

Neuronal and Electrophysiological Markers of Glioma

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Abstract

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The research performed in this thesis aims to improve our understanding about one of the most malignant tumors of the human brain – glioma. From the early stages of my career I was confronted with the cruel reality of losing patients due to this devastating disease. The studies performed over the last four years involve extensive data analysis in different clinical and laboratory settings. The direct application of different analysis methods and tools in order to investigate the glioma infiltration delineation has potentially lead to direct applications of our results in the clinical setting. The overall approach of the study is based on three primary outcome measures, i.e., neuronal, electrophysiological and genetic/molecular features for distinguishing infiltrated and non-infiltrated zones within specifically peritumoral tissue (PT) and, more extensively, across the radiologically-defined boundaries of healthy, peritumoral and tumoral tissues. As such, we propose for the first time an objective demarcation and characterization of the PT and we detail how the genetic and epigenetic alterations within the tumoral and peritumoral area are linked with macroscopic functional MRI results. We also describe scale-free features (power law exponent) as well as distinct spectral features and reactivity to external stimulus in the tumoral and adjacent tissue of patients and provide novel insights in terms of glioma's electrophysiology. The insights gained from these empirical studies further improve our understanding about the pathophysiology of this disease at micro- and macroscopic scales allowing us to envisage novel management methods for patients affected by glioma.

Cristina Diana Ghinda

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Contributions

Several people contributed to this research and my specific contribution was to design the studies, acquire and analyze the data and write the manuscripts for all papers discussed in this thesis. Gene sequencing was performed by the Genetron Health Laboratory (Beijing, China). The data presented in this thesis was acquired and analyzed at three sites: Huashan Hospital (Fudan University, Shanghai, China), The Ottawa Hospital (University of Ottawa, Ottawa, Canada) and Mind, Brain Imaging and Neuroethics Unit, Institute of Mental Health Research, (University of Ottawa, Ottawa, Canada). The study was approved by the Huashan Hospital ethics board (#KY201209) and by the Ottawa Hospital Review Ethics Board.

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Chapter 1 – INTRODUCTION

This chapter will provide a summary of the current knowledge regarding glioma and present the challenge in treating patients affected by this devastating disease. Subsequently, the series of enquiries that encompass our research will be presented. Chapter two, will outline a methodological overview of the different techniques used in our research. Chapters 3-5 represent specific projects as they have been published or submitted for publishing.

1.1 Glioma – an overview

Gliomas are the most frequent primary brain tumors and include a variety of different histological tumor types and malignancy grades. The most aggressive form, glioblastoma (GBM), represents one of the top malignant tumors in humans with a 5-year relative survival of approximately 5 % (Ostrom et al., 2015; Morgan et al., 2015). Mounting evidence suggests that more extensive surgical resection is associated with longer life expectancy for both low- and high-grade gliomas (Sanai & Berger, 2008; Wen and Kesari, 2008). Neurosurgeons use a variety of adjuncts such as intraoperative magnetic resonance imaging (MRI), neuronavigation, diffusion tract imaging (DTI) and macroscopic fluorescence to help guide surgical resection (Zehri et al., 2014). Nonetheless, the distinction of healthy and tumor cells remains challenging and a maximal resection is difficult to achieve due to the infiltrative nature of brain tumors and the need to preserve eloquent brain regions (Duffau, 2016). Also, in high-grade gliomas, early recurrence occurs frequently and in 80% of cases it occurs within 2-3 cm from the original lesion (Lemee et al., 2015). The delicate functional-oncological balance that needs to be maintained for the state of art management of glioma patients suggest the pressing need for novel and refined approaches englobing functional and structural markers.

Current imaging techniques assist the surgeon to define functional brain regions, navigate accurately during the surgical resection, identify critical structures, and maximize the extent of resection while preserving function (Tarapore et al., 2012). For instance, iMRI and functional neuronavigation can help maximize tumor resection, minimize neurological deficits in patients

with gliomas involving eloquent areas, and improve the survival time (Bai et al, 2015; Ghinda et al., 2017; Schucht et al., 2015). Among these techniques, intraoperative cortical mapping represents the standard method for monitoring functional cortex as it provides direct functional information of cortical and subcortical connectivity patterns.

Although functional MRI can equally provide a non-invasive assessment of the local and diffuse alterations occurring in the setting of glioma, its results can be inaccurate as the blood oxygen level dependent (BOLD) contrast enhancement can be “reduced or missing within or near gliomas, while neural activity is not significantly reduced” (neurovascular decoupling concept) (Parney *et al.*, 2010; Giussani et al., 2010; Aubert et al, 2002). Even though such non-invasive imaging studies outline a detailed non-invasive mapping of the macroscopic functional connectome, they provide just one view of the ‘complete’ brain connectome and cannot provide the direct neuronal activity flow available through electrophysiological techniques (Smith et al., 2011; Kennedy et al., 2013). While there is no established method for tracking *in vivo* neuronal connectivity, researchers already assessed the inter-area connections *in vivo* in the human language system using the “‘cortico-cortical evoked potentials’ (CCEPs) method and suggested the existence of a broader neuronal network surrounding previously recognized core region of this area. (Matsumoto et al., 2004).

As such, intraoperative mapping of brain functions using electrical stimulation in awake patients enables to maximize tumor removal, while preserving important cortical functions. Thereby, the focus is specifically on the zone between the tumor and healthy tissue, that is, peritumoral tissue, where the likelihood of cells infiltrating is the highest. Proper diagnosis and therapy requires thus accurate identification of the infiltrated peritumoral tissue.

Therefore, the goal of our research was to investigate the electrophysiological and structural changes occurring in the setting of glioma with a multimodal approach. By translating the most recent findings from the field of neurological science to the clinical setting, we hope to provide a better understanding of the interactions between the infiltrative and dynamic growth patterns of gliomas and the brain adaptation mechanisms. The proposed framework incorporates the neuroplasticity and network reorganization occurring in the setting of gliomas within a dynamic

individualized treatment strategy for patients affected by gliomas. The concept of neuro-oncology as a brain network surgery outlines the dichotomy between the neurological and oncological risk management. The ongoing feedback from the multimodal monitoring proposed in this project has major implications in terms of the clinical management and ensuing outcomes.

1.2 Network plasticity and intra-operative mapping for personalized multimodal management of gliomas

Neurosurgical resection remains the standard of care for gliomas and the extent of resection (EOR) is one of the most important factors affecting the patients' survival and quality of life for both high and low grade gliomas (Gonda et al. 2013; Sanai and Berger 2008; Yordanova, Moritz-Gasser, and Duffau 2011; Chaichana et al. 2014). The diffuse low-grade gliomas (DLGG) portray a distinct clinical and radiological behaviour and display particular gene expression signatures. DLGG is thought to represent a chronic invasive lesion that migrates along the white matter pathways, and eventually undergoes malignant transformation leading ultimately to death (Hugues Duffau and Taillandier 2015).

The concept of individualized surgery in neuro-oncological treatment of glial tumors is based on the goal of achieving a maximal tumor resection without inducing new neurological deficits. Analogously, for tumors located in proximity to critical functional areas the use of intraoperative cortical and subcortical electro stimulation mapping (IEM) during awake craniotomy evolved over time and allows a substantial increase in the survival and quality of life of patients (De Witt Hamer et al. 2012; Mandonnet, Sarubbo, and Duffau 2017).

The joint efforts of neuroscientists, researchers and clinicians have provided an unprecedented ability to precisely localize lesions and to assess the human brain function at the microscopic, mesoscopic and macroscopic scales (Mosayebi et al. 2012). The resultant array of invasive and noninvasive measures allowed surgeons to push the boundaries of safe surgical resection with subsequently improved clinical outcomes. As depicted by the extensive work performed by several research groups, the concepts of brain connectome and brain plasticity represent promising notions that advanced the neurosurgical treatments available for neurosurgical patients affected by DLGG.

