Altered Resting-State Functional Connectivity in the Hand Motor Network in Glioma Patients

Arka N. Mallela,^{1,2} Kyung K. Peck,^{1,3} Nicole M. Petrovich-Brennan,¹
Zhigang Zhang,⁴ William Lou,^{1,5} and Andrei I. Holodny^{1,6}

Abstract

To examine the functional connectivity of the primary and supplementary motor areas (SMA) in glioma patients using resting-state functional MRI (rfMRI). To correlate rfMRI data with tumor characteristics and clinical information to characterize functional reorganization of resting-state networks (RSN) and the limitations of this method. This study was IRB approved and in compliance with Health Insurance Portability and Accountability Act. Informed consent was waived in this retrospective study. We analyzed rfMRI in 24 glioma patients and 12 age- and sex-matched controls. We compared global activation, interhemispheric connectivity, and functional connectivity in the hand motor RSNs using hemispheric voxel counts, pairwise Pearson correlation, and pairwise total spectral coherence. We explored the relationship between tumor grade, volume, location, and the patient's clinical status to functional connectivity. Global network activation and interhemispheric connectivity were reduced in gliomas ($p < 0.05$). Functional connectivity between the bilateral motor cortices and the SMA was reduced in gliomas ($p < 0.01$). High-grade gliomas had lower functional connectivity than low-grade gliomas ($p < 0.05$). Tumor volume and distance to ipsilateral motor cortex demonstrated no association with functional connectivity loss. Functional connectivity loss is associated with motor deficits in low-grade gliomas, but not in high-grade gliomas. Global reduction in resting-state connectivity in areas distal to tumor suggests that radiological tumor boundaries underestimate areas affected by glioma. Association between motor deficits and rfMRI suggests that rfMRI may accurately reflect functional changes in low-grade gliomas. Lack of association between rfMRI and clinical motor deficits implies decreased sensitivity of rfMRI in high-grade gliomas, possibly due to neurovascular uncoupling.

Keywords: functional connectivity; glioma; primary motor cortex; resting state functional MRI; resting state network

Introduction

NEUROSURGICAL RESECTION REMAINS the mainstay treatment option for patients with many brain tumors (Freyschlag and Duffau, 2014). Preoperative functional MRI (fMRI) is rapidly becoming the standard of care as fMRI can accurately identify eloquent cortices to be avoided during resection of the brain tumor. fMRI can also identify reorganization of cortical function in the setting of brain tumors (Holodny et al., 2000; Holodny, 2008). Currently, in the clinical setting, the vast majority of preoperative fMRI studies are performed using paradigm-driven fMRI (Holodny, 2008). Resting-state functional MRI (rfMRI) is a variant of fMRI performed in a task-free manner. rfMRI examines low frequency blood oxygenation level-dependent (BOLD) oscillations across the brain

in the absence of paradigm-driven stimulation (Biswal et al., 1995). Several regions of the brain with correlated or coherent ("functionally connected") BOLD time courses form large-scale networks termed resting-state networks (RSNs). These RSNs correspond to known functional networks, including somatosensory, language, and vision (Biswal et al., 2010; Holodny, 2008; Lee et al., 2013; Salvador et al., 2005; Smith et al., 2013), and anatomical networks in animal models (Kelly et al., 2010; Margulies et al., 2009).

rfMRI appears to offer a number of potential advantages over traditional paradigm-driven fMRI (pdfMRI). First, rfMRI requires only a single scan from which multiple networks (e.g., motor, language) can be mapped, whereas each task-based paradigm requires a separate scanning session, which over multiple tasks can add up to 30–40 min (Lee et al., 2013).

¹Functional MRI Laboratory, Department of Radiology, Memorial Sloan-Kettering Cancer Center, New York, New York.

²Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania.

Departments of ³Medical Physics and ⁴Epidemiology and Biostatistics, Memorial Sloan-Kettering Cancer Center, New York, New York.

⁵Weill Cornell Medical College, New York, New York.

⁶Brain Tumor Center, Memorial Sloan-Kettering Cancer Center, New York, New York.

Second, paradigm-driven fMRI, unlike rfMRI, requires significant patient compliance, which can be difficult for neurologically impaired, unconscious, hearing impaired, or anesthetized patients (Smith et al., 2013). Third, rfMRI has the potential to map networks better than pdfMRI.

A known limitation of pdfMRI is the muting of the BOLD response adjacent to high-grade gliomas due to neurovascular uncoupling (Holodny et al., 2000; Pillai and Zacà, 2012; Ulmer et al., 2003). This decreased sensitivity of BOLD, which can lead to false-negative results, including “pseudo-reorganization” of neurological functions (Holodny et al., 2000; Ulmer et al., 2003), has undesirable clinical implications in the preoperative brain tumor setting. Previous studies using rfMRI have demonstrated an apparent decrease in functional connectivity in patients with brain tumors (Otten et al., 2012), altered BOLD oscillation spectra (Niu et al., 2014), and disturbance of functional brain networks (Huang et al., 2014). Since paradigm-driven fMRI and rfMRI rely on BOLD signal as a proxy for neural activity, neurovascular uncoupling, as seen in high-grade gliomas and other lesions, can potentially reduce the accuracy of both of these methods (Gabriel et al., 2014; Pillai and Zacà, 2012; Ulmer et al., 2003). Hence, it is unclear if the described findings occur due to changes in neural connectivity, a limitation of rfMRI as a method, or a combination of both factors (Lee et al., 2001; Ulmer et al., 2003; Villringer and Dirnagl, 1995).

