Potential role for magnetoencephalography in distinguishing low- and high-grade gliomas: a preliminary study with histopathological confirmation

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Gliomas are the most common form of tumor in the CNS and are exceptionally heterogeneous. Accurately characterizing gliomas, in terms of grade and type, is essential for predicting the rate of tumor progression. Histopathological grading and analysis based on biopsied tissue remains the gold standard, but non- and semi-invasive neuroimaging also plays a key role. Neuroimaging has been used to guide and optimize biopsies for several decades, but more recently molecular imaging and variants of MRI have shown promise in independently predicting glioma grade. Here we evaluated whether magnetoencephalographic (MEG) measurements of population-level physiology within the glioma space were predictive of the inherent grade of the tissue, based on definitive histopathological analyses. High-density MEG data were recorded from 11 patients who were undergoing functional mapping in preparation for resective surgery. The primary results indicated that glioma grade was positively correlated with the local amplitude of activity within the glioma space in the theta (4–7 Hz), alpha (8–14 Hz), and beta bands (14–30 Hz). Additionally, activity within the glioma was significantly elevated relative to the nonaffected homologue area in the same frequency bands. These results indicate that pathological levels of synchronization exist within the tumor space and that MEG may be a viable tool for noninvasively differentiating gliomas by their grade. Although these results should be considered preliminary and are only correlative in nature, these data suggest that MEG can potentially detect neurophysiological signatures or markers that predict the inherent grade of a glial tumor.

Keywords: glioma grade, MEG, theta, tumor histology, tumor physiology.

The incidence of primary brain tumors in the United States has increased, and the vast majority of these tumors are gliomas.1 These patients are generally diagnosed based on conventional neuroimaging (eg, MRI) and may subsequently undergo advanced imaging in an effort to identify key features of the tumor that can aid in treatment planning. Accurate characterization of the tumor, in terms of grade and type, is essential for predicting outcomes and determining appropriate therapeutic measures. Tissue histopathology remains the gold standard for glioma typing and grading, but non- and semi-invasive imaging measures also play an important role.2,3

More advanced neuroimaging techniques often utilize the inherent metabolic and/or histological features of the target tissue to potentially differentiate low- and high-grade gliomas, which is a crucial distinction for accurate treatment planning. For example, recent work has shown that diffusion-weighted imaging (DWI) and diffusion-tensor imaging (DTI) may hold substantial promise in distinguishing low- and high-grade gliomas. DTI is a relatively common procedure that provides detailed information about the course of white matter tracts, which in tumor cases has been utilized for presurgical planning and intraoperative guidance. However, several recent studies have shown that diffusion-based indices (eg, fractional anisotropy,
apparent diffusion coefficient) may also have the capacity to effectively distinguish between low- and high-grade gliomas based on the inherent tissue cellularity.3–5 In these studies, preoperative DTI-based predictions of glioma grade were compared with the postoperative histopathological diagnoses, and the results indicated that DTI could differentiate low- from high-grade with excellent specificity (87%–89%) and sensitivity (81%–92%).3–5 Another promising technique is MR perfusion, which provides quantitative and semiquantitative measurements of several different flow parameters. The most well-studied is relative cerebral blood volume (rCBV). Early studies indicated that the rCBV of tumors is strongly correlated with vascular-ity measures derived from histopathology and angiography in glioma patients.3,6,7 More recent studies have shown that rCBV is correlated with semiquantitative grading of the expression of vascular endothelial growth factor in biopsied gliomas8 and histological markers of glioma cell proliferation derived through the monoclonal-antigen MIB-1 labeling index.9 Studies using different approaches to MR perfusion imaging, such as arterial spin labeling, have also shown promise in glioma grading,10 but like all other MR perfusion measures, it still faces significant hurdles. For example, oligodendrogliomas inherently have higher rCBV values than do astrocytic tumors, which could lead to erroneous overgrading of oligodendrogliomas.11,12 Finally, there is evidence that magnetic resonance spectros-specroscopic (MRS) measures of the choline/N-acetyl aspartate (cho/NAA) ratio are correlated with cell density and cell proliferation indices from immunohistochemistry of biopsied tissue.13 However, to date, MRS has not shown a sensitivity to tumor grade that is equivalent to metrics derived from MR perfusion and diffusion-weighted methods,2,5 but the overall data also indicate that none of these MR techniques can, at least currently, accurately and consistently characterize tumor grade in individual patients.2,6,9

