Structural and Functional Imaging in Glioma Management

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The goal of glioma surgery is maximal safe resection in order to provide optimal tumor control and survival benefit to the patient. There are multiple imaging modalities beyond traditional contrast-enhanced magnetic resonance imaging (MRI) that have been incorporated into the preoperative workup of patients presenting with gliomas. The aim of these imaging modalities is to identify cortical and subcortical areas of eloquence, and their relationship to the lesion. In this article, multiple modalities are described with an emphasis on the underlying technology, clinical utilization, advantages, and disadvantages of each. functional MRI and its role in identifying hemispheric dominance and areas of language and motor are discussed. The nuances of magnetoencephalography and transcranial magnetic stimulation in localization of eloquent cortex are examined, as well as the role of diffusion tensor imaging in defining normal white matter tracts in glioma surgery. Lastly, we highlight the role of stimulated Raman spectroscopy in intraoperative histopathological diagnosis of tissue to guide tumor resection. Tumors may shift the normal arrangement of functional anatomy in the brain; thus, utilization of multiple modalities may be helpful in operative planning and patient counseling for successful surgery.

KEY WORDS: Low-grade glioma, Glioma, Imaging, Functional MRI, Magnetoencephalography, Transcranial magnetic stimulation, Diffusion tensor imaging, Stimulated Raman microscopy

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Low-grade gliomas present a surgical dilemma because of their poorly defined borders. Many studies, however, are congruent that extent of resection leads to improved patient outcome and indicate that extent of resection improves length of survival.[1-11] When the lesion is located near eloquent areas, the diffuse nature of gliomas makes it difficult to ascertain the functionality of adjacent brain, as normal cortical anatomy may not be reliably identified. Furthermore, it has been shown that in adjacent areas of damaged brain or tumor, the localization of eloquent areas may be shifted to accommodate the lesion while functionality remains preserved.[12,13] Thus, it is useful for appropriate preoperative planning to include functional imaging modalities so as to maximize the extent of resection while at the same time preserving areas of eloquence.[14-17] There is a myriad of functional neuroimaging modalities that have been developed to preoperatively elucidate eloquent cortex and white matter tracts in relation to tumor. This section discusses the methodology, use, and shortcomings of the most widely used methods of functional neuroimaging. We further discuss novel intraoperative histologic technologies that aim to discern normal tissue from residual tumor with the intent of maximizing safe surgical resection.

ABBREVIATIONS: 3D, three-dimensional; **BOLD,** blood oxygen level dependent; **CNS,** central nervous system; **DCS,** direct cortical stimulation; **DTI,** diffusion tensor imaging; **EMG,** electromyography; **FA,** fractional anisotropy; **FDi,** fiber density index; **fMRI,** functional MRI; **ICA,** Independent Component Analysis; **MD,** mean diffusivity; **MEG,** magnetoencephalography; **MEP,** motor evoked potential; **MRI,** magnetic resonance imaging; **nTMS,** navigated TMS; **rMT,** resting motor thresholds; **ROI,** region of interest; **rsfMRI,** resting state functional MRI; **SRM,** stimulated Raman spectroscopy; **TMS,** transcranial magnetic stimulation

FUNCTIONAL MAGNETIC RESONANCE IMAGING (FMRI)

Task-Based fMRI

Recognition of eloquent cortex allows the surgeon to maximize tumor resection while

limiting morbidity. Although the surgeon's knowledge of anatomy is vital, preoperative identification of eloquent cortex using structural magnetic resonance imaging (MRI) can be limited by multiple factors, including anatomic distortions (tumor), neuroplasticity (congenital abnormality),[18] and individual variability (speech localization).[19] Task-based fMRI has been used for the last 25 yr for preoperative identification of eloquent cortex.[16]

Task-based MRI uses specific tasks in order to increase focal cortical metabolism and thereby identify motor, sensory, and speech areas. As areas of neurons are activated, local oxygen metabolism is increased. There is a subsequent increase in local blood flow that increases the amount of available oxygen in the form of oxyhemoglobin relative to deoxyhemoglobin.[20,21] Diametric magnetic properties of oxy- and deoxyhemoglobin allows for T2-weighted sequences to detect changes in the oxy: deoxy ratio, known as blood oxygen level dependent (BOLD) contrast. A wide variety of specific tasks are used including finger tapping (motor), sentence completion (receptive language), and verb generation (expressive language).[22,23] Because of the subtle nature of the T2-weighted response to BOLD contrast, tasks are performed multiple times in order to produce a reliable signal. The accurate identification of eloquent cortex requires balanced postprocessing, which preserves the task-induced signal while eliminating background noise.[20]

Multiple studies have compared the accuracy of task-based MRI in patients with brain tumors against the gold standard of direct cortical stimulation (DCS).[17,24-27] Current task-based fMRI techniques using BOLD signal for identification of motor cortex have reported a sensitivity of 87.5% to 100% and specificity of 68.1% to 87.1%.[24,25] Results for language mapping have been more varied with a sensitivity of 59% to 78% and specificity of 67.7% to 97%.[24-26,28] Studies aimed at identifying expressive and receptive language areas separately show decreased fidelity in isolating Wernicke's area.[17,29]

Task-based fMRI has rapidly been adopted and is now commonly used for preoperative planning in cooperative patients with intracranial tumors (Figure 1). Although task-based fMRI has been a vital adjunct to preoperative planning, significant limitations exist. The primary criticism of the technique is that BOLD signal uses blood flow as a surrogate for neural activity. Tissue that is in the vicinity of a tumor may experience neurovascular uncoupling because of the inability to regulate perfusion. This has been identified as a source of false negatives.[30] BOLD contrast has been demonstrated to be significantly decreased in patients with high-grade glioma involving eloquent cortex.[31,32] Additional sources of error include head movement, physiological noise, and scanner-related noise.[33] Although task-based fMRI enables preoperative identification of at-risk cortex, the variable results, especially involving language, and potential for neurovascular uncoupling indicate that resection planned solely on the basis of task-based fMRI may lead to significant morbidity in some patients.