The purpose of the present study is to (1) examine the functional connectivity of the primary and supplementary motor areas (SMA) in glioma patients using rfMRI and to (2) compare rfMRI data with tumor characteristics and motor deficits to evaluate the abilities and limitations of this method to characterize functional reorganization of RSNs.

Materials and Methods

Subject selection

This study was approved by the institutional review board and was in full compliance with Health Insurance Portability and Accountability Act regulations. Informed consent was waived, as the study was retrospective. Twenty-four patients diagnosed with glioma of any grade that completed a routine MRI and rfMRI from July 2012 to December 2013 were included. Patients were selected consecutively. Pathology was confirmed by histological material in every case (Table 1). Any focal motor weakness (defined as less than 5/5 on clinical neurological examination) in patients was recorded. For comparison, 12 age, sex, and handedness-matched controls were obtained from the Nathan Kline Institute–Rockland Sample data set in the 1000 Functional Connectomes project (Nooner et al., 2012).

Image acquisition

RfMRI data were obtained using a 3T GE scanner with an eight-channel head coil. fMRI was obtained with a single-shot gradient echo echo-planar imaging sequence (TR = 4000 msec, TE = 35 msec, 128 × 128 matrix, 4.5 mm slice thickness). During the rfMRI scan, patients were instructed to relax, not move, and not think about anything. RfMRI data for control patients from the NKI-RS data set were obtained using a 3T Siemens Magnetom scanner with a 32-channel head coil. fMRI was obtained with a single-shot gradient echo-planar imaging

sequence (TR = 2500 msec, TE = 30 msec, 216 × 216 matrix, 3 mm slice thickness) (Nooner et al., 2012).

Matching T1-weighted (TR/TE = 400/14 msec, 256 × 256 matrix), FLAIR (TR/TE = 10,000/106 msec, inversion time 220 msec, 256 × 256 matrix), T2-weighted (TR/TE = 4000/102 msec, 256 × 256 matrix), and T1 postcontrast (TR/TE = 600/20 msec, 256 × 256 matrix) images were obtained. 3D T1-weighted anatomical images were acquired with a spoiled gradient recalled sequence (TR/TE = 22/4 msec, 256 × 256 matrix, 1.5 mm thickness). Matched MPRAGE (TR/TE = 2500/3.5 msec, 256 × 256 matrix) and T2-weighted (TR/TE = 2500/11 msec, 216 × 216 matrix) images were obtained for controls from the NKI-RS data set (Nooner et al., 2012).

Data analysis

Tumor volume and the distance from the tumor to the ipsilateral hand motor area were defined using BrainLab (BrainLAB AG, Feldkirchen, Germany). In enhancing tumors, the area of the tumors was defined by the rim of T1 contrast enhancement. In tumors with no enhancement, the area of the tumor was defined as the mass-like FLAIR abnormality. In mixed enhancing/nonenhancing tumors, the enhancing portion was used for analysis (Pronin et al., 1997). Distance was defined as the shortest length through space between the border of the ipsilateral primary motor cortex (PMC) regions of interest (ROI) and the border of the tumor on the imaging modalities described above. “Close” tumors were defined as those <25 mm and “far” tumors as ≥25 mm from the ipsilateral hand motor area, respectively, to create two comparable subsets with similar sample sizes. The centrally necrotic portion of high-grade lesions was included in volume analysis. A board-certified neuroradiologist with a certificate of added qualification in neuroradiology and 20 years experience in fMRI supervised all results.

RfMRI analyses were performed using Analysis of Functional Neuroimaging (AFNI) (Cox, 1996). Processing and analysis were performed in native rather than standard space to remove any risk of error propagation during analysis and due to the presence of large tumors, which occasionally can make registration to standard space precarious. Motion correction, spatial smoothing (Gaussian filter with 4 mm full width of half maximum), and linear trend removal were performed. Data were filtered using a band-pass of 0.01–0.08 Hz to remove physiological high-frequency noise. ROI for the left and right hand homunculi in the PMC and the SMA were hand-drawn using anatomic landmarks (PMC—hand omega, SMA—bilateral, paramedian, anterior to leg PMC) for guidance for each subject and control. The SMA ROI was drawn bilaterally as previous work in the literature suggests SMA activation is bilateral in the hand motor network (Lyo et al., 2015; Peck et al., 2009). Averaged time series were obtained from the ROIs and then applied as a regressor within the data set. The data were analyzed with (i) voxel count analysis and (ii) correlation analysis and coherence analysis.

Voxel count analysis. Network voxels were defined as voxels in a given hemisphere that correlated to the averaged ROI time signal at $p < 10^{-7}$ (corrected). For hemispheric analysis, only the frontal and parietal lobes were included (i.e., those parts involved in the hand motor network). This analysis yielded three ROIs in two hemispheres for a total of six voxel count regions. For controls, the three ROIs were defined as left, right, and SMA. For patients, the three ROIs were defined as tumor-side PMC, nontumor side PMC, and SMA.