PET is another imaging method that has been widely employed to better characterize gliomas. The first and most widely used PET radiotracer was the glucose analogue [18-F] fluoro-deoxyglucose (FDG), and early studies showed a clear correlation between FDG uptake and tumor cell density (in biopsied tissue), overall tumor grading, and malignancy.15–17 However, larger studies later showed that greater FDG uptake was not consistent for high-grade compared with low-grade tumors and that [18-F] FDG PET was useful for diagnosing gliomas but not for tumor grading.18,19 Given these limitations, several groups have explored using radiolabeled amino acids in PET studies of gliomas. Two of the most popular amino acid labels are t-methyl-[11-C]-methionine (MET) and O-(2-[18-F] fluoroethyl)-1-tyrosine (FET; see la Fougeré20 for a review). Studies using [11-C] MET and/or [18-F] FET have shown increased amino acid uptake in 72% to 76% of low-grade and 95% to 100% of high-grade gliomas for both labels and demonstrated high sensitivity (79% positive predictive value) in differentiating nonneoplastic lesions and low-grade gliomas.18,19,21 Furthermore, a recent [18-F] FET study utilized a new type of dynamic analysis, which enabled the researchers to distinguish low- and high-grade gliomas with high diagnostic power (94% sensitivity and 100% specificity),22 and a follow-up study demonstrated that the kinetics of [18-F] FET uptake were robustly correlated with individual histopathological features.23 Such dynamic analyses of amino acid PET data may have a major impact on glioma management in the future, but much research remains before it can be used in actual clinical decision making.24

In this study, we investigate whether preoperative magnetoencephalographic (MEG) measurements of ongoing neurophysiology can inform noninvasive grading of tumor tissue in patients with gliomas. MEG noninvasively measures magnetic fields that naturally emanate from postsynaptic currents in parallel-oriented pyramidal cells of the neocortex. Thus, the technique is a direct measure of neurophysiology with millisecond temporal resolution and a spatial precision on the order of 3–5 mm in the cortex. To our knowledge, no previous electrophysiological investigations (i.e., electro-encephalography, electrocorticography, MEG, etc) have examined in vivo tumor physiology, and as a corol-lary, no studies have evaluated whether physiological parameters are in any way related to the inherent grade of a tumor. Given the high metabolic activity within tumors, neuronal (and glial) physiological parameters are almost certainly perturbed within the tumor proper and potentially in surrounding tissues. Very little is known about the neurophysiological impact that gliomas have on individual neurons and especially populations of neurons. Presumably, tumor infiltration would affect activity in all cells immediately surrounding and within the gliomic area, but whether or not such neuronal activity is modulated in any way that is predictive of glioma grade remains to be demonstrated. Thus, in this preliminary study, our goal was simply to identify a potential neurophysiological marker that varied as a function of glioma grade. Ultimately, such a marker could be invaluable for treatment planning and monitoring, as MEG measurements are entirely noninvasive. To this end, we recorded spontaneous brain activity in patients who were undergoing preoperative functional mapping in preparation for resective surgery. Our primary hypothesis was that MEG indices of local spectral amplitude, within the glioma space, would correlate with the grade of the particular glioma derived through histopathological analyses of the excised tissue.

**Methods and Materials**

**Subject Selection**

We studied 11 adults (5 females) with a glioma type of primary brain tumor who were undergoing MEG functional mapping as part of their neurosurgical workup. Mean age of patients was 35.9 years at the time of recording. Additional demographic information and histopathological summaries are provided in Table 1.
Note that 1 patient was excluded from the glioma grade correlation analyses due to incomplete clinical histology information about the type/grade of glioma. Exclusionary criteria included any medical illness affecting CNS function, neurological disorder, history of head trauma, and current substance abuse. All procedures were performed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and the standards of the University of Nebraska Medical Center’s Institutional Review Board.