A dynamic concept: tumor growth and functional neuroplasticity

The survival benefit associated with an increased EOR has been demonstrated for both high and low grade gliomas; however, such oncological benefits need to take into account the risks of inducing neurological deficits. Although it is acknowledged that in DLGG, tumor infiltration follows the white matter tracts beyond the boundaries visualized on standard neuroimaging techniques, current treatment thresholds are still based on static radiological perceived boundaries. For instance, conventional radiotherapy protocols target a 2 cm conventional Euclidean distance around the macroscopically visible tumor, without taking into account the infiltrative and dynamic growth patterns of the lesion, thus equally radiating “non-cancerous brain tissue that could not only cause neurological deficits but also restrict the residual plasticity potential while leaving alive cancerous cells in other areas” (Mosayebi et al. 2012). Also, tumor cell dissemination and heterogeneity represent important aspects that should be taken into account in order to improve the medical and surgical therapeutic regimens. Computational models attempt to simulate the functional consequences associated with brain tumor growth by incorporating the tumor induced plastic compensatory mechanism along with the structural and biological heterogeneity of gliomas (Szaliszno et al. 2013). Furthermore, there are promising future perspectives for performing in vivo and in vitro models that assess the biological and pathological characteristics of different populations of tumor cells as well as their microenvironment.

Cerebral plasticity represents the “continuous process allowing short-term, middle-term and long-term remodeling of neuronal synaptic maps, to optimize the functioning of brain networks” (Hugues Duffau 2005). The concept of adult neuroplasticity exemplifies the strong interplay between the cortex and other structures provided by the myriad of cortical and subcortical connections. The underlying mechanisms for this functional reorganization and brain plasticity are not fully elucidated, and multiple theories have been proposed such as modulation of synaptic efficacy, neurogenesis, cortical hyperexcitability, redistribution, unmasking of latent networks and establishment of new functional connections (H. Duffau 2001; Castellanos et al. 2011; J. Martino et al. 2009; Desmurget, Bonnetblanc, and Duffau 2007). Although mounting evidence depict functional reorganization in the setting of a surgical intervention, the concept of brain plasticity in the context of DLGG is still controversial (Zadeh et al. 2015). Our current understanding of the morphological, biochemical and connectivity changes occurring in the setting of a tumour is still

in its infancy and long-term large multicenter studies incorporating longitudinal multimodal investigations will likely allow us to gather more objective evidence and improve our understanding of the underlying mechanisms.

One of the main proposed mechanisms of adult plasticity reposes on the connectome concept where the brain processing relies on “dynamic large-scale, parallel subcircuits able to interact and to compensate themselves following cerebral injury” (De Benedictis and Duffau 2011; Hugues Duffau 2014). As depicted by the recent advancements achieved in terms of brain plasticity, the disease may also induce changes in large-scale functional connectivity that should be taken into account by the surgical approach (Douw et al. 2008). As such, DLGG infiltrating classical ‘Broca’ and ‘Wernicke’ areas in the dominant left hemisphere can be resected with no functional consequences given the complex organization of language and the network reorganization occurring in the setting of a slowly growing tumor (H. Duffau 2014). Thus, understanding the underlying neuromodulation principles governing the neurosynaptic networks could lead to new methods for functional restoration (Mandonnet and Duffau 2014; H. Duffau 2014; Hugues Duffau, Moritz-Gasser, and Mandonnet 2014; Herbet et al. 2014, 2016). Furthermore, as brain plasticity is equally involved in the development of long-lasting recurrent seizures, understanding brain reorganization mechanisms also has important clinical implications for controlling surgically refractory seizures (Ji et al. 2015).

This approach facilitates the concepts of “functional neurooncology” and of “preventive glioma surgery” in order to achieve earlier and more complete resections, while giving the patients the opportunity to enjoy a normal life. Consequently, understanding the individual organization of the cortical and subcortical connectivity is essential to optimize the risk–benefit ratio of glioma surgery (H. Duffau 2013). Prominent experts in this field suggest an integration of the conceptual achievements in the neuroscience, neuroimaging and genetic fields, in order to create a holistic personalized treatment strategy incorporating “the course of this chronic disease, reaction brain remapping, and onco-functional modulation elicited by serial treatments” (Hugues Duffau and Taillandier 2015).

Connectomics and glioma surgery

Functional connectivity (FC) is a measure used to express the degree of communication between brain areas and thus to describe brain networks (Reijneveld et al. 2007). The concept of the brain

connectome depicts the dynamical structural and functional neural networks that form at multiple spatial and temporal scales (Smith et al. 2013a). While it is possible to portray structural networks delineating anatomical connectivity with deterministic tractography-derived fiber tracts (Gong et al. 2009), ‘functional networks’ are derived from statistical estimates of time series data such as resting-state functional MRI (fMRI) (van den Heuvel and Sporns 2013). Although such non-invasive imaging studies outline a detailed non-invasive mapping of the macroscopic functional connectome, it provides just one view of the ‘complete’ brain connectome and cannot provide the direct neuronal activity flow available through electrophysiological techniques (Smith et al. 2013b; Kennedy, Knoblauch, and Toroczkai 2013).

Brain tumors alter the normal structural and functional connectivity of the brain, consequently impacting the normal functioning of the brain. Altered FC in patients with brain tumors affects not only the tumor area, but also other brain areas, as demonstrated through different imaging modalities (Bartolomei et al. 2006; H. Zhang et al. 2016; Osswald et al. 2015; Harris et al. 2014; Maesawa et al. 2015; Esposito et al. 2012). For instance, changes in resting state networks (RSNs) in patients with tumors localized in the left hemisphere, were observed in the contralateral side and correlated with alterations in some cognitive functions even before the onset of major symptoms (Maesawa et al. 2016). Intrinsic FC measures can also predict surgical outcome, and thereby could “provide information regarding the residual presence of function and also could define the extent of brain tumor invasion that may not be evident on structural MRI” (J. Martino et al. 2011).

As described by De Benedictis and Duffau, the classical “topological” representation ought to be replaced by a “hodotopical” framework which takes into account the changes occurring in the large-scale networks of the brain (De Benedictis and Duffau 2011). Only by acknowledging the complex cortico-subcortical network of the brain, the clinicians could further understand and take into account the dynamical neural processes occurring at distinct spatial and temporal scales (Sporns 2011). The functional connectome framework refined our understanding of functional localization as evidenced through the contemporary concepts of language organization, namely that neuronal groups participate as components of a network allowing reorganization and recruitment of parallel circuits in the setting of injury (Hugues Duffau 2015; Matsumoto et al. 2004; Ding et al. 2015; Babajani-Feremi et al. 2016).

Considering glioma surgery as “brain networks surgery” has led not only to a dramatic decrease of permanent neurologic impairment (<2% in series using intraoperative cortico-subcortical mapping) but also to improvement of higher-order functions such as working memory, neurocognitive functions as well as emotions and behavior, as evidenced by postoperative neuropsychological assessments following surgery (Hugues Duffau 2012). Therefore, the concept of neuro-oncology as a brain network surgery has major implications in terms of the clinical management and ensuing outcomes, as indexed by the increased survival and quality of life of patients managed using such an approach.