TABLE 1. PATIENT DEMOGRAPHICS, MOTOR DEFICITS, AND TUMOR INFORMATION

Age (years)	Sex	Hand	Motor deficits	Tumor side	Lobe	Close to PMC (<25 mm)	Tumor volume (cm ³)	Tumor type
50	F	RH	None	Left	Frontal	Close	28.9	Oligodendroglioma (Grade II)
51	M	RH	None	Left	Frontal/Temporal/ Insular	Far	44.3	Oligodendroglioma (Grade II)
22	M	RH	Left face/arm partial seizure	Left	Temporal	Far	15.2	Mixed low-grade glioma
35	M	RH	None	Left	Temporal	Far	87.1	Diffuse astrocytoma (Grade II)
31	M	RH	None	Left	Frontal	Close	34.6	Mixed low-grade glioma
43	M	RH	None	Right	Frontal/Temporal/ Insular	Far	105.5	Oligodendroglioma (Grade II)
29	M	RH	None	Left	Parietal	Close	23.3	Diffuse astrocytoma (Grade II)
35	M	RH	Left pronator drift	Bilateral	Temporal	Close	L (113.0) R (184.0)	Diffuse astrocytoma (Grade II)
58	M	RH	None	Left	Frontal/Temporal/ Insular	Far	25.0	Diffuse astrocytoma (Grade II)
42	F	RH	None	Left	Parietal	Close	12.6	Anaplastic astrocytoma (Grade III)
29	M	LH	None	Left	Frontal	Close	4.6	Anaplastic astrocytoma (Grade III)
60	M	RH	None	Left	Insular	Far	5.6	Anaplastic astrocytoma (Grade III)
43	M	RH	None	Left	Frontal/Temporal/ Insular	Far	25.4	Anaplastic astrocytoma (Grade III)
64	M	RH	None	Left	Frontal	Close	10.1	Glioblastoma multiforme (Grade IV)
84	F	RH	None	Left	Temporal	Far	24.4	Glioblastoma multiforme (Grade IV)
64	F	RH	None	Left	Frontal/Temporal/ Insular	Far	50.5	Gliosarcoma (Grade IV)
42	F	LH	Right hand weakness	Left	Frontal	Close	16.6	Glioblastoma multiforme (Grade IV)
69	F	LH	Gait instability	Right	Temporal	Far	35.0	Glioblastoma multiforme (Grade IV)
51	F	LH	None	Right	Temporal/Parietal	Far	30.0	Glioblastoma multiforme (Grade IV)
57	F	RH	None	Left	Temporal/Parietal	Close	36.6	Glioblastoma multiforme (Grade IV)
59	M	RH	Right pronator drift	Left	Frontal/Temporal/ insular	Close	77.4	Glioblastoma multiforme (Grade IV)
68	M	RH	None	Left	Temporal	Far	13.3	Glioblastoma multiforme (Grade IV)
61	F	RH	Left arm/leg rigidity, weakness	Right	Parietal	Close	64	Glioblastoma multiforme (Grade IV)
51	M	RH	None	Left	Frontal/Insular	Far	4.8	Mixed high-grade glioma

PMC, primary motor cortex.

To measure the level of network activation, voxel percent (%) activation was calculated by dividing the number of network voxels in a given hemisphere by the total voxels in that hemisphere. To compare interhemispheric connectivity, network voxel ratios were obtained by dividing the network voxels from a given ROI-hemisphere pair with the voxels from another ROI-hemisphere pair.

Network Voxel Ratio

$$= \frac{\text{Voxels in Hemisphere A activated by ROI 1}}{\text{Voxels in Hemisphere B activated by ROI 2}}$$

Ideally, RSNs are bilateral and symmetric: the amount of activation in one ROI hemisphere pair should be the same as the activation in another ROI hemisphere pair (Smith et al., 2013). Therefore, the network voxel ratio should be 1. The ratio significantly less than 1 (for example, in a tumor pa-

tient) implies that the number of voxels in the network from an ROI in a hemisphere (the strength of the connection) is weaker than the controls. This ratio is labeled as “ROI 1 to Side A: ROI 2 to Side B.” Voxel% activation and voxel count ratio from patients were compared to controls.

Correlation and coherence analysis. The strength of connectivity (value of correlation and coherence) was calculated from the averaged time series. For correlation analysis, the three averaged time series (tumor PMC, nontumor PMC, and SMA for patients and left PMC, right PMC, and SMA for controls) were Pearson correlated, producing three r^2 values.

For coherence analysis, the spectral coherence function was calculated from pairs of the three time series and integrated from 0.01 to 0.08 Hz. This value was normalized on a 0–1 scale, producing three values proportional to total spectral coherence in the frequency band.

$$\text{Total Coherence}_{ROI\ 1, ROI\ 2} \propto \int_{0.01\ Hz}^{0.08\ Hz} \text{Coherence}\{ROI\ 1, ROI\ 2\}(f)df$$

$f = \text{frequency}$

Association between functional connectivity and tumor grade, location, and volume was calculated quantitatively.

Statistical analysis

As control individuals should have bilaterally symmetric networks, (Smith et al., 2013) the left side of control was arbitrarily chosen as the comparison for the tumor side in subjects and the right in controls for the nontumor side in subjects. Differences between patients and controls and between patient subgroups, when adequately powered, were tested using the Mann–Whitney *U*-test at significance levels $p < 0.05$, with medians and semi-interquartile ranges reported. To correct for multiple comparisons, Benjamini–Hochberg method was used to control the false discovery rate (Benjamini and Hochberg, 1995).