Resting-State fMRI

As mentioned in the section above, task-based fMRI is a commonly utilized technique that allows for the noninvasive identification of eloquent cortex by measurement of the neurovascular coupling. As patients perform specific tasks, changes in local perfusion are detected using BOLD signal.[20] During the use of task-based fMRI, changes in BOLD signal were also noted in patients while at rest.[34] Biswal et al[34] first reported the finding in 1995. While imaging patients at rest, they reported low-frequency fluctuations of BOLD signal within sensorimotor cortex, which had a high temporal correlation with BOLD signal in multiple other areas associated with motor function. It was determined that the changes in BOLD signal represented the functional connectivity of the brain.[34] Since that time, the technique has been used to reliably identify multiple other functional networks such as the visual, auditory, attention, language, and executive.[35]

The data acquisition time for resting-state fMRI is typically 6 to 12 min.[36,37] Data may be acquired in the awake, asleep, or anesthetized patient.[38] As opposed to task-based fMRI, resting state functional MRI (rsfMRI) data acquisition is rapid, does not require patient participation, and can be completed by standard MRI technicians. Data processing is a critical step and can be performed using multiple methods. The most common methods include Independent Component Analysis (ICA) and region of interest (ROI) or seed-based approaches. ICA is an automatic data-driven approach that will analyze BOLD contrast throughout the brain to identify all detectable resting-state networks, whereas the ROI method will only detect regions that are associated with seed region activity.[39] The seed-based rsfMRI is a manual means of placing a "seed," essentially delineating an area of interest on the MRI, to an anatomically established portion of cortex involved in motor or language processing and analyzing the data in relation to the chosen area of interest. This is reliant on healthy, nonlesional tissue, such as placing the seed on healthy Rolandic cortex. Although well-established networks, such as sensorimotor, are usually clearly identified, the detection of more complex networks, such as language,[40] is heavily influenced by the type of data processing.[37,41] Some authors have proposed the use of a seed-based approach to identify areas of interest within the motor system while reserving ICA processing for instances when pathology obscures usual anatomy or to avoid bias when trying to localize or lateralize language.[37] At this time, it is not clear which method of data processing produces the most reliable results.

Multiple studies have validated the results of rsfMRI with DCS.[42-45] Similar to the outcomes for task-based fMRI, results of language mapping with rsfMRI have been less reliable than motor mapping.[42,43] Cochereau et al[42] reported a series of 98 patients with diffuse low-grade gliomas who underwent preoperative rsfMRI with ICA detection for sensorimotor and language mapping. Of the 98 patients, 90 (92%) were found to have overlapping motor network detection with DCS, whereas only 41 (42%) were noted to have overlapping language networks

