model ANOVA with CRT and Exercise as the between-subjects measures and Training Stage as the within-subjects (repeated) measure. As all animals did not achieve the same level of performance by the time training was suspended, analysis was restricted to only those stages that all animals advanced through. Thus, criterion performance during training was assessed during the first 5 stages. Outcomes of 5-CSRTT testing time points (6, 9, and 12 mo) were averaged across the 5 days of testing and analyzed by 2-way ANOVAs with CRT and Exercise as independent variables. All imaging data at 12 months post-CRT were also analyzed using 2-way ANOVAs. Significant interactions were followed up with Bonferroni comparisons. Distance run over 7 weeks in exercised rats was analyzed using a 2-way repeated measures ANOVA comparing the effects of CRT over time. Significance was gauged against a P-value of 0.05.

Detailed methods on connectome statistical analysis can be found in the Supplementary material.

Results

Body Weight Differences

Weights of animals were recorded weekly through the 12 months post-CRT testing time point. SHAM and CRT groups separated early after radiation, and body weights dipped in response to food restriction associated with 5-CSRTT training and testing at 6, 9, and 12 months (Supplementary Figure S2). For final body weight at 12 months post-CRT (Fig. 1), there was a significant effect of CRT, F(1, 37) = 248.1 (P < 0.001). Cranial irradiation, regardless of exercise, resulted in a lower body weight.

Exercise Distance

There was a significant CRT × Week effect on distance run, F(6, 77) = 245.9, P < 0.0001. Post hoc tests showed that irradiated rats ran a significantly greater distance than shams during the final 2 weeks of exercise (week 6, P = 0.0003; week 7, P < 0.0001) (Supplementary Figure S3).
5-CSRTT

Analysis of performance during training on the 5-CSRTT, 3 months after radiation and more than 1 week after the end of exercise, showed that there was a significant effect of CRT, $F(1, 40) = 9.59$, $P < 0.01$, and Exercise, $F(1, 40) = 6.67$, $P < 0.05$, on number of trials to reach criterion during stages 1–5 (Fig. 2). Specifically, radiation impaired performance, whereas exercise improved it. (As Mauchly’s test of sphericity indicated that the assumption of sphericity had been violated, $\chi^2(9) = 152.02$, $P < 0.001$, a Greenhouse–Geisser correction was used for tests of within-subjects effects.) There was a significant effect of Training Stage, $F(1.4, 55.99) = 21.44$, $P < 0.001$, and a significant Training Stage × CRT effect, $F(1.4, 55.99) = 4.61$, $P < 0.05$, on performance. Post hoc analysis revealed that number of trials to criterion was significantly higher in irradiated animals relative to shams at stage 5 ($P < 0.05$). At the 6-month testing time point, there was a significant CRT effect, $F(1, 39) = 4.89$, $P < 0.05$, and a significant CRT × Exercise effect, $F(1, 39) = 5.74$, $P < 0.05$, on Accuracy (Fig. 3A). Bonferroni-corrected post hoc tests showed that S-CRT animals were significantly impaired relative to S-SHAM animals ($P < 0.05$) (Fig. 3A). There was no significant effect of CRT or Exercise seen on Accuracy at the 9-month testing time point (Fig. 3B); however, analyses also revealed a significant effect of CRT, $F(1, 36) = 6.58$, $P < 0.05$, on Accuracy at the 12-month testing time point (Fig. 3C). For % Premature Responses, there was a significant effect of Exercise at 6 months, $F(1, 39) = 11.67$, $P < 0.01$ (Fig. 3D), 9 months, $F(1, 39) = 16.45$, $P < 0.001$ (Fig. 3E), and 12 months, $F(1, 36) = 8.19$, $P < 0.01$ (Fig. 3F). There was a significant effect of CRT on Latency to Correct at 6 months, $F(1, 39) = 18.75$, $P < 0.001$ (Fig. 3G), and 12 months, $F(1, 36) = 9.03$, $P < 0.01$ (Fig. 3H), but no effect at 9 months (Fig. 3I). There was also a significant effect of Exercise on Latency to Correct at 6 months, $F(1, 39) = 10.75$, $P < 0.01$ (Fig. 3G), and 9 months, $F(1, 39) = 8.55$, $P < 0.01$ (Fig. 3H), but not at 12 months post-CRT (Fig. 3I).