Results

Reduced network voxel activation in the hand motor network in glioma patients

For controls, the connectivity network is very strong, symmetrical, and bilateral (Fig. 1, *bottom*). Patients with gliomas demonstrated decreased activation and interhemispheric connectivity (Fig. 1, *top*). On visual (qualitative) inspection, brain tumor patients had reduced activation in the hand motor RSN and displayed lateral bias in activation to the seed ROI side (Fig. 1, *top*). We observed reduced global activation in the hand RSNs in patients (compare Fig. 1 *top* to *bottom*). Quantitatively, brain tumor patients had significantly reduced proportion of network voxels ($p < 0.001$) in all brain regions (tumor side hemisphere, nontumor hemisphere, whole brain) when compared to healthy controls (Fig. 2).

Network voxel ratios demonstrate reduced interhemispheric connectivity

The ratios comparing symmetric connections were not significantly different than controls (tumor PMC to tumor hemisphere: nontumor PMC to nontumor hemisphere: 1.10 ± 0.59 vs. 1.09 ± 0.07 ; tumor PMC to nontumor hemisphere: nontumor PMC to tumor hemisphere: 1.00 ± 0.50 vs. 1.02 ± 0.09) (Fig. 3, left row).

Glioma patients had significantly more output from a given PMC to the ipsilateral hemisphere than to the contralateral hemisphere (ratio > 1) when compared to controls. In other words, ratios comparing the output of a seed ROI to the ipsilateral versus output to the contralateral hemispheres were significantly higher in the glioma group than in controls (tumor PMC to tumor hemisphere: tumor PMC to nontumor hemisphere: 1.34 ± 0.56 vs. 1.05 ± 0.09 , $p < 0.05$; nontumor PMC to nontumor hemisphere: nontumor PMC to tumor hemisphere: 1.58 ± 0.80 vs. 1.00 ± 0.05 , $p < 0.01$) (Fig. 3, middle row).

The nontumor hemisphere received more input from the ipsilateral PMC than from the contralateral (tumor) hemisphere in glioma patients, when compared to controls (nontumor PMC to nontumor hemisphere: tumor PMC to nontumor hemisphere: 1.29 ± 1.03 vs. 0.97 ± 0.03 , $p < 0.05$). The ratio measuring input to the tumor hemisphere was not significant (tumor PMC to tumor hemisphere: nontumor PMC to tumor hemisphere: 1.39 ± 1.64 vs. 1.05 ± 0.07 , $p = 0.067$). (Fig. 3, right row). For the controls, these connectivity ratios approached 1:1, implying the bilaterally symmetric connectivity.

Reduced functional connectivity between key regions in the hand motor RSN

Functional connectivity between motor RSN regions (tumor side PMC, nontumor PMC, SMA) is significantly reduced in glioma patients versus controls. This was true for both correlation

FIG. 1. Hand motor resting-state network (RSN) in a representative patient and control. The horizontal axis refers to seed regions of interests (ROIs) to which voxel signals were correlated. Seed ROIs in the hand motor primary motor cortex (PMC) (hand omega) and supplementary motor area (SMA) are highlighted in blue. (top) Hand RSN in a 64-year-old right-handed woman diagnosed with a gliosarcoma (WHO Grade IV) in the left frontal/temporal/insular region. (bottom) Hand RSN in matched control. Scale bar shows Pearson correlation ranges. Color images available online at www.liebertpub.com/brain

FIG. 2. Reduced network activation in glioma patients. Diagrams showing the percent of network voxels involved in the hand RSN, a measure of global network activation, in patients and controls. Voxel% activation was reduced in tumor patients in all network segments, regardless of source ROI and target hemisphere. ** $p < 0.001$.

(tumor PMC to nontumor PMC -0.44 ± 0.25 vs. 0.79 ± 0.08 , $p < 0.001$; tumor PMC to SMA -0.47 ± 0.15 vs. 0.79 ± 0.07 , $p < 0.001$; nontumor PMC to SMA -0.54 ± 0.21 vs. 0.78 ± 0.11 , $p < 0.001$) and spectral coherence (tumor PMC to nontumor PMC -0.64 ± 0.25 vs. 0.90 ± 0.10 , $p < 0.01$; tumor PMC to SMA -0.69 ± 0.12 vs. 0.89 ± 0.05 , $p < 0.01$; nontumor PMC to SMA -0.72 ± 0.21 vs. 0.90 ± 0.07 , $p < 0.01$). The reduction occurs in all regions, even in connections not directly containing the tumor (nontumor PMC to SMA), again illustrating global connectivity loss in patients. When comparing the two approaches, spectral coherence remained higher than correlation in brain tumor patients. Figure 4 displays weighted graphs representing this functional connectivity in all patients, high-grade gliomas patients, and controls.