**Structural MRI**

High-resolution anatomic images were acquired using a Philips Achieva 3T X-series scanner. The T1-weighted sagittal images were obtained with an 8-channel head coil using a 3D fast field echo sequence with the following parameters: field of view, 24 cm; slice thickness, 1 mm with no gap; in-plane resolution, 1.0 × 1.0 mm; sense factor, 1.5. The structural volumes were aligned parallel to the anterior and posterior commissures and were used for MEG coregistration.

**MEG Experimental Paradigm and Data Acquisition**

Once positioned inside the MEG recording chamber, each participant completed an approximately 10-min block of an awake eyes-closed-rest exercise. Patients were instructed to relax and remain awake with their eyes closed throughout the recording. With an acquisition bandwidth of 0.1–330 Hz, neuromagnetic responses were sampled continuously at 1 kHz using a Neuromag system with 306 magnetic sensors (Elekta). Using MaxFilter (v2.1.15; Elekta), MEG data from each session and subject were individually corrected for head motion, coregistered to structural MRI, and subjected to noise reduction using the signal space separation method with a temporal extension.

Prior to the MEG measurement, 4 coils were attached to the patient’s head, and the locations of these coils, together with the 3 fiducial points and scalp surface, were determined with a 3D digitizer (Fastrak 3SP0002, Polhemus Navigator Sciences). Once the subject was positioned for MEG recording, an electric current with a unique frequency label (eg, 322 Hz) was fed to each of the coils. This induced a measurable magnetic field and allowed each coil to be localized in reference to the sensors throughout the data acquisition session. Since coil locations were also known in head coordinates, all MEG measurements could be transformed into a common coordinate system. With this coordinate system (including the scalp surface points), each participant’s MEG data were coregistered with the participant’s structural T1-weighted MRI data prior to source analyses.

**MEG Source Analyses**

Following signal space separation with temporal extension and head-motion correction, the entire magnetic time series was transformed into a 29-node regional source model via inverse spatial filtering (see Fig. 1). Essentially, a 29-point grid with dual orthogonal orientations per point was constructed, and each orientation was used as an inverse spatial filter on the continuous 306-sensor time series data of a 6-min recording period per patient. After transformation into source space, the current-amplitude (nAm) time series for each of the 2 orthogonal orientations per source was divided into epochs of 4096-ms duration (4096 points). Artifact rejection was based on a threshold method supplemented with visual inspection. For each participant, artifact-free epochs were transformed into the frequency domain using Fourier analyses (ie, 4096 data points per window). Average spectra across the 6-min recording were then computed for each orientation per brain region by averaging the approximately 90 Fourier-transformed epochs. Subsequently, for each of the 29 regional sources, the amplitude per band was summed across the 2 orthogonal orientations to yield the total current-amplitude per frequency band for the particular brain region. We focused on regional sources centered in the glioma space based on areas of hypointensity on T1-weighted images and hyperintensity on T2-weighted images, as well as the homologue brain area in the nonaffected hemisphere, and examined the local spectral amplitude of neuronal activity within 7 frequency bands.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Tumor Location</th>
<th>Tumor Grade</th>
<th>Tumor Type</th>
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<tr>
<td>P01</td>
<td>38</td>
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<td>Right frontal parietal</td>
<td>II</td>
<td>Oligodendroglioma</td>
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<tr>
<td>P02</td>
<td>47</td>
<td>M</td>
<td>Left frontal temporal</td>
<td>–</td>
<td>Low-grade glioma</td>
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<td>I</td>
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<tr>
<td>P11</td>
<td>29</td>
<td>M</td>
<td>Left temporal</td>
<td>III</td>
<td>Oligodendroglioma</td>
</tr>
</tbody>
</table>
Operative Procedure and Histopathological Analysis

All patients underwent surgery. Four patients had a gross total or near total resection, 6 had subtotal resection, and 1 had open biopsy. With the exception of Patient 2, all pathology was reviewed at our institution by one of our neuropathologists. CNS tumors were identified according to current WHO type and grade classifications (see Table 1).