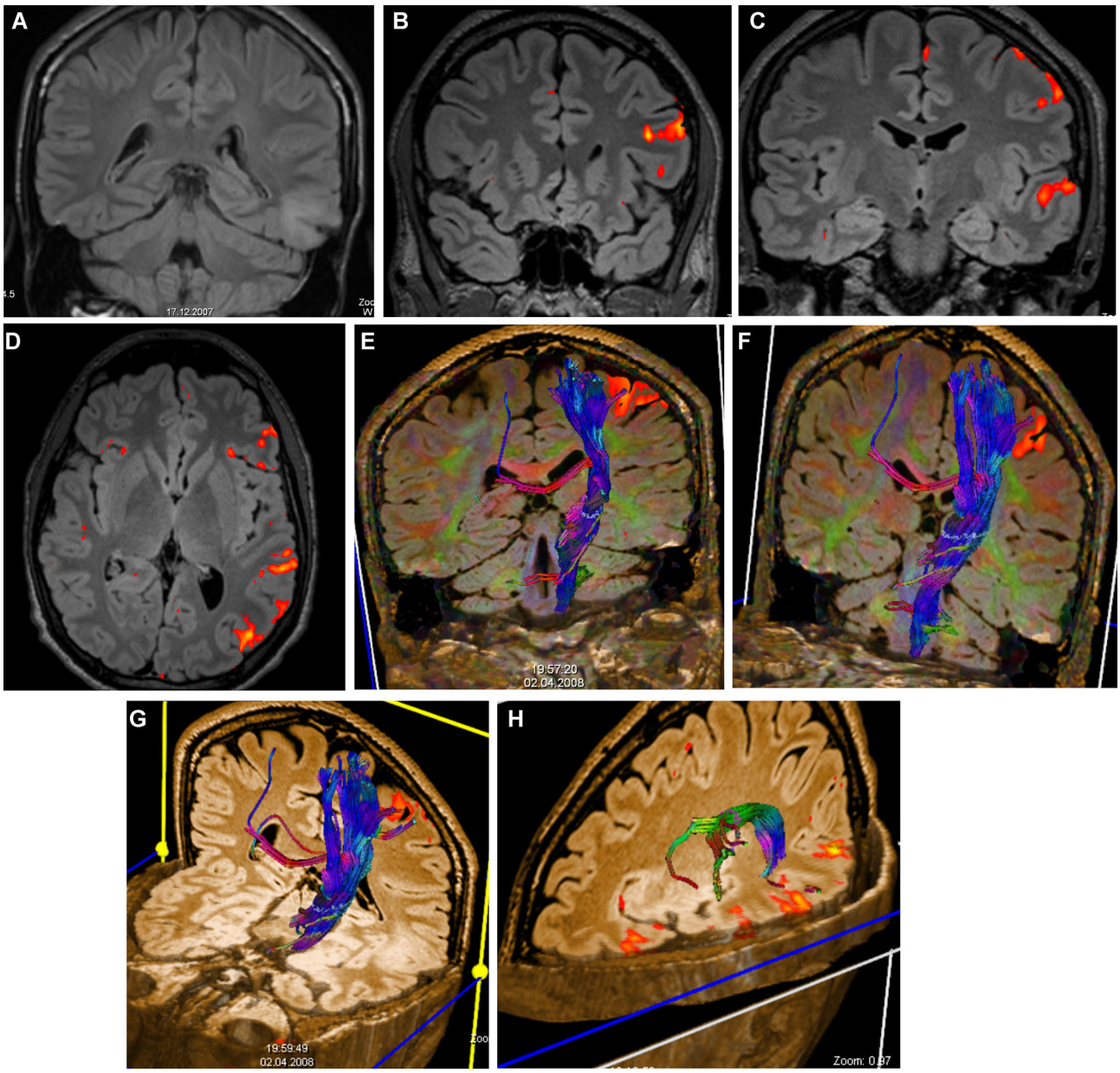

FIGURE 1. *Coronal FLAIR* **A**, *coronal* **B** *and* **C**, *and axial* **D** *fMR images and diffusion tensor images* **E-H** *are shown.* **A**, *A cortical/subcortical FLAIR signal abnormality is noted in the left posterior temporal lobe. fMRI localizes the language function to the left cerebral hemisphere in the inferior frontal gyrus (Broca's area) and superior temporal gyrus (Wernicke's area).* **B-D**, *Activation for motor cortex is also shown with right thumb tapping paradigm.* **E-G**, *DTI merged to fMRI delineates motor fibers in relation to the areas of eloquence. The superior longitudinal fasciculus, which is a large association fiber bundle for language, is shown in* **F**.

with DCS responses. A mean of 84 ± 24% of sensorimotor stimulation points were located within 5 mm from the rsfMRI map. However, a mean of 70 ± 41% of language stimulation points were located within 5 mm the rsfMRI map. This produced an overlap of resected cortex with the mean motor network of 3.1 ± 5.8% and overlap of resected cortex with the mean language map of 15 ± 17%. The authors concluded that use of rsfMRI for resection near language areas without DCS would expose the patient to considerable risks.[42]

Resting-state fMRI is a compelling technique, as it allows for the rapid identification of multiple networks without the need for patient cooperation. The technique has been

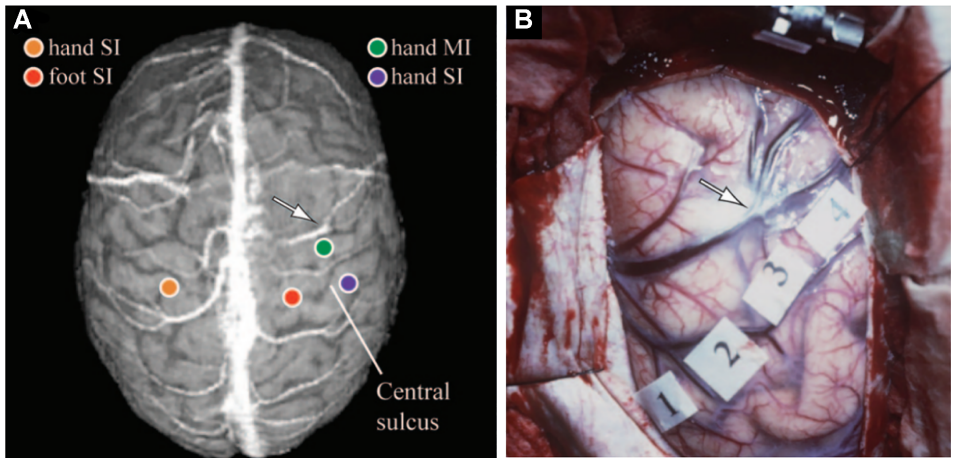

FIGURE 2. **A**, *Preoperative 3D surface renderings with superimposed veins of the brain of a patient with a right parasagittal frontal glioma. Owing to edema, the anatomic landmarks were poorly visible in this patient. Sources of responses to right and left median (hand SI) and left tibial nerve stimulation (foot SI) and source of MEG-electromyography (EMG) coherence for the left wrist extension (hand MI) are superimposed on the surface rendering.* **B**, *Intraoperative photograph showing venous structure and stimulation sites used to define the motor cortex location. The arrows in the MRI surface rendering and in the photograph indicate the same vein bifurcation. The distance between the estimated source of maximum MEG-EMG coherence and the site producing hand movement in stimulation was 6 mm. Reprinted from Mäkelä JP, Forss N, Jääskeläinen J, Kirveskari E, Korvenoja A, Paetau R. Magnetoencephalography in neurosurgery. Neurosurgery. 2006;59(3):493-511, by permission of the Congress of Neurological Surgeons.*