Awake craniotomy and intraoperative mapping

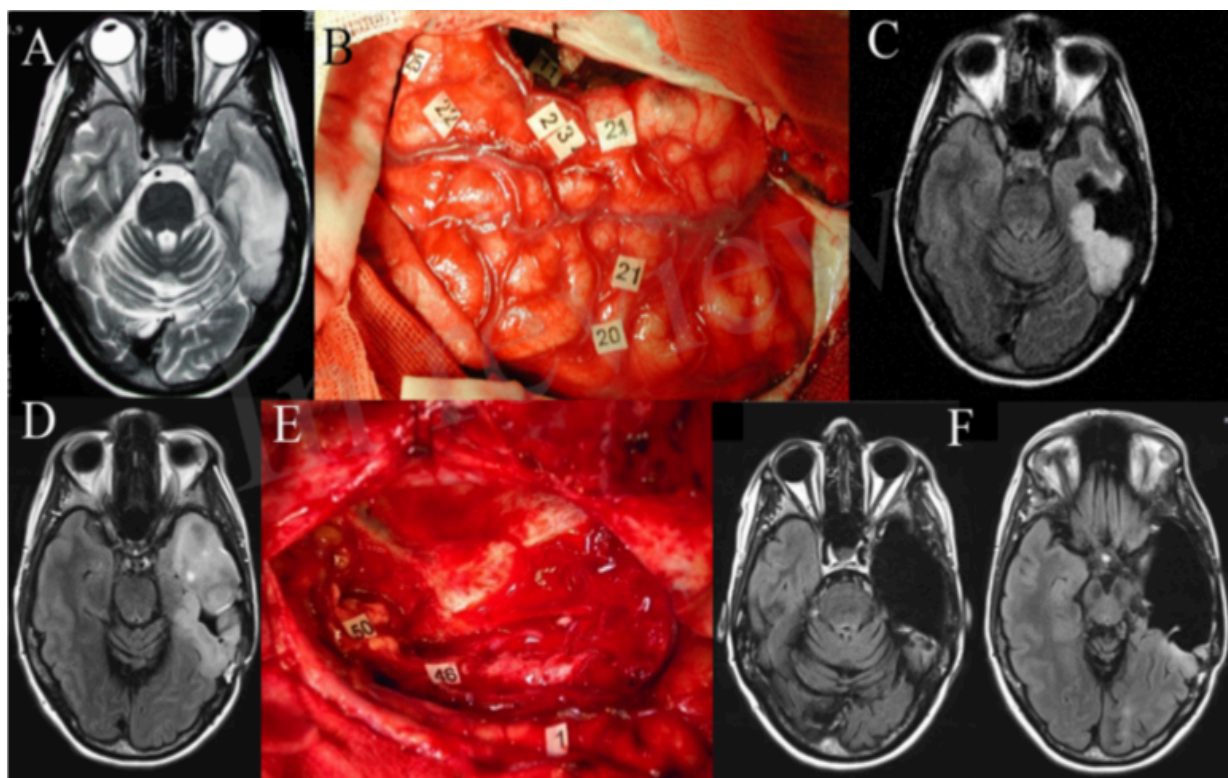
Although the art and science of brain mapping was once the purview of epilepsy surgeons, the use of this technique in the neuro-oncological field had seen an exponential increase over the last decades. Proper choice and execution of brain mapping techniques has improved the precision and safety of the surgical treatment for some of the most challenging cases and can currently allow a more radical surgical resection than indicated by presumed pre-operative functional localization. This entails an optimization of the intraoperative tests selection based on the functional cortico-subcortical networks expected to be encountered as well as on the specific preoperative neurological and neuropsychological assessments of each patient (De Witte et al. 2015; Coello et al. 2013; Teixidor et al. 2007).

Although many promising brain mapping techniques are currently being refined, the large inter-individual differences between healthy and diseased brain preclude the ability to reliably identify standard imaging-based biomarkers for functional connectomics (Zuo et al. 2014; Hugues Duffau 2017). As such, cortical and subcortical mapping via direct electrical stimulation continues to remain the most reliable approach for accurate localization of highly functional centers specific to each patient; the usefulness of this technique being described even for children (Balogun et al. 2014). Furthermore, the continuous assessment of cognitive and neurophysiological parameters provides the neurosurgeon with immediate feedback on the impact of his/her intervention.

The concept of “eloquent” cortex depends on the view that, although all cortical areas are “capable of being engaged in useful function, some brain regions are clearly more critical than others” (Boiling and Olivier 2016), causing some degree of functional decline if resected or disconnected. This framework shift has direct implications in the clinical practice as the presumed eloquence

represents a modifiable risk factor for survival (Jakola et al. 2012; Hugues Duffau 2013a). Although a detailed knowledge of both cortical and subcortical anatomical structures represents the cornerstone of neurosurgery, understanding the underlying functional correlations provide the fine details of the relationship between the lesion to be managed and the healthy brain (Campero et al. 2014). Figure 1.1 shows an illustrative case depicting the importance of performing intra-operative mapping of cortical and subcortical fibers in a patient with a left temporal DLGG. As portrayed, the respect of functional boundaries during the first surgery allowed the patient to enjoy a normal life for ten years whereas the language network reorganization occurring in the setting of a slowly growing tumor allowed subsequent resection of tumoral tissue at sites where functionality prevented tumour resection initially (Figure 1.1).

Figure 1.1 Example of the epileptic seizure history for a patient with a left temporal diffuse low-grade glioma.



Axial FLAIR-weighted MRI (A) showing a left temporal DLGG in a 36-year-old patient who presented with isolated seizures and no neurological deficits. Intraoperative photograph during the first awake surgery (B), after resection was performed according to individual functional boundaries. Stimulation mapping demonstrated the persistence of eloquent cortical areas in the temporal lobe (tags 22, 23, 24, 25) as well

as subcortical fibers (tag 11, corresponding to the inferior longitudinal fascicle) still critical for language function. Postoperative axial FLAIR-weighted MRI (C) revealing a partial resection, with a posterior residual tumor voluntarily left for functional reasons. The diagnosis of DLGG was confirmed and the patient resumed a normal familial, social and professional life. Ten years later, epileptic seizures recurred concomitantly with an imaging progression as demonstrated on the axial FLAIR-weighted MRI (D). Reoperation was proposed to the patient. Intraoperative photograph (E) during the second awake surgery, after resection was performed according to the new individual functional boundaries. Electroocortical stimulation mapping revealed brain reorganization, allowing the achievement of a significantly wider resection compared to the first surgery. Of note, at the subcortical level, stimulation of the left inferior fronto-occipital fascicle (IFOF) (46 and 50) elicited semantic paraphasia when stimulated at the end of surgery. Thus, resection of the anterior part of the inferior longitudinal fascicle was possible given the compensation provided by the direct ventral pathway represented by the left IFOF. Postoperative axial FLAIR-weighted MRI (F) performed three months after the second surgery showing a complete resection, made possible due to mechanisms of neuroplasticity, in a patient who returned to a normal life with no permanent neurological deficit (from Ghinda and Duffau, 2017).

Despite the fact that the precise influence on the electrophysiological state of brain's networks and the biophysical modelling of the electrode-tissue interface is not well elucidated (Mitchel S. Berger and Rostomily 1997), direct electrical stimulation represents a highly reliable and reproducible technique (Mandonnet, Winkler, and Duffau 2010; Keller et al. 2014; Desmurget et al. 2013; Borchers et al. 2012; Hugues Duffau et al. 2005). IEM has equally allowed to increase the quality of life for patients affected by a glioma in the non-dominant hemisphere by testing functions such as spatial awareness (de Schotten et al. 2005) or even mentalizing (Herbet et al. 2015) to avoid injuring the networks involved in those functions. Furthermore, IEM during awake craniotomy allows the unique opportunity to assess and validate the anatomo-functional connectivity for multimodal systems such as sensorimotor, language, visuo-spatial and sociocognitive systems (Hugues Duffau 2015) providing a real-time understanding of the individual organization of both cortical epicenters and subcortical connectivity (Hugues Duffau 2011).

Several manuscripts provide a comprehensive overview of the methods and technical nuances proposed for a maximal safe resection during awake brain tumor surgery (Dziedzic and Bernstein 2014; Shawn L. Hervey-Jumper et al. 2015; S. L. Hervey-Jumper and Berger 2015). There is increasing evidence that this technique allows to improve the outcomes by maximizing the extent of resection while preserving functional cortex in both low and high grade gliomas (Brown et al. 2013; Chacko et al. 2013; Yordanova, Moritz-Gasser, and Duffau 2011; Sanai and Berger 2008). Other benefits associated with this technique are shorter hospital stay, less blood loss, shorter operative time, reduced pain and anxiety, cost effectiveness as well as lower complications and

morbidity (Milian, Tatagiba, and Feigl 2014; Paldor et al. 2016). Nonetheless, careful pre-operative planning by a dedicated multidisciplinary team with an informed patient remains an important pre-requisite for a successful awake craniotomy (Balogun et al. 2014).