Diffusion Tensor Imaging Data

Analysis of DTI data, 12 months post-CRT, showed a significant effect of CRT, $F(1,17) = 61.03$, $P < 0.0001$, and Exercise, $F(1,17) = 52.91$, $P < 0.0001$, on total brain volume where irradiated brains were significantly smaller than SHAM brains, and exercised brains were significantly larger compared with sedentary brains (Fig. 4A, B). Interestingly, the changes in volume arose differently when comparing CRT and Exercise. There was a significant effect of CRT, $F(1,16) = 243.4$, $P < 0.0001$, and Exercise, $F(1,16) = 8.403$, $P = 0.0105$, on brain length (Fig. 4C). However, for brain width, there was only a significant effect of Exercise, $F(1,16) = 58.28$, $P = 0.0001$ (Fig. 4D). These results imply that radiation affected the growth of the brain in only one direction and that exercise improves the overall size in both the width and length of the brain. Looking at specific brain regions, we saw a significant change in volume in a number of regions that included the cerebellum, hindbrain, midbrain, and the ventricles compared with S-SHAM or S-CRT (Supplementary Table S1). There were also qualitative changes in fiber number, as shown in Fig. 4E. There is a visually observed change in fiber density within the corpus callosum of the brain. Once quantified, we observed an overall effect of Exercise, $F(1, 17) = 14.69$, $P = 0.0013$, on the number of fibers in the entire brain, again demonstrating the benefits of exercise (Fig. 4F). Visually, there appeared to be changes in FA in the CRT rats compared with SHAM (Supplementary Figure S4a); however, when we measured whole brain FA, there was only an effect due to Exercise, $F(1, 17) = 8.398$, $P = 0.01$, and no effect due to CRT (Supplementary Figure S4b). The FA
values in fiber-rich regions (white matter) such as the optic pathway (0.571 ± 0.034), fimbria (0.401 ± 0.047), and corpus callosum (0.400 ± 0.046) were higher than gray matter regions such as the hypothalamus (0.192 ± 0.023), isocortex (0.191 ± 0.016), and the pituitary gland (0.142 ± 0.016) (see Supplementary Table S2). When we compared the S-CRT versus S-SHAM groups using a *t*-test, we observed significantly higher FA values in the pituitary gland (*P* = 0.0289), pre-optic area (*P* = 0.0299), and substantia nigra (*P* = 0.0052), and lower values in the pineal gland (*P* = 0.0164) (see Supplementary Table S2). However, when we compared them using a 2-way ANOVA, including the E-SHAM and E-CRT groups, there was no significant change in FA values between the S-CRT and S-SHAM except in the pineal gland, which was significantly higher in the S-CRT animals than in the S-SHAM animals (Supplementary Table S2).

Further, we conducted a higher-level analysis using connectomics. All connectomes demonstrated expected small-world characteristics across measured densities defined as small-worldness index greater than one\(^26\) (Fig. 5A). CRT was associated with higher connectome assortativity (*P* = 0.02), characteristic path length (*P* = 0.005), mean local efficiency (*P* = 0.02), modularity (*P* = 0.04), and normalized clustering coefficient (*P* = 0.03) as well as lower global efficiency (*P* = 0.009; Fig. 5B). Lower connectivity was observed in multiple brain regions in S-CRT mice compared with S-SHAM (*P* = 0.001; Supplementary Table S3). Exercise alone was associated with lower characteristic path length (*P* = 0.03), mean...
local efficiency ($P = 0.01$), normalized clustering coefficient ($P = 0.03$), and modularity ($P = 0.03$; Fig. 5C) but had a subthreshold effect on regional connectivity ($P = 0.08$). In CRT-treated rats, exercise appeared to improve assortativity ($P = 0.006$) and characteristic path length ($P = 0.04$; Fig. 5D) as well as a majority of the regional damage associated with CRT ($P = 0.03$; Supplementary Table S3).

Immunohistochemistry and ELISA

Twelve-month immunohistochemical analysis of myelination in the corpus callosum showed change in the width of the myelinated fibers in the corpus callosum (Fig. 6A). There was a significant effect of CRT, $F(1,23) = 9.652$, $P = 0.005$, and CRT $\times$ Exercise, $F(1,23) = 9.333$, $P = 0.0056$ (Fig. 6B). Staining for GFAP in the cortex of rats showed no
significant effect of CRT or Exercise. There was, however, a trend toward increased GFAP stain seen in S-CRT rats compared with S-SHAM (Supplementary Figure S5). In the separate cohort, proliferation and stem cell density measured using Ki67 and CD133, respectively, demonstrated a clear trend toward increased staining in the E-CRT compared with the S-CRT (Supplementary Figure S6). BDNF plasma levels of E-CRT were significantly higher ($P < 0.05$, Student’s $t$-test) compared with S-CRT animals at 21 days post-irradiation (Supplementary Figure S7).
Discussion