rapidly adapted in clinical neurosurgery for preoperative planning,[36] prognostication,[46] and intraoperative monitoring of eloquent networks.[47] Although the acquisition of data is straightforward relative to task-based studies, rsfMRI shares many of the same sources of potential error such as the neurovascular uncoupling in the presence of pathology, movement artifact, and environmental noise.[30,37,48] Additionally, it is not yet clear as to the ideal form of data processing nor the clinical implication of a resting network. Further studies are needed to correlate involvement within a resting network and the morbidity associated with its disruption in order to further instruct the surgeon in clinical application of resting-state fMRI.

MAGNETOENCEPHALOGRAPHY (MEG)

MEG is a means of recording the brain's magnetic signals, intimately related to its electrical activity. Electrochemical currents in neurons, primarily postsynaptic potentials, generate a magnetic field. These small bioelectric currents on the magnitude of pico- or femtotesla are detected by specialized superconductive sensors within the confines of an insulated magnetic environment.[12,49] Synchronized to task-based activities and coregistered to conventional MRI, MEG has been applied to localize the central sulcus, primary auditory and visual cortex, motor cortex, and language lateralization.[50] The utility of MEG has been validated for use in glioma surgery by several studies. MEG has also been shown to localize the motor cortex and central sulcus in studies with superiority to fMRI.[50-52] Furthermore, MEG merging to high-resolution MRI has advanced intraoperative neuronavigation to include eloquent areas, effectively providing "functional neuronavigation."[53,54] Resting-state MEG has also shown promise in its ability to predict postoperative outcomes. By evaluating the preoperative functional connectivity utilizing MEG and by studying the functionality of tissue surrounding tumors, the authors were able to correlate this to postsurgical outcome.[55] In a study by Ganslandt et al,[56] 119 patients with gliomas inclusive of, or near eloquent sensorimotor, visual or speech areas underwent preoperative MEG. Surgical resection was not pursued in 55 (46.2%) patients because of invasion of tumor into functional cortex as defined by MEG, whereas of the 64 patients who were deemed resective candidates by MEG, only 4 suffered postoperative neurological decline.

The major advantages of MEG are the fact that this is a noninvasive technique that does not rely on traditional radiation or the injection of contrast, which may be harmful to the patient. Furthermore, unlike other functional imaging modalities like fMRI, which has high spatial resolution, MEG does not rely on hemodynamic changes to detect increased neuronal activity, and thus has greater temporal resolution. MEG has been applied to the preoperative identification of the visual and auditory cortex, language lateralization, and the localization of the motor cortex.[57-60] MEG is a powerful, noninvasive adjunct that can be utilized for the preoperative localization of eloquent areas to aid in the planning of surgical resection of gliomas (Figure 2). Although

it is a great tool in the neurosurgeon's armamentarium for preoperative planning, MEG mapping is a financially costly and not readily accessible modality, which has limited both its clinical utility and has led to limited data in tumor resections.[14,50,55,61]

TRANSCRANIAL MAGNETIC STIMULATION

Prior studies have shown that cortical reshaping because of the presence of intraparenchymal pathology occurs and that classical locations of eloquent areas may not be applicable in these patients.[62-65] Transcranial magnetic stimulation (TMS) is based on electromagnetic induction of the underlying cortical neurons and was first developed in 1985 by Barker et al.[66] In brief, a current is generated within a figure of 8 coils extracranially creating a magnetic field that passes through the scalp, skull, and dura mater, which then generates a secondary current in the underlying cortex. This current may activate cortical interneurons, in turn leading to activation of motor neurons in the corticospinal tract (CST) leading to an increase in the amplitude of a compound muscle action potential (CMAP).[67-69] During stimulation, the patient's head is registered to a structural MRI with subsequent placement of EMG electrodes into the muscles of interest depending on tumor location or patient specific factors. Resting motor thresholds (rMT) are obtained by application of TMS while changes in location of the probe, coil tilt, and coil rotation are made. The rMT is then defined as the lowest stimulation intensity that evokes motor evoked potentials (MEPs) in at least 5/10 trials.[70] Once this is defined, stimulation is performed in areas of concern for motor activity at 110% of the rMT. A TMS hotspot is then defined as the location on the MR sequence that elicited the greatest MEP response. These "hotspots" are then plotted on structural MRI available to the surgeon intraoperatively as areas of high concern for containing underlying motor cortex or CST (Figure 3). Tarapore et al[71] performed preoperative navigated TMS (nTMS) for localization of the motor cortex and compared its accuracy with both DCS and MEG imaging. In a cohort of 24 patients who underwent craniotomy with DCS confirmation of motor cortex, the mean distance between nTMS identified motor cortex and DCS was 2.13 and 4.71 mm between TMS and MEG imaging motor sites. There were also no patients in which a motor site was found by DCS, but not nTMS.[71] A number of studies have shown that use of TMS over traditional preoperative planning may increase progression free survival, the number of resective surgical candidates, and the probability of gross total resection achieved while also decreasing postoperative neurological deficit. However, it is the surgeon's discretion with intraoperative findings, including intraoperative cortical mapping, that ultimately dictate operative course.[72-74] Advantages of this method are chiefly in its ability to provide electrophysiological data about patient specific motor areas as opposed to metabolic data via methods such as fMRI.[75-78] Krieg et al[70,74,75] compared nTMS to fMRI data and DCS and found nTMS data to be near equivalent to DCS and superior to the metabolic fMRI data. A more recent technique