Conclusion

The paradigm shift encouraging the translation of the most recent findings in the field of neurological science to the clinical setting allowed a better understanding of the interactions between the infiltrative and dynamic growth patterns of DLGG and brain adaptation mechanisms (such as neuroplasticity and network reorganization). Using multimodal imaging studies and different neurophysiological tools does not take the place of a meticulous surgical technique and an extensive knowledge of the functional-structural anatomy in order to protect the cortical and subcortical functional connectivity. The concept of awake craniotomy as a brain network surgery allows neurosurgeons to adequately assess the dichotomy between the neurological and oncological risk management. It also highlights the delicate functional-oncological balance that needs to be maintained, as it will ultimately reflect on the quality of life and overall survival rate of the patients. A joint multidisciplinary approach where the emerging advancements from different fields are integrated in clinical practice in a personalized dynamic approach using ongoing feedback from clinical-radiological monitoring could provide more effective treatment options for patients affected by DLGG as already demonstrated by the increased survival and quality of life of patients treated using such a treatment strategy.

Article 1. Network Plasticity and Intraoperative Mapping for Personalized Multimodal Management of Diffuse Low-Grade Gliomas

1.3 Objectives and Hypothesis

The overall approach of the study was to investigate the peritumoral area delineation by multimodal imaging. We formulated three objectives that are based on the *three primary outcome measures*, i.e., *neuronal*, *electrophysiological* and *genetic/molecular*, for distinguishing peritumoral tissue (PT) in relation to the radiologically-defined boundaries of the tumoral tissue. Table 1 summarizes the detailed hypotheses based on our data.

Table 1.1 Objectives and hypotheses of investigating markers for distinguishing between infiltrated and non-infiltrated peritumoral tissue (PT) as distinguished from both tumor tissue (TT) and healthy tissue (HT).

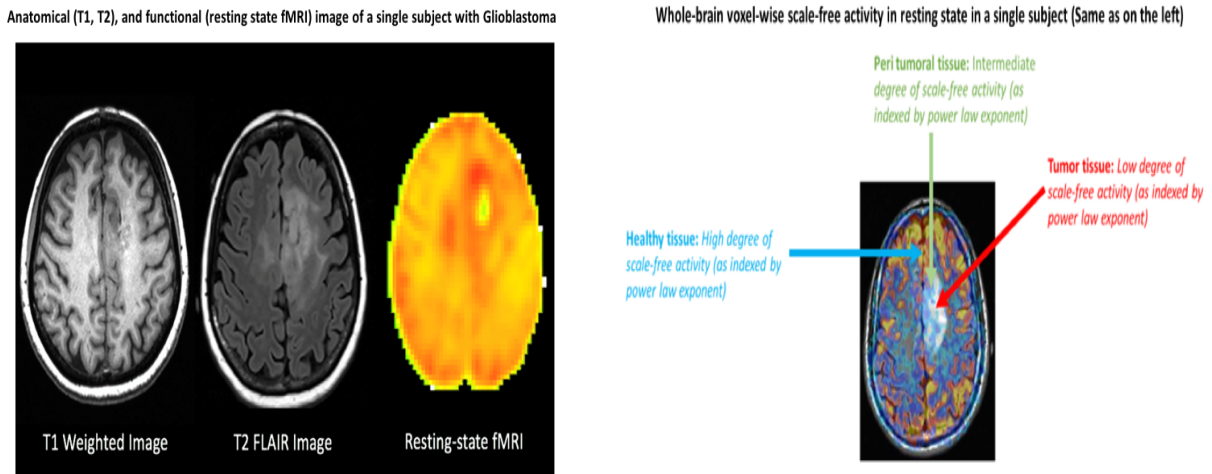
Objectives/Means	Prior Evidence	Hypothesis	Results
First Objective: Neuronal markers - fMRI resting state	Changes in functional connectivity in TT, no data available for distinguishing infiltrated PT from non-infiltrated PT and HT	Infiltrated PT show distinct changes that could be compared with TT and HT	Changes in mean BOLD signal are observed in PT as distinguished from TT and microscopical HT. Changes in temporal features (scale-free activity) are equally observed (Figure 1.2)
Second Objective: Electrophysiological markers ECoG resting state	Previous studies showed power increase in slow (1-4Hz) and decrease in faster (5-40Hz) frequencies in brain tumor (which mostly included glioma)	Power increase in delta (1-4Hz) and decrease in beta (13-30Hz) and gamma (30-40Hz) frequency in infiltrated PT (and TT) as distinguished from non-infiltrated PT and HT	Power increase in delta (1-4Hz) and decrease in beta (13-30Hz) and gamma (30-40Hz) frequency in TT while PT shows intermediate values between TT and HT (Figure 1.3)
ECoG task-evoked activity	No data available for ECoG marker of PT in comparison to TT and HT	Decrease in alpha (8-13Hz) and beta (13-30Hz) pre-stimulus activity and absent/decreased reduction in TTV in TT and infiltrated PT	Decrease in alpha (8-13Hz) and beta (13-30Hz) pre-stimulus activity and absent/reduced reduction in TTV in TT and infiltrated PT (Figure 1.4)
Third Objective: Genetic and molecular markers of peritumoral tissue	No specific study concentrated only on PT zone specifically; no data available for GABA in TT when compared to PT and HT	Different GABA related gene mutations are present in infiltrated PT and TT as distinguished from non-infiltrated PT and HT in patients presenting with seizures.	We found significant differences in the genome and transcriptome between TT and HT, which allowed for a robust classification of the degree of infiltration of the PT (Figure 1.5)

Abbreviations: trial-to-trial variability (TTV), blood-oxygenation level dependent (BOLD), γ -aminobutyric acid (GABA).

The first objective is to investigate neuronal markers of infiltrated tissue within PT specifically across the radiologically-defined macroscopic boundaries of TT and HT in glioma on the regional and network level using fMRI. The literature (D. C. Ghinda et al. 2018) shows abnormal resting state functional connectivity in TT, but without demarcating it from PT. Our own pilot data extend these observations by demonstrating that temporal measures of resting state neural activity like scale-free activity (and neuronal variability; as successfully used in psychiatric disorders (M. Martino et al. 2016)) can well demarcate PT and, more specifically, distinguish infiltrated and non-infiltrated zones within PT (as distinguished from TT and/or HT) (Figure 1.2 below). We therefore

hypothesize that temporal markers of neuronal activity in resting state fMRI like scale-free activity (Z. Huang et al. 2014, 2017) and blood-oxygenation level dependent (BOLD) signal are abnormal on a voxel-based gradient ranging from TT over infiltrated to non-infiltrated PT to higher values in HT (see Figure 1.2 below).

Figure 1.2 Resting state functional MRI in a patient with glioma

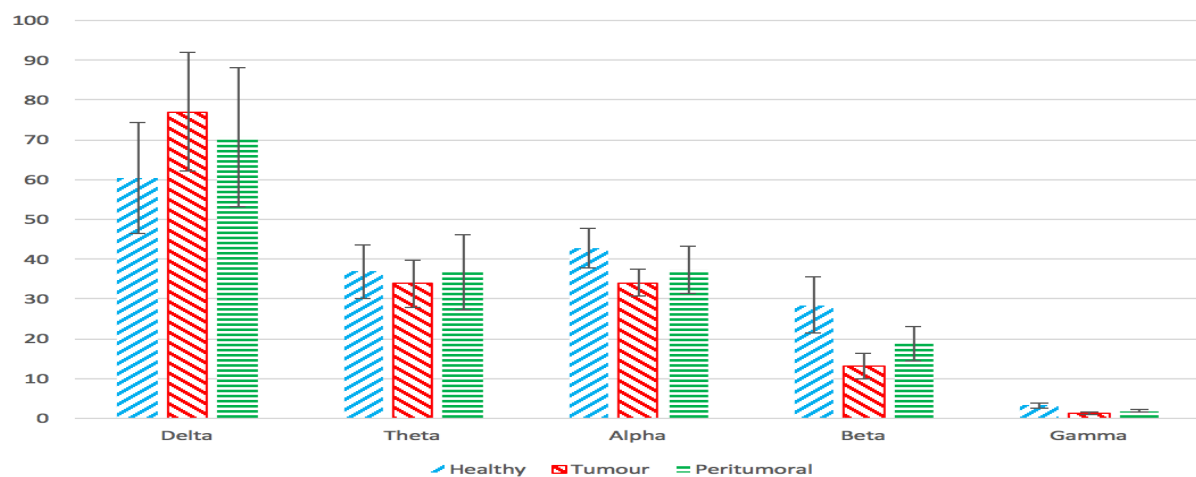


LEFT: Single subject with GBM shown in T-1 and T2 anatomical images (first and second left) and functional MRI image (resting state fMRI, right). The figure clearly shows the tumor demarcation in the resting state fMRI image. **RIGHT:** same subject now with whole-brain voxel-wise measurement of scale-free activity (as indexed by power law exponent); the figure clearly shows a gradient in scale-free activity from tumor tissue (strong white) over peritumoral (weak white/bluish) to healthy (blue/yellow) tissue: the more blue and ultimately yellow, the higher the degree of scale-free activity (as measured by power law exponent; see Huang et al. 2014, 2017 for method of measuring power law exponent in fMRI), and the more healthy the tissue.