The objective of this study was to establish a translational animal model of cognitive impairment following irradiation of the juvenile brain and evaluate voluntary exercise as a potential mitigator. We have observed that CRT resulted in early and lasting impairments in task acquisition, accuracy, and latency to correct response, as well as caused stunting of growth and changes in brain volume and axonal density and integrity. CRT in both sedentary and exercised groups resulted in a lower body weight; however, CRT animals exercised almost as much as their heavier sham counterparts except on week 7 of the exercise regimen, when they ran more. While we and others have previously reported on this effect of CRT on body weight, length, and the effect of exercise and CRT on behavioral parameters pertaining to open field, Barnes maze, and Morris water maze, we are not aware of any other study on the effect of exercise on 5-CSRTT parameters following CRT in rodents. Exercising after irradiation improved acquisition, behavioral control, and processing speed. Interestingly, exercise blocked the stunting of brain size and increased brain fiber numbers compared with sedentary-CRT values. Interestingly, exercise partially restored global connectome organization and appeared to remodel regional connections by increasing connectivity between alternate pairs.

Irradiation and exercise both independently affected acquisition of 5-CSRTT as assessed by the number of trials animals took to reach criterion during training. Irradiation impaired acquisition, particularly as task difficulty increased. In contrast, exercise reduced trials to criterion, irrespective of CRT. These results reflect an early indication of difficulty in learning in irradiated animals and the potential for mitigation by exercise. It is important to note here that 5-CSRTT usually involves training of animals to a predetermined stage prior to administering the insult or drug. In our study, because of the young age of the animals at the time of insult, the training phase occurred post-CRT and as a result, the “training” phase was now a readout or test compared with previous studies.

Of the behavioral measures obtained at the 6-, 9-, and 12-month post-CRT time points, accuracy and latency to correct responses conveyed the most valuable information regarding neurocognitive deficits after CRT. For measures of accuracy, post hoc comparisons indicated that the S-CRT group was significantly less accurate than the S-SHAM group at 6 months. In other words, the S-CRT group made fewer correct responses and more incorrect responses, leading to a lower accuracy level than SHAM. Also, measures of accuracy showed a significant interaction of radiation and exercise at 6 months post-CRT, indicating that the effect of radiation depended on exercise. It is important to note that accuracy of the E-CRT group was not statistically different from the 2 SHAM groups, indicating that exercise preserved accuracy. By 9 months, accuracy was not impacted by either radiation or exercise. This recovery or settling continued through the 12-month measurement, though it appears that at 12 months, radiation had a negative impact on accuracy albeit not significantly.

Our data further show that exercise improved behavioral control by reducing impulsivity in both sham and irradiated rats at all 3 time points. Gapin et al found that daily moderate-to-vigorous exercise was associated with improved performance in a cognitive planning task that assessed executive function in children with ADHD, a common disorder associated with impulsivity. It was also reported by Wigal et al that physical activity breaks before and during class positively impact attention and classroom performance. The detrimental effect of CRT on attentional processing is further elucidated through changes in latencies to correct response. The latency to correct response in 5-CSRTT is a measure of processing speed in the task. At 6 months post-CRT, there were main effects of both radiation and exercise. These effects were diluted at the later time points. Overall, our results indicate that CRT groups took longer to process the stimuli and yield a correct response, which is consistent with the literature on processing speed deficits after cranial irradiation in children. Exercise not only enhanced frontal lobe function in naïve animals, but also helped protect against CRT-induced deficit.

Previous studies have shown the neuroprotective effects of exercise. One study found that long-term exercise protected the prefrontal cortex against the effects of aging. This information is consistent with improvements that we see in the 5-CSRTT in the E-CRT group compared with S-CRT. As 5-CSRTT is heavily frontal and prefrontal cortex dependent, neuroprotection arising from exercise may contribute to ameliorating deficits due to CRT. Our data also show that within the separate cohort of rats, there was an increase in Ki67 and CD133 staining in the cortex. We also measured, in the same animals, a significant increase in BDNF plasma levels. BDNF has been shown to mediate improvements in executive function in humans, following exercise intervention. It is important to note that these molecular measurements were conducted following a shorter exercise regimen (2 wk), while our exercise regimen, employed in the cognitive and imaging studies, occurred over a longer period (7 wk). Its effects, therefore, would be more rigorous and appear to have persisted through testing time points of 6, 9, and 12 months, which highlights the sustained effect of exercise during a developmental phase and its potential long-term impact for patients treated with CRT.