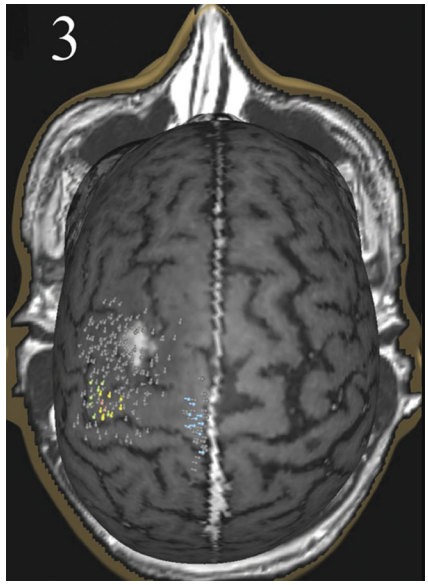

FIGURE 3. *Example of a complete navigated TMS somatotopic map. Each stimuli point is colored according to the electromyographic channel with the highest amplitude motor evoked potential output. Peeling depth of the 3D MRI was set to 25 mm to visualize the tumor with the central sulcus. Green, abductor pollicis brevis; yellow, abductor digiti minimi; pink, first dorsal interosseous; blue, tibialis anterior; gray, no response. Reprinted from Picht T, Schmidt S, Brandt S, et al.[70] Preoperative functional mapping for Rolandic brain tumor surgery: comparison of navigated transcranial magnetic stimulation to direct cortical stimulation. Neurosurgery. 2011;69(3):581-588; discussion 588, by permission of the Congress of Neurological Surgeons.*

involves combining nTMS and diffusion tensor imaging (DTI) fiber tracking by using nTMS-derived motor mapping points as seeding the ROIs for DTI. This may provide more reliable resolution of the CST in a patient that has exhibited cortical rearranging secondary to tumor formation.[50,79,80] Other advantages of nTMS over fMRI include less need for cooperation with the examination and thus a higher likelihood to be tolerated by patients.[17] Although fMRI data may be compromised by above mentioned changes associated with tumor neurovascular uncoupling, nTMS does not have this issue.[74] Disadvantages to the system include brain shift occurring after durotomy making true spatial registration inaccurate, need for experienced operators to perform the mapping preoperatively, and availability of the TMS device.[74,81]

DIFFUSION TENSOR IMAGING

Advanced MRI techniques like DTI can play a role in better delineating affected brain parenchyma.[82] DTI is a noninvasive form of diffusion weighted MRI that relies on physiological water