SMA connectivity in glioma patients

Lateral bias in SMA connectivity (i.e., stronger SMA connectivity to the nontumor hemisphere) was not observed. The voxel ratio of the tumor side to the nontumor side for SMA seed ROI (0.93 ± 0.26) and was not significantly different than controls (1.02 ± 0.10). In glioma patients, correlation and spectral coherence were not significantly different between the SMA to tumor PMC versus SMA to nontumor PMC connections (correlation -0.47 ± 0.15 vs. 0.54 ± 0.21 ; coherence -0.69 ± 0.12 vs. 0.72 ± 0.21).

Lower functional connectivity with high-grade than low-grade gliomas

The strength of the tumor side PMC to contralateral PMC and tumor side PMC to SMA connections was reduced in higher

grade tumors. Correlation of tumor side PMC to nontumor PMC (0.22 ± 0.23 vs. 0.64 ± 0.22 , $p < 0.05$) and tumor PMC to SMA (0.34 ± 0.09 vs. 0.66 ± 0.06 ; $p < 0.01$) correlation was significantly decreased in high-grade gliomas versus low-grade gliomas. There was no significant difference in nontumor PMC to SMA connectivity (0.50 ± 0.24 vs. 0.58 ± 0.08) between high-grade gliomas and low-grade gliomas.

Changes in functional connectivity not associated with tumor location and tumor volume

Functional connectivity between these regions, as measured by correlation, was not significantly different when “close” and “far” subgroups were compared (tumor PMC to nontumor PMC -0.36 ± 0.33 vs. 0.44 ± 0.22 , $p = 0.80$; tumor side PMC to SMA -0.35 ± 0.19 vs. 0.52 ± 0.11 , $p = 0.55$; nontumor PMC to SMA -0.55 ± 0.27 vs. 0.54 ± 0.17 , $p = 0.93$). We found no correlation between tumor volume and loss of functional connectivity in the hand motor RSN ($r^2 < 0.05$ for all measures).

Functional connectivity, tumor grade, and motor deficits

The correlation between motor RSN regions in low-grade glioma patients with motor deficits was lower than in those without deficits (tumor PMC to nontumor PMC—deficit: mean = 0.19, no deficit: mean = 0.71; tumor side PMC to SMA—deficit: mean = 0.56, no deficit: mean = 0.69; nontumor PMC to SMA—deficit: mean = 0.20, no deficit: mean = 0.66). Functional connectivity in high-grade glioma patients with deficits did not show the same association with motor deficits (tumor PMC to nontumor PMC—deficit: mean = 0.44, no deficit: mean = 0.29; tumor side PMC to SMA—deficit:

FIG. 3. Reduced inter-hemispheric connectivity in glioma patients. Voxel count ratios demonstrating inter-hemispheric connectivity in patients and controls. Diagrams illustrate the connections examined in the ratio—the strength of the red connection divided by the strength of the blue connection. The red horizontal line on plots is the ideal ratio of 1:1 (no bias in connectivity). For the control subjects, the left side is taken as equivalent to the tumor side and the right as equivalent to the nontumor side, as discussed in the methods. * $p < 0.05$, ** $p < 0.01$. Color images available online at www.liebertpub.com/brain

mean=0.39, no deficit: mean=0.32; nontumor PMC to SMA—deficit: mean=0.46, no deficit: mean=0.38).

Discussion

We report that both functional connectivity between hand motor RSN regions and interhemispheric connectivity are significantly reduced in glioma patients. Furthermore, we demonstrate that overall activation of the hand motor RSN is decreased, irrespective of seed ROI placement and measured hemisphere. Interestingly, SMA resting-state connectivity is globally disrupted, but does not seem to be weaker to

the tumor side hemisphere when compared to the nontumor side. These findings suggest that gliomas have disruptive effects beyond the abnormalities identified on routine MR imaging.

While measures of functional connectivity, correlation, and spectral coherence were reduced in glioma patients, correlation was decreased more than coherence. One possible explanation for this is that coherence is insensitive to phase shifts in the time variable unlike Pearson correlation (i.e., $\sin(x)$ and $\cos(x)$ have perfect spectral coherence, but poor correlation). This difference suggests that the phase of BOLD signal should be analyzed in future work.

We further found that high-grade gliomas cause greater disruption in functional connectivity than low-grade gliomas. The result is expected, given the increased infiltration, angiogenesis, and alterations in brain autoregulation in high-grade gliomas (Canoll and Goldman, 2008; Harris et al., 2014; Wang et al., 2012).

Surprisingly, we did not find significant difference in functional connectivity in the hand motor network when comparing between tumors close to the PMC (<25 mm) and those far from it (>25 mm). This again suggests that functional effects of the tumor extend further than the radiographic boundaries seen on routine MR. Other investigators have found that small-world brain network topology is globally disturbed by brain tumors (Huang et al., 2014). These effects of this network disruption may extend past tumor boundaries. In addition, a recent study utilizing coregistration of histology and MRI demonstrated infiltration of tumor cells beyond conventional radiographic boundaries (Zetterling et al., 2016). As such, effects on RSNs may also extend past these boundaries.

We found no association between the radiographic volume of the tumor and loss of functional connectivity in the motor RSN, similar to previous findings that tumor volume does not associate with loss of functional connectivity in the default mode network (Harris et al., 2014). While this finding may at first appear counterintuitive, it suggests that radiological measures of volume do not represent the maximum extent of the functional effects of tumor infiltration.