The second objective is to investigate electrophysiological markers of infiltrated tissue across the radiologically-defined macroscopic boundaries of TT and HT in glioma on the regional level using electrocorticography (ECoG). The literature only shows few exploratory ECoG studies in brain tumor which demonstrated shifts towards slower frequencies (delta) (and decrease in faster frequencies like beta and gamma) in glioma patients (Boussen et al. 2016). This was replicated and extended in our data in a larger sample that shows analogous power changes in TT (increased slower/delta: 1-4Hz and decreased faster/beta: 13-30Hz, gamma: 30-40Hz frequencies) when compared to HT while PT has an intermediate feature (Figure 1.3). We therefore hypothesize that resting state power is abnormally shifted towards slower frequencies (like delta) with concurrent

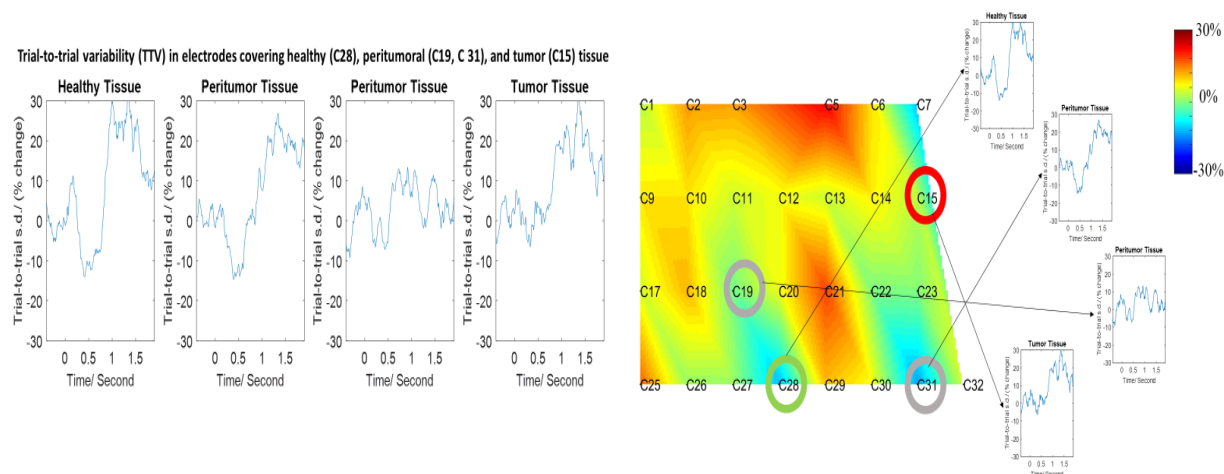
decrease in faster (beta and gamma) frequencies in infiltrated tissue within infiltrated tissue of specifically PT (and TT) whereas such pattern cannot be observed in non-infiltrated tissue as in both non-infiltrated PT and HT. Resting state ECoG is complemented by task-evoked stimulation. Our data shows that specifically the trial-to-trial variability (TTV) in electrodes covering PT may show values that are either similar to the ones in TT or HT – this, as we suppose, allows distinguishing infiltrated (PT = TT) and non-infiltrated (PT = HT) tissues within PT (Figure 1.4). We therefore hypothesize that pre-stimulus power spectrum and post-stimulus TTV allow for distinguishing infiltrated and non-infiltrated tissue within PT specifically; such demarcation can serve to guide the exact location for subsequent biopsy for verifying the tumoral infiltration.

Figure 1.3 ECoG data on the power spectrum in resting state activity in the different tissues of patients with glioma



The figure shows the power spectrum for the three types of tissue in the resting state (eyes closed, 5min) in patients with glioma. The tumor tissue (red bars) show differences are mainly in the faster frequencies that show decreased power in faster frequencies like beta and gamma (see on the very right of the figure). Healthy tissue (blue bars) shows stronger power in faster frequencies while peritumoral tissue (green bars) shows intermediate values.

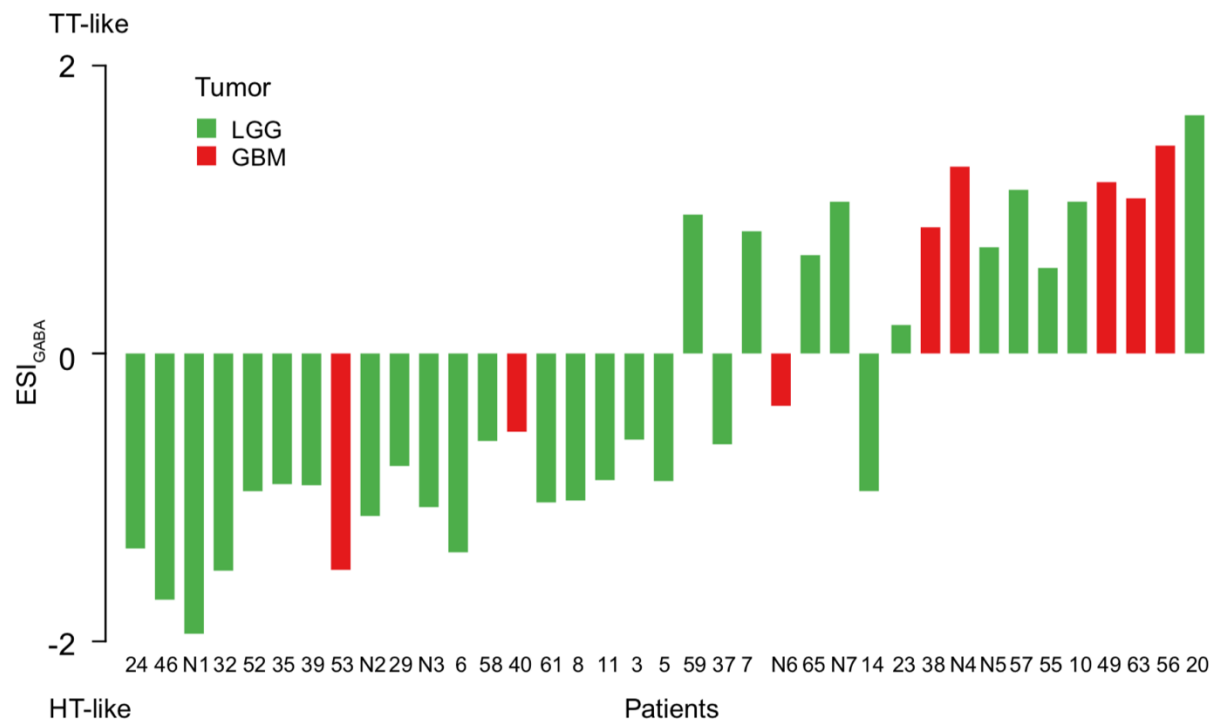
Figure 1.4 ECoG data from task-evoked activity (listening to patient's own and other matched name) in a single subject



LEFT: the trial-to-trial variability (TTV) after stimulus onset; the data show that the healthy tissue (left in panel) shows TTV reduction 400-600ms after stimulus onset while the tumor tissue (right) does not. Importantly, one peritumoral electrode shows TTV reduction (like healthy tissue) (middle left) while another does not (like tumor tissue) (middle right). **RIGHT:** distribution map for all electrodes in the same subject with the electrodes from the left panel marked with their respective TTV; the map shows that the two peritumoral electrodes from the left panel stand in different spatial location to the tumor electrode. This figure shows the feasibility of TTV serving as marker for distinguishing different peritumoral tissues, e.g., infiltrated (no TTV reduction) and non-infiltrated (TTV reduction).