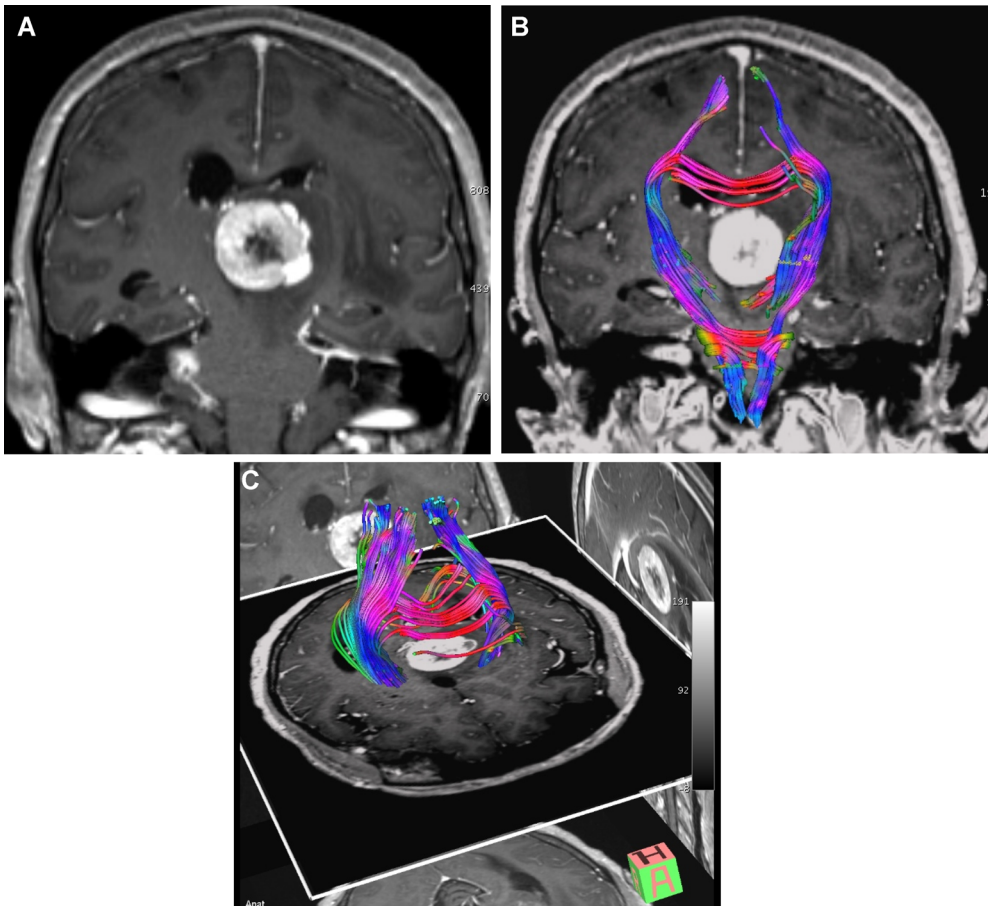

FIGURE 4. *Coronal postcontrast T1-weighted image,* **A**, *shows an enhancing centric/left gangliothalamic mass. Diffusion tensor tractography merged with postcontrast T-weighted coronal,* **B**, *and axial,* **C**, *images depict the CSTs bilaterally. Note the close proximity of the mass with the left CST.*

motion and directionality for delineation of the white matter tracts of the central nervous system (CNS).[83,84] Water diffusion is relatively unimpeded in the direction parallel to the axonal fiber orientation in white matter tracts; however, diffusion is highly hindered in the directions perpendicular to the fibers as a result of the myelin sheath. This entity represents "anisotropy," and the information is known as "the diffusion tensor," a three-dimensional (3D) ellipsoid model of water diffusion. Total 3 major eigenvectors that are orthogonal to each other represent the direction of diffusion. DTI data provides knowledge on diffusion anisotropy and fiber orientation and has 2 main metrics: fractional anisotropy (FA) and mean diffusivity (MD). Fiber tract trajectories of the CNS white matter tracts can be computed in Vivo from diffusion tensor MRI data. White matter tracts are estimated by a starting location, so-called "the seed point." The direction of propagation and moving a small distance in that direction is called tract integration. Also, tracts may be constituted and constrained by using more regions of interest. Most algorithms use the major eigenvector to estimate the trajectory of the white matter tract.[85]

Depiction of white matter tracts provides better guidance for surgical approach and resection, especially for tumors that are in or close to eloquent areas. The identity of the tract is predicted from its course and location. DTI also shows the position of the tract with respect to the tumor, eg, superior, medial, etc, allowing the surgeon to decide on the best surgical approach to the lesion. It has been demonstrated that white matter fibers are incorporated within the tumor mass, especially in low-grade tumors, and destroyed in high-grade tumors (Figures 4 and 5).[82,86,87] The use of DTI to distinguish between high-grade gliomas and metastases has also been investigated. Several studies have found mean FA values of T1 and T2 abnormalities, and the normal appearing white matter adjacent to the T2 abnormality to be lower than those in contralateral normal white matter in both

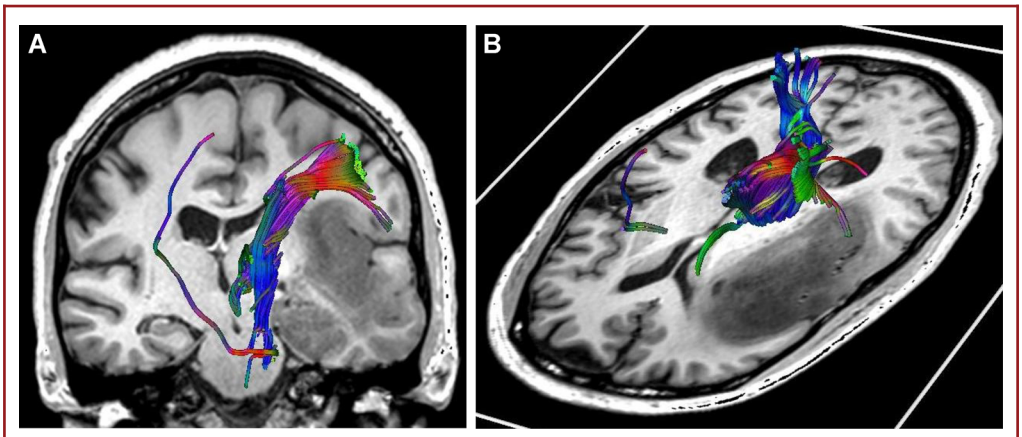

FIGURE 5. *Coronal,* **A**, *and axial,* **B**, *sections of diffusion tensor tractography depicting medial displacement of the CSTs in a patient with a left insular low-grade glioma.*

high-grade gliomas and metastases. A total of 14 studies were analyzed in a recent meta-analysis to investigate the diagnostic performance of diffusion weighted imagin and DTI for differentiating high-grade glioma from solitary brain metastasis with a wide range of individual sensitivities/specificities and only a moderate diagnostic performance.[88] Several other studies have also shown that the fiber density index (FDi) and FA values could be used to differentiate between high-grade and low-grade gliomas.[89] FDi is a qualitative measure of the density of fiber tracts, and it has been shown that FDi values are higher in low-grade gliomas compared with high-grade gliomas. Another study by Chen et al[90] demonstrated FDi ratios with FA thresholds of 0.25 that were significantly different between patients with high-grade and low-grade gliomas. On the other hand, Sinha et al[91] demonstrated that MD values can be used to differentiate normal white matter, edematous brain, and enhancing tumor margins, whereas diffusion anisotropic data added no benefit to tissue differentiation.