Finally, we examined the relationship between tumor grade, motor deficits, and loss of functional connectivity. Neuronal connection loss is associated with reduction in functional connectivity and causes motor deficits (Fontaine et al., 2002; Guye et al., 2003; Jiang et al., 2010; Sporns, 2010; Wang et al., 2012). This was confirmed in our study, as low-grade glioma patients with motor deficits did have lower functional connectivity than those without motor deficits. By contrast, we did not observe an association between the presence of motor deficits and the loss of functional connectivity in patients with high-grade gliomas.

This lack of association implies another factor that is not seen in low-grade gliomas. It is known that high-grade gliomas demonstrate a muted BOLD response in paradigm-driven fMRI due to the presence of abnormal neovascularity (Holodny et al., 2000; Ulmer et al., 2003). Since both paradigm-driven fMRI and rfMRI probably rely on the same BOLD mechanism, it would appear that this known liability of paradigm-driven fMRI also affects rfMRI. This reasoning implies that the apparent loss of functional connectivity seen on rfMRI studies in high-grade gliomas may be caused by a combination of the true-positive changes in neuronal connections and by false-negative changes of neurovascular uncoupling. The artifactually decreased connectivity due to an altered hemodynamic response should not produce motor deficits. The combination of an actual loss of connectivity and an altered hemodynamic response seen in high-grade glioma patients probably leads to the lack of difference in our observed results in those with motor deficits and those without motor deficits.

Our results are consistent with previous reports in the literature that demonstrate altered cerebral autoreactivity and neurovascular uncoupling utilizing paradigm-driven fMRI, breath-holding BOLD imaging, and resting-state fMRI (Agarwal et al., 2016, 2016; Iranmahboob et al., 2015; Pillai and Mikulis, 2015). It also is possible that this neurovascular uncoupling extends past conventional MR boundaries as there is histologic evidence of tumor spread past these limits (Zetterling et al., 2016). Taken together, these data suggest a cautious approach when interpreting resting-state fMRI and other functional imaging in high-grade glioma patients.

The literature is mixed as to the effects of gliomas on RSNs. Otten et al. (2012) reported that only patients with motor deficits demonstrate weaker networks. Contrastingly, two recent reports have indicated that the default mode network is weaker (Harris et al., 2014) and that Pearson correlation between the left and right PMC is reduced in all brain tumor patients (Niu et al., 2014). Niu et al. also demonstrated that spectral power is decreased in the BOLD time

signal from brain tumor patients. This may contribute to our finding of decreased spectral coherence.

These findings have positive and negative implications for the use of rfMRI in preoperative surgical planning. This study demonstrates that brain tumors may disrupt RSNs with anatomic substrates distal to glioma borders on T1 contrast and FLAIR. This suggests that while conventional imaging misses the full effects of gliomas, rfMRI can characterize such changes in connectivity and can guide a more appropriate surgical approach. This is consistent with current reports in the literature that have utilized rfMRI to characterize the language network (Tie et al., 2014). However, we also demonstrated that global rfMRI signal is reduced. Such a reduction could result in false negatives, that is, a lack of activation in eloquent cortices, which may lead to overaggressive resection. Moreover, our data suggest that neurovascular uncoupling may limit the sensitivity of rfMRI in high-grade glioma cases. These limitations of the method suggest that caution is required when using rfMRI clinically. To use rfMRI as an independent tool for preoperative planning, more insight into the mechanisms of signal reduction and neurovascular uncoupling is required.

This study has limitations. First, our sample size ($N=24$), while sufficient for patient versus control comparisons, was smaller than ideal for subgroup analysis. We identified several preliminary results based off subgroup analysis, but a larger population is required to make firm judgments.

Second, while voxel counts allow measurement of network activation across the entire brain, the method has limitations. First, voxels represent an arbitrary parcellation of the brain. Furthermore, the significance cutoff for network voxels—correlated to the averaged ROI time signal at $p < 10^{-7}$ (corrected)—though stringent, likely has a nonlinear, but monotonic, relationship to the counts of network voxels. While this approach is consistent with the literature (Otten et al., 2012), we acknowledge that changing this cutoff may change the counts of network voxels. When used in combination with functional connectivity metrics, as in this report, voxel counts can provide additional valuable insight into whole brain activation and interhemispheric connectivity.

Third, the control patients in this study were obtained from a publically available database. As such, although both our study subjects and controls had single-shot EPI rfMRI protocols, the scan parameters are different (patients: TR/TE 4000/35 msec, controls: TR/TE 2500/30 msec). Current reports in the literature show no difference in the functional connectivity in RSNs between scans performed at TR of 2500 and 5000 (Van Dijk et al., 2010). As such, we do not expect the difference in TR in our study (Controls: 2500, Subjects: 4000) to affect our results. Moreover, all of the statistics reported here were calculated within each study arm and either was normalized to whole brain activation (voxel counts) or involved comparing time series obtained from the same scan. Due to both of these reasons, the difference in scan parameters should not significantly affect the results of this study. Fourth, independent components analysis may be a more powerful tool to explore RSNs than seed based on analysis alone (Smith et al., 2013). This analysis allows simultaneous exploration of multiple brain networks, providing more insight into pathological changes caused by gliomas.