Statistical Analyses

Spearman’s rho correlation analyses were performed using the local spectral amplitude within the glioma space (per frequency band), and the diagnostic glioma grade was based on histological analyses. In addition, we evaluated differences in local spectral amplitude between the glioma space and the homologue brain region using a repeated-measures analysis of variance (ANOVA) with tissue type (2 levels; tumor, homologue) and frequency band (7 levels) as within-subjects factors. Significant interaction effects were followed up using appropriate \( t \)-tests. All statistical tests were 2-tailed and conducted in SPSS.

Results

Glioma-Grade Correlation Analyses

To examine the relationship between neurophysiological activity and glioma grade, we computed a series of Spearman’s rho correlation coefficients using the local spectral amplitude estimates (per band) corresponding to the center of the glioma space and the tumor grading information from the histological analyses. Note that 1 patient was excluded due to incomplete histological information. These analyses indicated that tumor grade was positively correlated with neuronal activity in the theta band (4–7 Hz; \( r(10) = 0.66, P < .05 \)), alpha band (8–14 Hz; \( r(10) = 0.744, P < .05 \)), and beta band (14–30 Hz; \( r(10) = 0.63, P < 0.05 \)). Descriptive statistics for these amplitude-by-grade values were as follows, in nAm, mean (SEM): theta: grade II = 38.3 (1.39), grade III = 67.4 (18.4), grade IV = 82.03 (28.76); alpha: grade II = 95.67 (7.88), grade III = 112.0 (9.80), grade IV = 163.73 (94.53); beta: grade II = 132.0 (4.65), grade III = 186.96 (19.94), grade IV = 273.0 (57.62).

The local amplitudes of physiological activity in the other frequency bands were not statistically related to glioma grade (see Fig. 2).

Amplitude Analyses of Gliomic and Nonaffected Homologue Area

The repeated-measures ANOVA for local spectral amplitudes revealed a main effect of tissue type \( F(1,10) = 4.98 (P < .05) \), a main effect of frequency band \( F(6,60) = 29.82 (P < .001) \), and a tissue-by-frequency interaction effect \( F(6,60) = 2.53 (P < .05) \). The main effect of tissue type indicated that across all frequency bands, local spectral amplitude was significantly stronger in the glioma space compared with the homologue brain region. We did not further evaluate the main effect of frequency, as it indicated that across both tissue types, neuronal activity was stronger in some frequency bands compared with others, which is not relevant to our primary hypothesis. Finally, the interaction term indicated that the effect of tissue type varied by frequency band; thus, we conducted follow-up testing to fully evaluate this interaction. These post hoc \( t \)-tests indicated that diagnostic activity within the glioma space was stronger in the delta (1–4 Hz; \( t(10) = 2.35, P < .05 \)), theta (4–7 Hz; \( t(10) = 2.23, P < 0.05 \)), alpha (8–14 Hz; \( t(10) = 2.33, P < 0.05 \)), and beta bands (14–30 Hz; \( t(10) = 2.21, P < 0.05 \)).
compared with that in the nonaffected homologue regions (see Fig. 3). Local spectral amplitude in the ultra-low band (∼1.0 Hz) and gamma band ranges (30–56 Hz and 64–82 Hz) did not statistically differ between the glioma space and the nonaffected homologue cortices.

Discussion

We evaluated spontaneous neurophysiological activity within the glioma space using high-density MEG in patients undergoing functional mapping in preparation for resective surgery. These data were combined with definitive histopathological analyses of glioma type and grade to decipher whether quantitative MEG spectral data can potentially be used to noninvasively distinguish glioma grade. Our primary results indicated that the local spectral amplitude of physiological activity in the theta, alpha, and beta bands within the glioma space is positively correlated with the inherent grade of the glioma. In other words, higher grade gliomas were associated with stronger neurophysiological activity.

Our other main findings indicated that the local amplitude of physiological activity in the delta, theta, alpha, and beta bands was stronger in the glioma space relative to that in the homologue brain area. Below, we discuss the implications of these findings for understanding population-level neurophysiological activity within gliomas and the overall viability of using high-density MEG to noninvasively inform tumor-grading estimates.