There are several limitations in using tractography. First, the local tract directions are sensitive to eddy currents causing misregistration of the diffusion tensor images. Ghosting because of motion and signal loss because of susceptibility variations can affect computation of the fiber tract. Because most algorithms are based on the major eigenvector, the crossing white matter pathways may not be resolved when a voxel contains nonuniformly distributed fibers, curved fibers, or 2 or more interdigitating fiber populations. A study in which 8 international institutions were tasked with tractography of the pyramidal tract in the same neurosurgical patients with a glioma near the motor cortex revealed a high rate of variability.[92] New diffusion imaging methods may be able to better resolve intersecting and crossing white matter regions more accurately. However, these methods require higher diffusion weighting (typically 3000-15 000 s/mm^2), resulting in longer acquisition times.[84]

STIMULATED RAMAN MICROSCOPY

Stimulated Raman microscopy (SRM) is a spectrographic method based on laser light scattering intrinsic to the molecular qualities of a particular tissue.[93] The current technology has been adapted as an intraoperative technique for the pathological diagnosis of normal vs abnormal tissue differentiation during tumor margin resection. SRM is based on the Raman effect, described in the early 1900s, whereby a photon scattering yields a small fraction of energy at a frequency either higher or lower than the initial photon, essentially producing a spectrum that is specific to different tissue compositions.

SRM has shown promising results in maximizing extent of resection in non-neurosurgical pathologies such as breast cancer, certain GI pathologies, and cervical neoplasias.[94-96] Tumor specimen is taken and placed on a slide, then run through SRM in the operating room, providing a real-time image that can be interpreted for markers of glioma such as nuclear atypia, hypercellularity, and microvascular proliferation. Initially validated with in Vivo animal models of glioblastoma, studies have evaluated technology ex Vivo in human studies.[97,98] In Vivo human studies have been developed utilizing a Raman-based probe in order to detect abnormal tissue with a sensitivity and specificity of greater than 90%.[99] This allows for tissue analysis around a resection cavity without the need for further resection for tissue sampling. This shows promise particularly for low-grade gliomas, in which tumor margins are more difficult to discern under typical light microscopy. Consequently, this is also the population of patients in which extent of resection correlates with survival benefit.[2]

Further in Vivo human studies are still needed to provide robust evidence for utilization of SRM during glioma surgery. The major advantages of SRM include the lack of need for dyes or injections to determine pathological brain tissue, which is likely unable to delineate normal tissue from low-grade glioma with an intact blood brain barrier.[98] Limitations of the technology include