Finally, resting-state fMRI is limited, as it does not directly measure changes in white matter structure. Long white matter tracts are the anatomic substrates of RSNs, and tumors likely affect RSNs by both disrupting these tracts and directly

encroaching on gray matter. In our study, none of the gliomas crossed the midline or involved the corpus callosum. However, it is likely that the effect of these gliomas on intrahemispheric white matter tracts in part mediated the changes observed in the hand motor network. The literature contains some reports on paradigm-driven (task-based) fMRI utilized in conjunction with diffusion tensor imaging (Bailey et al., 2015; Tantillo et al., 2016). To date, the relationship between diffusion tensor imaging and resting-state fMRI has not been explored in the brain tumor population. This remains an active area of research for our group.

Conclusions

Gliomas weaken functional connectivity, interhemispheric connectivity, and overall activation in the hand motor RSN. Furthermore, while tumor grade is associated with functional connectivity loss, tumor location may not have such association. These findings suggest that gliomas induce global changes in brain connectivity and have positive and negative implications for the use of rfMRI as a tool in preoperative planning. Additional study is required to elucidate the mechanisms of connectivity loss and to determine the clinical uses of rfMRI.

Acknowledgments

The authors are grateful to Michael Milham, Bennett Leventhal, and colleagues at the Nathan Kline Institute for submitting their data to the 1000 Functional Connectomes project.

Author Disclosure Statement

No competing financial interests exist.

References

- Agarwal S, Sair HI, Airan R, Hua J, Jones CK, Heo H-Y, et al. 2016. Demonstration of brain tumor-induced neurovascular uncoupling in resting-state fMRI at ultrahigh field. *Brain Connect* 6:267–272.
- Agarwal S, Sair HI, Yahyavi-Firouz-Abadi N, Airan R, Pillai JJ. 2016. Neurovascular uncoupling in resting state fMRI demonstrated in patients with primary brain gliomas. *J Magn Reson Imaging JMRI* 43:620–626.
- Bailey PD, Zacà D, Basha MM, Agarwal S, Gujar SK, Sair HI, et al. 2015. Presurgical fMRI and DTI for the prediction of perioperative motor and language deficits in primary or metastatic brain lesions. *J Neuroimaging* 25:776–784.
- Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Methodol* 57:289–300.
- Biswal B, Yetkin FZ, Haughton VM, Hyde JS. 1995. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med* 34:537–541.
- Biswal BB, Mennes M, Zuo X-N, Gohel S, Kelly C, Smith SM, et al. 2010. Toward discovery science of human brain function. *Proc Natl Acad Sci U S A* 107:4734–4739.
- Canoll P, Goldman JE. 2008. The interface between glial progenitors and gliomas. *Acta Neuropathol* 116:465–477.
- Cox RW. 1996. AFNI: software for analysis and visualization of functional magnetic resonance imaging. *Comput Biomed Res* 29:162–173.
- Fontaine D, Capelle L, Duffau H. 2002. Somatotopy of the supplementary motor area: evidence from correlation of the