To begin, it is crucial to discuss the neurobiological elements that contributed to our main finding of increased physiological activity in higher grade gliomas and in pathological tissue compared with nonaffected homologue tissue, including the type of cells that underlie the increased physiological activity and the direction of the change (increased/decreased). In healthy participants, it is generally accepted that MEG not only is sensitive primarily to parallel-oriented pyramidal cells of the cortex but also measures activity in cortical interneurons and neuronal activity in deeper structures with somewhat coarser resolution.30–34 Although there is plenty of evidence that glial cells such as astrocytes are electrically active, there is no evidence that MEG is actually sensitive to such activity. Thus, we assume that the neurophysiological responses reported here reflect excitatory and perhaps inhibitory neural cells, but not glial cells. In regard to the direction of the change, we propose that the increased neurophysiological activity reflects a pathological synchronization of neurons within the glioma space. No previous studies have evaluated electrophysiological activity within infiltrative tumors, but given that infiltration is quite extensive, it is likely that the physiology is significantly disturbed. Potentially, the higher grade tumors are more destructive, and this process may cause increased cell death amongst inhibitory interneurons. If cell death were more selective to interneurons compared with pyramidal cells, this could perturb the excitatory/inhibitory balance within local neuronal networks, which would eventually lead to widespread synchronization of neurons within the tumor and surrounding tissues. Such pathological synchronization would certainly explain the current findings both of increased physiological activity in the glioma space compared with nonaffected homologue tissues and of the robust correlations we observed between the amplitude of neural activity and glioma grade. However, it must be emphasized that very little is known about the
neurophysiological impact that gliomas have on individual neurons and especially populations of neurons, thus our interpretation should be considered speculative and taken with caution. It is worth noting that the mechanism(s) underlying our key findings could be largely clarified through a series of exquisite experiments using induced tumors in an animal model.

The primary aim of the current study was to evaluate whether high-density MEG-based neurophysiological metrics have the potential to predict histopathological glioma grading results. In this regard, our main findings provided clear preliminary evidence that MEG-based methods have at least some utility in distinguishing low- and high-grade gliomas in applicable patients. However, further MEG studies are certainly warranted to confirm these preliminary findings and to examine whether specific ranges of neurophysiological activity can be derived using the current metrics or related MEG indices, which are specifically indicative of a particular grade of glioma. It is also possible, given the multidimensionality of the MEG signal, that a different marker or neurophysiological signature exists that is more specific than the metrics we examined in this preliminary study. Nevertheless, there is a tremendous interest in using newer imaging methods to assist more broadly in tumor grading and biopsy guidance in the near term, and ultimately it is hoped that these methods will be useful for assessing both progression and therapeutic response to various interventions. To date, MR-based imaging methods are not sufficient for tumor grading independent of confirmatory histological analyses following biopsy. This is unfortunate, as even though histology is the gold standard for tumor grading, biopsies can be associated with morbidity and rarely mortality. Furthermore, needle biopsies can be inconclusive or not fully accurate due to the small sample size or nonrepresentative tissue sampling. Such sampling errors can be especially problematic in this realm, as gliomas are inherently heterogeneous tumors and the sample may not be representative of the grade. Biopsy-based analyses are also ill-suited for tracking treatment responses in glioma patients undergoing radiotherapy, chemo- or other pharmacotherapies due to sampling errors, the potential risk of complications, and other, more practical problems. Understanding responses to a given treatment is essential to improving the efficacy of available interventions, but imaging-based observation methods alone are not currently capable of accurately tracking treatment responses, and this remains a relatively distant goal. Thus, new techniques are clearly needed to improve non- and semi-invasive methods for the assessment and monitoring of gliomas and other brain tumors. The recent advances of amino acid PET imaging in this arena are very promising, but further research is needed before these methods are widely applicable. Furthermore, given the short half-life of relevant radiolabels, it is unclear whether these methods will ultimately be available at treatment centers not equipped with a cyclotron (ie, the vast majority of centers). Regardless, amino acid PET imaging will be an important player in the future of advanced neuroimaging for gliomas, and the PET, MR-based, and now electrophysiological (eg, MEG) methods are in dire need of a larger-scale and more controlled clinical research effort.

Conflict of interest statement. None declared.

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References