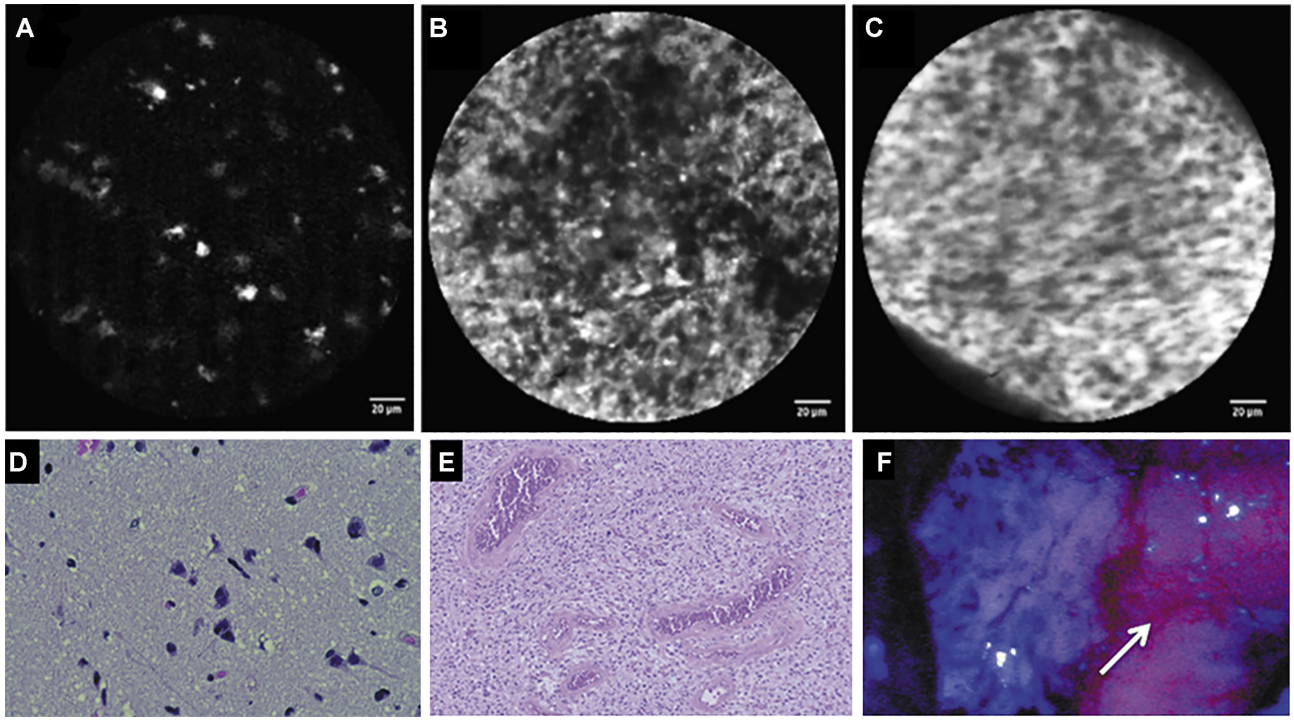

FIGURE 6. *Probe-based confocal laser endomicroscopy imaging, using 5-ALA (5-aminolevulinic acid).* **A**, *Normal cortex.* **B** *and* **C**, *Distinct patterns of solid glioblastoma tumoral tissue.* **D**, *Normal cortex histology.* **E**, *Solid glioblastoma histology.* **F**, *High rate of macroscopic fluorescence of solid glioblastoma's tumoral tissue (arrow). Reprinted from Pavlov V, Meyronet D, Meyer-Bisch V, et al.*[107] *Intraoperative probe-based confocal laser endomicroscopy in surgery and stereotactic biopsy of low-grade and high-grade gliomas: a feasibility study in humans. Neurosurgery. 2016;79(4):604-612, by permission of the Congress of Neurological Surgeons.*

the relatively small fields of view that can be examined which include a typical depth of approximately 100 μm.[97] There also exist challenges of incorporating SRM into the workflow of the operating room; however, its implementation still holds potential major promise for maximizing the extent of safe resection in glioma surgery.

CONFOCAL MICROSCOPY

For many years, the resection of gliomas has been reliant on preoperative imaging and white light microscopy. Recent advances such as that of 5-aminolevulinic acid (5-ALA) and neuronavigation have increased resection margins, a desired outcome which has been linked to improved patient outcomes.[1,7,100] However, the inherent difficulty in low-grade glioma resection remains in distinguishing normal parenchyma from tumor-invaded tissue. Confocal microscopy and variations of the technology including confocal laser endomicroscopy or confocal laser scanning microscopy, in combination with high-definition white light microscopy have allowed for a greater degree of tissue visualization at the microscopic level to distinguish normal tissue from that of gliomas.[101]

Confocal microscopy is an optical fluoresce imaging modality that utilizes a specific laser light wavelength that excites fluorophores of tissue. This excitation causes emission of photons that pass through a confocal aperture, or pin hole, that will only allow light of certain frequencies to pass through. The laser moves through the specimen, scanning different portions of the tissue. Then, software is utilized to combine a final image. With adjustment of the confocal aperture, different axial wavelengths may also be detected, giving the ability of software to produce a 3D image.[101,102] In terms of transitioning this to intraoperative use, recent advances have allowed for a miniature, probe-based, and handheld instrument capable of being utilized in Vivo, leading to the so-called endomicroscope. The technology does rely on a fluorescent agent to be used for the best accuracy, the most commonly including 5-ALA, fluorescein, indocyanine green (ICG), and methylene blue (Figure 6).[103-107]

Confocal laser endomicroscopy investigators found sensitivity of 90% and a specificity of 93% for low-grade glioma; however, this is in a small sample size of only 10 specimens.[108] Similar studies with the technology have shown 93% accuracy rates of intraoperative, in Vivo tissue analysis when compared with neuropathology review.[109] Although 5-ALA is avidly taken up in high-grade glioma, thus producing macroscopic tumor

fluorescence, this is not the case with low-grade glioma. However, fluorescence has been seen in low-grade tumors at the microscopic, cellular level when performed in conjunction with confocal microscopy, which may aid in tumor margin resection, but full results are still pending larger trials.[110] It must be noted that the utilization of this technology is dependent on being able to visualize tumor tissue, and in this respect, there is a limitation to the technology for lesions under normal cortex.

Although much larger studies are needed to show the accuracy of the technology, there is much promise in its in Vivo application for tumor margin resection. Confocal laser endomicroscopy technology is still in early stages and perhaps years away from standard of care in glioma surgery; however, the ability of the surgeon to obtain real-time intraoperative information regarding tumor margins will undoubtedly increase the extent of tumor resection. The major disadvantages of the technology include a learning curve in order to interpret the data, parameters for deciding which features constitute abnormal tissues, although some have already been proposed.[108] The handheld versions of the confocal laser endomicroscopes have a relatively low field of view, in the order of 400 μm, and may be prone to sampling errors.[106] With larger trials currently underway, confocal laser endomicroscopy shows promise as the next intraoperative tool in glioma surgery.

CONCLUSION

Functional neuroimaging is a cornerstone of preoperative evaluation for patients with any brain mass felt to be in the area of eloquent cortex. In the case of gliomas, mounting evidence shows that the functional connectivity of the brain itself may be altered, and that strictly anatomic studies may be insufficient for surgical planning. Functional imaging modalities may provide the surgeon with vital information to the location of eloquent tissue in relation to a tumor. This is useful in guiding the surgeon's operative plan and extent of resection. Even more so, it gives surgeons information to better counsel patients of operative risks. Intraoperative histologic diagnosis with the aid of SRM and confocal microscopy show promise as the next step in achieving maximal surgical resection. Although still in very early stages of utilization, these technologies hold promise to become an integral part of the neurosurgeons set of tools.

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