- extent of surgical resection with the clinical patterns of deficit. *Neurosurgery* 50:297–305.
- Freyschlag CF, Duffau H. 2014. Awake brain mapping of cortex and subcortical pathways in brain tumor surgery. *J Neurosurg Sci* 58:199–213.
- Gabriel M, Brennan NP, Peck KK, Holodny AI. 2014. Blood oxygen level dependent functional magnetic resonance imaging for presurgical planning. *Neuroimaging Clin N Am* 24:557–571.
- Guye M, Parker GJM, Symms M, Boulby P, Wheeler-Kingshott CAM, Salek-Haddadi A, et al. 2003. Combined functional MRI and tractography to demonstrate the connectivity of the human primary motor cortex in vivo. *NeuroImage* 19:1349–1360.
- Harris RJ, Bookheimer SY, Cloughesy TF, Kim HJ, Pope WB, Lai A, et al. 2014. Altered functional connectivity of the default mode network in diffuse gliomas measured with pseudo-resting state fMRI. *J Neurooncol* 116:373–379.
- Holodny AI. 2008. *Functional Neuroradiology: A Clinical Approach*. New York, NY: CRC Press.
- Holodny AI, Schulder M, Liu WC, Wolko J, Maldjian JA, Kalnin AJ. 2000. The effect of brain tumors on BOLD functional MR imaging activation in the adjacent motor cortex: implications for image-guided neurosurgery. *AJNR Am J Neuroradiol* 21:1415–1422.
- Huang Q, Zhang R, Hu X, Ding S, Qian J, Lei T, et al. 2014. Disturbed small-world networks and neurocognitive function in frontal lobe low-grade glioma patients. *PLoS One* 9:e94095.
- Iranmahboob A, Peck KK, Brennan NP, Karimi S, Fiscaro R, Hou B, et al. 2016. Vascular reactivity maps in patients with gliomas using breath-holding BOLD fMRI. *J Neuroimaging Off J Am Soc Neuroimaging* 26:232–239.
- Jiang Z, Krainik A, David O, Salon C, Troprès I, Hoffmann D, et al. 2010. Impaired fMRI activation in patients with primary brain tumors. *NeuroImage* 52:538–548.
- Kelly C, Uddin LQ, Shehzad Z, Margulies DS, Castellanos FX, Milham MP, et al. 2010. Broca's region: linking human brain functional connectivity data and non-human primate tracing anatomy studies. *Eur J Neurosci* 32:383–398.
- Lee MH, Smyser CD, Shimony JS. 2013. Resting-state fMRI: a review of methods and clinical applications. *AJNR Am J Neuroradiol* 34:1866–1872.
- Lee YJ, Chung TS, Yoon YS, Lee MS, Han SH, Seong GJ, et al. 2001. The role of functional MR imaging in patients with ischemia in the visual cortex. *AJNR Am J Neuroradiol* 22:1043–1049.
- Lyo JK, Arevalo-Perez J, Petrovich Brennan N, Peck KK, Holodny AI. 2015. Pre-operative fMRI localization of the supplementary motor area and its relationship with postoperative speech deficits. *Neuroradiol J* 28:281–288.
- Margulies DS, Vincent JL, Kelly C, Lohmann G, Uddin LQ, Biswal BB, et al. 2009. Precuneus shares intrinsic functional architecture in humans and monkeys. *Proc Natl Acad Sci U S A* 106:20069–20074.
- Niu C, Zhang M, Min Z, Rana N, Zhang Q, Liu X, et al. 2014. Motor network plasticity and low-frequency oscillations abnormalities in patients with brain gliomas: a functional MRI study. *PLoS One* 9:e96850.
- Nooner KB, Colcombe SJ, Tobe RH, Mennes M, Benedict MM, Moreno AL, et al. 2012. The NKI-Rockland sample: a model for accelerating the pace of discovery science in psychiatry. *Front Neurosci* 6:152.
- Ottens ML, Mikell CB, Youngerman BE, Liston C, Sisti MB, Bruce JN, et al. 2012. Motor deficits correlate with resting state motor network connectivity in patients with brain tumours. *Brain* 135:1017–1026.
- Peck KK, Bradbury M, Psaty EL, Brennan NP, Holodny AI. 2009. Joint activation of the supplementary motor area and presupplementary motor area during simultaneous motor and language functional MRI. *Neuroreport* 20:487–491.
- Pillai JJ, Mikulis DJ. 2015. Cerebrovascular reactivity mapping: an evolving standard for clinical functional imaging. *AJNR Am J Neuroradiol* 36:7–13.
- Pillai JJ, Zacà D. 2012. Comparison of BOLD cerebrovascular reactivity mapping and DSC MR perfusion imaging for prediction of neurovascular uncoupling potential in brain tumors. *Technol Cancer Res Treat* 11:361–374.
- Pronin IN, Holodny AI, Petraitkin AV. 1997. MRI of high-grade glial tumors: correlation between the degree of contrast enhancement and the volume of surrounding edema. *Neuroradiology* 39:348–350.
- Salvador R, Suckling J, Coleman MR, Pickard JD, Menon D, Bullmore E. 2005. Neurophysiological architecture of functional magnetic resonance images of human brain. *Cereb Cortex* 15:1332–1342.
- Smith SM, Vidaurre D, Beckmann CF, Glasser MF, Jenkinson M, Miller KL, et al. 2013. Functional connectomics from resting-state fMRI. *Trends Cogn Sci* 17:666–682.
- Sporns O. 2010. *Networks of the Brain, 1st Edition*. Cambridge, Mass: The MIT Press.
- Tantillo G, Peck KK, Arevalo-Perez J, Lyo JK, Chou JF, Young RJ, et al. 2016. Corpus callosum diffusion and language lateralization in patients with brain tumors: a DTI and fMRI study. *J Neuroimaging Off J Am Soc Neuroimaging* 26:224–231.
- Tie Y, Rigolo L, Norton IH, Huang RY, Wu W, Orringer D, et al. 2014. Defining language networks from resting-state fMRI for surgical planning—a feasibility study. *Hum Brain Mapp* 35:1018–1030.
- Ulmer JL, Krouwer HG, Mueller WM, Ugurel MS, Kocak M, Mark LP. 2003. Pseudo-reorganization of language cortical function at fMR imaging: a consequence of tumor-induced neurovascular uncoupling. *AJNR Am J Neuroradiol* 24: 213–217.
- Van Dijk KRA, Hedden T, Venkataraman A, Evans KC, Lazar SW, Buckner RL. 2010. Intrinsic functional connectivity as a tool for human connectomics: theory, properties, and optimization. *J Neurophysiol* 103:297–321.
- Villringer A, Dirnagl U. 1995. Coupling of brain activity and cerebral blood flow: basis of functional neuroimaging. *Cerebrovasc Brain Metab Rev* 7:240–276.
- Wang L, Chen D, Olson J, Ali S, Fan T, Mao H. 2012. Re-examine tumor-induced alterations in hemodynamic responses of BOLD fMRI: implications in presurgical brain mapping. *Acta Radiol* 53:802–811.
- Zetterling M, Roodakker KR, Berntsson SG, Edqvist P-H, Latini F, Landtblom A-M, et al. 2016. Extension of diffuse low-grade gliomas beyond radiological borders as shown by the coregistration of histopathological and magnetic resonance imaging data. *J Neurosurg* 1–12. DOI: 10.3171/2015.10.JNS15583.

Address correspondence to:

Arka N. Mallela

Functional MRI Laboratory

Department of Radiology

Memorial Sloan-Kettering Cancer Center

1275 York Avenue

New York, NY 10065

E-mail: amallela@mail.med.upenn.edu