The third objective is to identify genome wide alterations and epigenetic changes occurring in the setting of glioma with specific focus on the peritumoral area and its relevance to tumour-related epilepsy development. Based on previous reports, we also discovered distinct alterations in the inhibitory pathways as indexed by GABA related genes (Figure 1.5).

Figure 1.5 Genetic results



The peritumoral tissue was classified as TT-like/HT-like if the expression pattern of γ -aminobutyric acid ($GABA$)_A receptors genes and the classification of PT was in accordance with those inferred from differential expressed genes (P -value = 5×10^{-7} , one tailed binomial test).

Chapter 2 – TECHNIQUES

In this chapter, the different aspects of the techniques used to acquire and analyze the data are described.

2.1 ECoG IN GLIOMA

2.1.1 Current state

Cortical strip electrodes and direct cortical and subcortical stimulation are used to localize eloquent regions and ensure the integrity of cortical and subcortical tracts. Until recently, this has been achieved primarily through electrocortical stimulation mapping (CSM), during which a weak electrical current is used to stimulate the cortex and localize the eloquent area. Although this procedure is still considered to be the gold standard for predicting post-operative functional impairments (De Witt Hamer et al., 2012; Roux et al., 2003), CSM is time consuming and can cause electrically-induced seizures that can endanger the patient and prevent completion of the brain mapping (Bauer et al., 2013). Given the limitations of CSM, other functional mapping approaches have been developed, including high gamma electrocorticography (hgECoG), functional MRI, and transcranial magnetic stimulation (TMS) (Crone et al., 2006; Picht et al., 2013).

Electrophysiological recordings (e.g., EEG, ECoG, and MEG) are potentially capable of identifying the cortical regions activated during different stages of task performance while avoiding the CSM-related complications. Such techniques could equally delineate changes of brain's bioelectric activity in the setting of a localized pathology such as glioma. ECoG or intracranial EEG, is a type of electrophysiological monitoring that uses electrodes placed directly on the exposed surface of the brain to record electrical activity. ECoG signals are thought to be composed of synchronized postsynaptic potentials, and the spatial and temporal resolution are much higher than EEG where the electrical signals must be conducted through the skull.

Besides facilitating the localization of the ictal onset zone, intraoperative ECoG is used in the

surgical management of patients with medically refractory epilepsy to guide the extent of surgical resection and for functional mapping of speech, sensory or motor areas (Gallentine & Mikati, 2009). Local task-specific changes of the high gamma oscillations' power have been shown to be a reliable marker of local task-related cortical activation and have provided promising results for functional mapping (Kamada et al., 2014; Cervenka et al., 2013; Crone et al., 2006), also reproducible in realtime (Gupta et al., 2014). Functional mapping through ECoG has several advantages over CSM, most notably the elimination of the risk of producing seizures and a reduction of the time required for functional mapping. Several investigators have utilized hgECoG changes during language tasks to localize eloquent cortex and have reported good concordance between hgECoG and CSM (Sinai et al., 2005; Wang et al., 2016; Bauer et al., 2013; Cervenka et al., 2011; Wu et al., 2010; Babajani-Feremi et al., 2016; Miller et al., 2011; Genetti et al., 2015; Arya et al., 2015; Greenlee et al., 2014; Kubanek et al., 2013).

Furthermore, although most ECoG studies focus on seizure assessment or brain-computer interface (BCI) experiments, a relatively non-explored domain represents ECoG use for assessment of electrophysiological changes occurring during tumour infiltration. In a systematic review performed on this subject, we found only nine articles using ECoG signals in the glioma patients for tumour delineation assessment (see Chapter 3 for details).

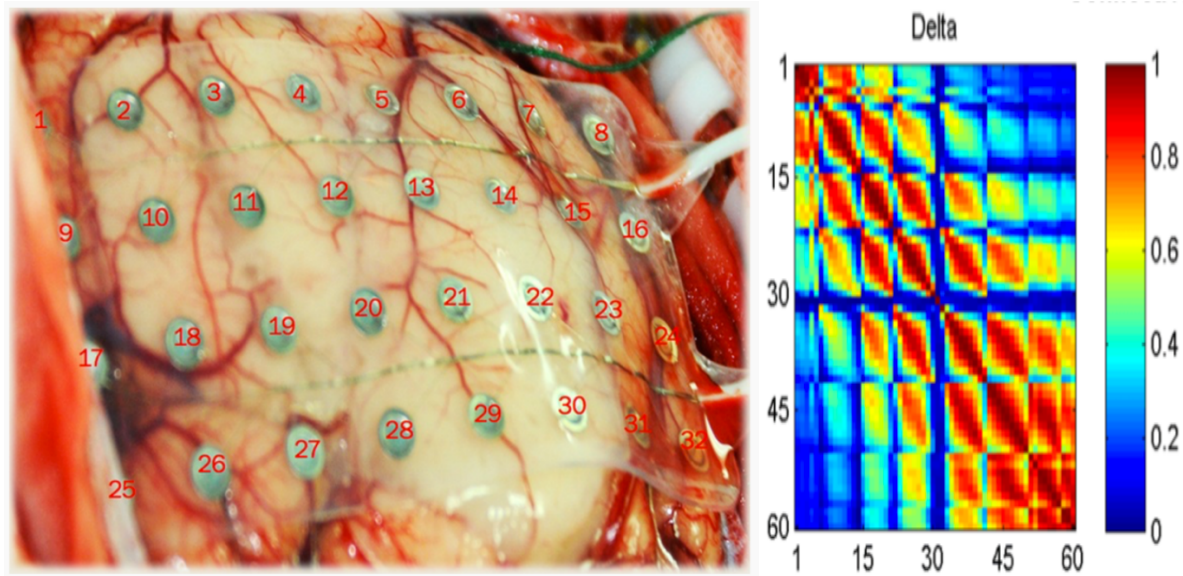
Different studies suggest that gliomas have a diffuse infiltration displaying a connectivity pattern at the cellular level. In vivo imaging of glioma cell's membrane tube development over time revealed that the microtube-connected tumour cells create a multicellular anatomical network that serve as routes for brain invasion, proliferation, and communication over long distances (Osswald et al., 2015, Osswald et al., 2016). ECoG could allow us to understand the electrophysiological changes occurring at the mesoscopic scale following tumoral infiltration while equally allowing us to delineate the functional connectivity that needs to be preserved during the surgical resection.

2.1.2 Implications

Utilizing ECoG as a novel technique to portray distinct tumoral electrophysiological features could allow the development of a new method for intra-operative tumour mapping (Figure 2.1). This technique could equally be utilized for delineation of the cortical and subcortical connectivity

changes occurring in the setting of glioma allowing a more precise and individualized management of these patients.

Figure 2.1 Electrocorticography (ECoG) grid used intra-operatively and corresponding 2D connectivity matrix view displaying the coherence between all channel pairs for the Delta frequency.



2.2 RESTING STATE MRI IN GLIOMA

2.2.1 Current state

Resting state functional MRI (rs-fMRI) measure the spontaneous, low-frequency neuronal oscillations of the brain. These networks can be measured indirectly by rs-fMRI as initially demonstrated by Biswal and his colleagues (Biswal et al., 1995). While task-based fMRI assesses the response elicited in a particular brain area by a specific task, rs-fMRI can be used to investigate the functional connectivity (FC) of different brain areas. FC is a measure used to express the degree of communication between brain areas and thus to describe brain networks and the related neuroplasticity (Reijneveld et al., 2007). One of the main proposed mechanisms of adult plasticity repose on the connectome concept where the brain processing relies on “dynamic large-scale, parallel subcircuits able to interact and to compensate themselves following cerebral injury” (De Benedictis and Duffau, 2011, Duffau, 2014). While it is possible to portray structural networks

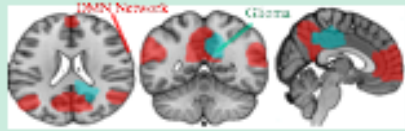
delineating anatomical connectivity with deterministic tractography-derived fiber tracts (Gong et al., 2009), ‘functional networks’ are derived from statistical estimates of time series data (van den Heuvel and Sporns, 2013).