Using MRI, we observed that brain size was affected by not only radiation, but also exercise. Interestingly, these changes arose differently: Brain length was reduced by radiation, while brain width was increased by exercise. Brain volume has been used to diagnose phenotypical changes caused by Alzheimer’s and Parkinson’s disease. Similarly, in our study, whole brain volume of CRT groups was significantly lower, while whole brain volume of exercised groups was not. We also observed a CRT-induced decline in cerebellar, hindbrain, midbrain, and ventricle volumes. The addition of exercise abrogated the volume effect in the case of the cerebellum and midbrain. Our results are consistent with literature where Alzheimer’s disease patients have shown increases in hippocampal volume after undergoing an established exercise paradigm. Although the affected region is different (hippocampal versus cerebellum, etc), these results are consistent with our data, where exercise recovers size deficits due to disease/CRT insult.
As expected, our results demonstrated a lower FA value in gray matter and CSF compared with white matter. We also observed an overall increase in FA between S-SHAM and E-CRT rats, and a trend toward increase in FA between sedentary and exercise rats; however, this may be explained by the significant effect of exercise on fiber number. Similarly, in specific brain regions, we did not detect changes in FA caused by radiation and observed only a positive effect of exercise on the cerebellum, olfactory structures, and ventricles, which indicate higher fiber formation caused by exercise. Wang et al showed minute changes (~3%) in FA in their rat model of radiation therapy; however, their study utilized adult female rats and a single collimated dose of 25 or 30 Gy. While there are sex, dose, and imaging differences between our studies and theirs, both studies measure FA changes, demonstrating the importance and difficulty of interpreting these changes. Also, Buddo et al showed that gliosis can contribute to increases in diffusion tensor anisotropy, another potential explanation for the increases seen in FA.

Mulhern et al found that pediatric brain tumor survivors who were treated with CRT had more calcification and significantly less volume of CNS white matter in the brain, which could contribute to the deficits in intelligence. A later study by the same group found that the main dysfunction as a result of decreased white matter in the brain was attention, which could be related to decreases in IQ and poor academic achievement in survivors. Finally, our observed imaging changes related to exercise agree with that of Herting et al, who demonstrated a correlation between white matter and connectivity and fitness levels in adolescent males, while Tseng et al showed an inverse correlation between physical fitness and white matter hyperintensity levels in the brain, again pointing toward the benefits of exercise.

In terms of connectome organization, CRT was associated with global and local damage to network connectivity. Assortativity was higher in S-CRT compared with S-SHAM rats, indicating that nodes having high connectivity did not properly interact with low connectivity nodes. Increased assortativity has been observed in demyelinating disease such as MS. S-CRT rats also demonstrated high characteristic path length and lower global efficiency, which reflect disconnection within the network, resulting in disrupted network integration and information exchange. Elevated characteristic path length is a consistent finding in patients with Alzheimer’s disease. Higher mean local efficiency and normalized clustering coefficient in CRT-treated mice indicate abnormal network segregation and suggest that local clusters or subnetworks may function in a more isolated manner. Higher modularity indicates a fragmented network with atypical functional subnetwork organization.

Exercise appeared to partially restore global connectome organization, including assortativity and characteristic path length. Exercise did not increase connectivity between the specific regional areas that were affected by CRT but appeared to remodel these connections by increasing connectivity between alternate regional pairs.

Several limitations of our design are worth noting. The duration of the 5-choice training, yearlong testing period, exercise regimen, and DTI imposed practical limitations on our design. This research would have benefited from a larger sample size, by using untested groups at every time point, and from another behavioral testing paradigm together with 5-CSRTT. Finally, our exercise paradigm could be extended, and/or started at an earlier time point to increase its benefits. Nevertheless, despite these limitations, exercise showed clear benefits after CRT and supports the animal research in this domain that has been established thus far.

At present, there is no effective treatment to alleviate neurocognitive deficits and impaired quality of life that survivors of pediatric brain tumors sustain. Physical activity presents a promising way to promote brain health after CRT and may prove to be a means, alone or as adjuvant therapy, through which to ameliorate cognitive late effects of pediatric brain tumor treatment.

### Supplementary Material

Supplementary material is available at Neuro-Oncology online.

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### Conflict of interest statement

No conflicts.

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