The utility of task-based MRI has been explored by multiple research teams (Barone et al, 2014; Clarkson et al., 2008; Morrison et al, 2016; Szaflarski et al., 2017) and is currently increasingly employed in the clinical practice to attempt a pre-operative non-invasive localization of a critical functional area (Kosteniuk et al., 2016; Azar et al., 2015). Nonetheless, task-based fMRI is mainly used to localize eloquent functional areas (i.e., sensorimotor, language) while overlooking other issues such as precise tumour localization and large-scale effective connectivity changes.

Over the last decade, resting-state MRI has emerged as a simple and short technique (time scan in the order of minutes) based on spontaneous task-independent fluctuations of the blood-oxygenation level dependent (BOLD) signal (Northoff et al., 2014; Biswal et al., 2010; Greicius et al., 2009). In a systematic review (Ghinda et al., 2017) assessing the use of rs-fMRI for glioma patients, 43 studies attempted to use this technique for this patient population and most of them aimed to delineate functional areas as a surrogate to task-based MRI (Table 2.1).

Brain tumors alter the normal structural and functional connectivity of the brain, consequently impacting the normal functioning of the brain (see Figure 1.2 above for example of the structural and functional MRI data). Distorted FC in patients with brain tumors involve not only the tumor area, but also other brain areas, as demonstrated through different imaging modalities (Bartolemei et al., 2006, Esposito et al., 2012, Maesawa et al., 2015, Harris et al., 2014, Zhang et al., 2016). For instance, in patients with tumors localized in the left hemisphere, changes in resting state networks in the contralateral side correlated with alterations in cognitive functions before the onset of major symptoms (Maesawa et al., 2015).

Table 2.1 Functional networks changes in glioma patients as identified with resting-state fMRI.

Default Mode Network	Sensorimotor Network	Language Network (LN)
		
Decreased FC in the DMN (Mallela et al., 2016, Esposito et al., 2012, Maesawa et al., 2015) with connectivity changes also occurring in the contralateral side (Maesawa et al., 2015)	Decreased inter-hemispheric FC (Otten et al., 2012, Niu et al., 2014, Mallela et al., 2016, Zhang et al., 2016)	FC is reduced within seed regions of the LN especially in the left TPJ node (Briganti et al., 2012)
DMN lateralization to the hemisphere contralateral to low-grade gliomas (Esposito et al., 2012)	Decreased FC on the tumour side versus the unaffected side (Manglore et al., 2013)	
DMN connectivity in the left side of the brain may be more fragile to insults by lesions (Ghumman et al., 2016)	FC is displaced but preserved in the patients without epilepsy while it is decreased or absent in the patients with epilepsy (Poliakov et al., 2012)	
In the dominant hemisphere, the parietal lobe tumours have a more impaired DMN compared with tumours located in the frontal lobe (Harris et al., 2014)		
FC is displaced but preserved in the patients without epilepsy while it is decreased or absent in the patients with epilepsy (Poliakov et al., 2012)		

Intrinsic FC measures can also predict surgical outcome, and thereby could “provide information regarding the residual presence of function and also could define the extent of brain tumor invasion that may not be evident on structural MRI” (Martino et al., 2011). Nonetheless, although rs-fMRI could outline a detailed noninvasive mapping of the macroscopic functional connectome, it provides just one view of the ‘complete’ brain connectome and cannot provide the direct neuronal activity flow available through electrophysiological techniques (Smith et al., 2013, Kennedy et al., 2013).

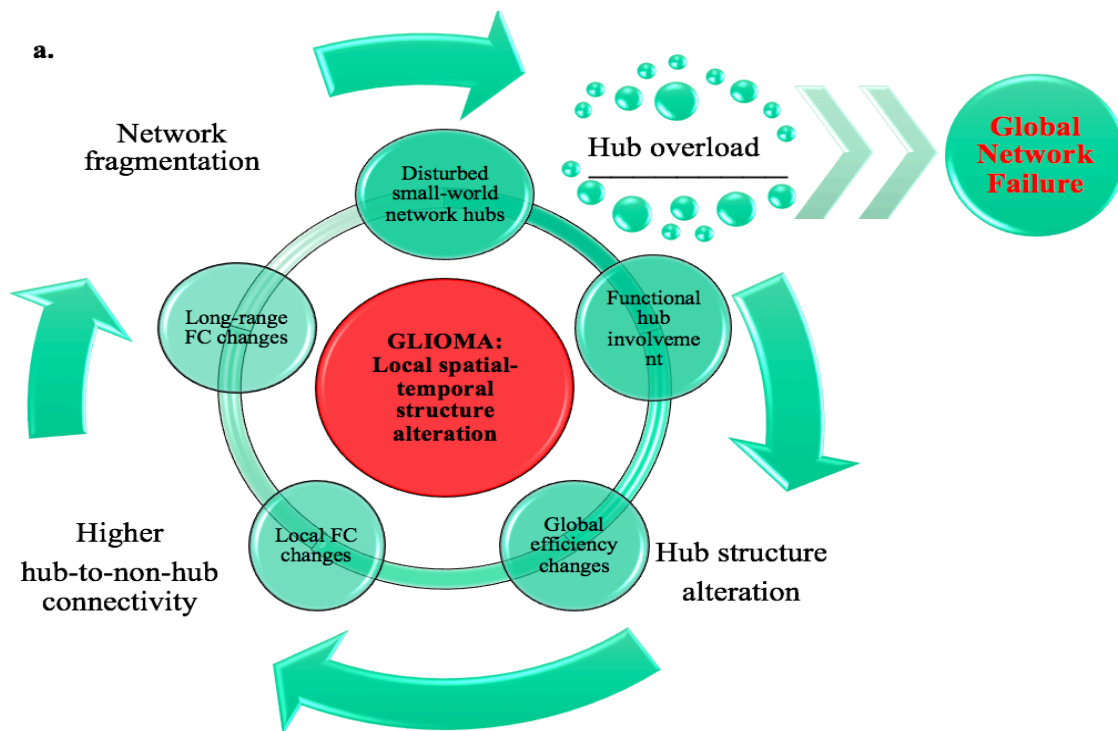
2.2.2 Implications

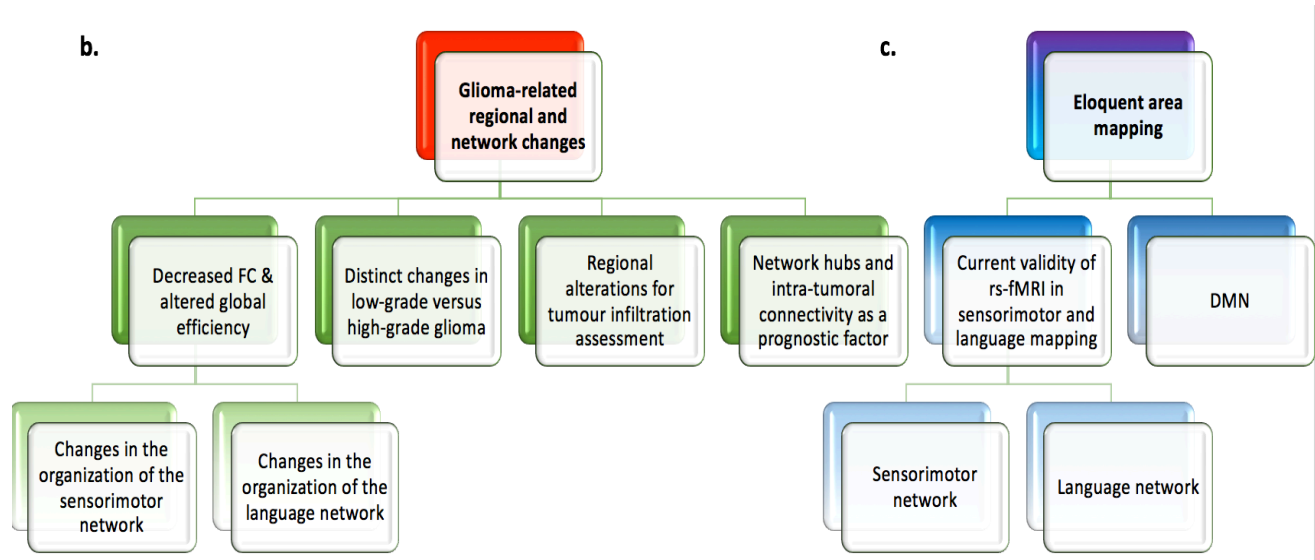
Capturing the characteristics of the large-scale cortico-subcortical dynamical networks forming the human connectome could thus allow us to understand the physiology and functional consequences of glioma within this framework. While the structural connectivity typically reflects the white matter fiber bundles and thus the structural integrity of the brain, FC refers to the “temporal correlations or statistical dependences between observed neurophysiologic events in spatially remote brain areas” (Zhijiang Wang et al. 2015). Combining the structural-functional

relationship with the clinical symptomatology allows a dynamic assimilation of the physical topological constraints with the functional network patterns across both local and global scales (Ziyi Wang et al. 2016). A theoretical model for the diffuse network changes observed in the setting of gliomas is illustrated in Figure 2.2.

Identifying the functional and structural large-scale brain network disruptions occurring in the setting of gliomas represents a critical step allowing us to understand the glioma's pathophysiology. Using resting-state fMRI for assessment of the changes occurring in the setting of glioma in conjunction with the electrophysiological modalities mentioned previously could overcome the limitations of spatial coverage with electrophysiological measures (subdural grids, cortical stimulation). A complete review and comprehensive assessment of the underlying mechanism in glioma with the use of rs-fMRI along with a proposed pathophysiology is detailed in Article 2 in Appendix.

Figure 2.2 a. Theoretical model for the diffuse connectivity changes observed in glioma patients. b. Schematic overview of the glioma-related regional and network changes. c. Schematic overview of the rs-fMRI eloquent area mapping.





Article 2. How much is enough—Can resting state fMRI provide a demarcation for neurosurgical resection in glioma?

2.3 GENETIC AND EPIGENETIC MARKERS IN GLIOMA

2.3.1 Current state

Recent advances in the genetic field significantly improved the diagnostic criteria as well as the prognostic relevance of the available genetic markers for patients with gliomas. As evidenced in the updated 2016 edition of World Health Organization (WHO), the new classification attempts to incorporate new clinical, prognostic, and genetic data largely derived from gene expression analysis and next-generation sequencing. Also, the Cancer Genome Atlas (TCGA) and other groups have recently classified gliomas into gene expression-based subtypes (TCGA, 2015; Phillips et al. 2006; TCGA 2008; Verhaak et al. 2010). On the other hand, epigenetic alterations in brain tumors include “CpG island hypermethylation associated with tumor suppressor gene silencing, gene-specific hypomethylation associated with aberrant gene activation, and genome-wide hypomethylation potentially leading to loss of imprinting, chromosomal instability, and cellular hyperproliferation” (Nagarajan et Costello, 2009; Kondo et al., 2014; Jones and Baker, 2014).

The exact origin of gliomas is still unknown, however it is thought to represent a complex phenomenon involving both genetic and epigenetic alterations with a suspected cellular origin from a neural stem cell or an oligodendrocyte precursor cell (Darlix et al., 2016; Lei et al., 2011; Riemenschneider et al., 2010). While multiple studies assessed the presence of tumorigenic stem cells in high-grade lesions, the occurrence of those cells has equally been reported in patients harboring LGG (Bourkoula et al., 2014). These cells are highly resistant to conventional chemotherapeutic drugs and could equally mediate tumor recurrence following radiation therapy (Tamura et al., 2013; Murat et al., 2008; Bao et al., 2006).

2.3.2 Implications

Further research concentrating on this aspect could allow a more precise delineation of the genetic and epigenetic profile associated with infiltrating peri-tumoral tissue in respect to healthy and tumoral tissue. Providing more specific markers of tumour related epilepsy could have a significant impact in the quality of life of patients suffering from glioma and glioma related epilepsy as particular alterations could predict tumor response to therapy, aggressiveness and patient survival.

Chapter 3 - Awake Craniotomy in Glioma

3.1 Combined intraoperative electrophysiology and awake craniotomy

As mentioned in the previous chapters, the current paradigm shift considers glioma management in a comprehensive perspective that takes into account the intricate connectivity of the cerebral networks. This allowed significant improvement in the outcome of patients with lesions previously considered inoperable. The article below summarizes the current theoretical framework integrating the adult human brain plasticity and functional reorganization within a dynamic individualized treatment strategy for patients affected by diffuse low-grade gliomas.

This clinical study aimed to assess the clinical efficiency of combined awake craniotomy with 3-Tesla intraoperative MRI (iMRI)–guided resection of gliomas adjacent to eloquent cortex. It also sought to explore the contribution of iMRI to surgeons’ learning process of maximal safe resection of gliomas. We demonstrated that combined awake craniotomy and iMRI is a safe and efficient technique allowing maximal safe resection of eloquent area gliomas with possible subsequent OS and PFS benefits. Although there is a learning curve for applying this technique, it can also improve the surgeon’s ability to perform eloquent glioma surgery.

3.1.1 Introduction

The natural history of brain gliomas has been extensively studied. Multiple studies have demonstrated the benefits of radical surgery in terms of overall outcome and recurrence-free survival (Capelle et al. 2013; G. Evren Keles, Anderson, and Berger 1999; G. E. Keles, Lamborn, and Berger 2001; Marko et al. 2014; Sanai and Berger 2009, 2008; Barone, Lawrie, and Hart 2014). Although there is currently a lack of Class I evidence, these studies suggest a survival benefit derived from maximal resection for both low-grade gliomas (LGG) and high-grade gliomas (HGG). Current technological improvements, such as 5-aminolevulinic acid fluorescence and intraoperative MRI (iMRI), circumvent the brain shift that occurs during the surgical procedure. They also enhance the rate of complete tumor resection by providing surgeons with updated information, allowing them to precisely localize tumor remnants intraoperatively and guide their resection accordingly (Liang and Schulder 2012; Maldaun et al.

2014; Nimsky, Ganslandt, and Fahlbusch 2006; Senft et al. 2010; Wu et al. 2014). Specifically, the application of iMRI for patients with glioma continues to expand because it can enable maximal resection while maintaining the integrity of uninvolved brain tissue (Liang and Schulder 2012; J.-F. Lu et al. 2013; Wu et al. 2014). Nonetheless, as depicted in a recent Cochrane Review, first-level evidence for the clinical applications of such expensive technologies is still lacking and there is no clear answer with regard to the overall benefit in terms of survival and quality of life (QOL) (Barone, Lawrie, and Hart 2014). A prospective, randomized, triple-blinded study is currently being performed at our center in an attempt to answer these critical questions (Wu et al. 2014).

Although the extent of resection (EOR) represents a crucial factor in terms of clinical outcome, the ultimate goal of the surgery is a maximally safe resection. As such, radical surgery of infiltrative glioma might compromise the patient's QOL if the procedure causes new neurological sequelae. Currently, the functional and electrophysiological mapping remains the gold standard for identifying and preserving eloquent structures (De Witt Hamer et al. 2012; Hugues Duffau 2016).

The potential role and benefit of combining iMRI and awake craniotomy have been discussed in few studies to date, with no clear results in terms of the clinical benefit obtained by the use of iMRI-guided resection during awake craniotomies (J. Lu et al. 2013; Nabavi et al. 2008; Leuthardt et al. 2011). Our group has previously demonstrated the safety of combining awake functional mapping with high-field (3-T) iMRI for the resection of gliomas located in proximity to language areas (J. Lu et al. 2013). Herein, we present the outcome of a consecutive series of 106 patients with eloquent gliomas who underwent an awake craniotomy in an iMRI-integrated neurosurgical suite.

Besides describing the clinical and radiological outcomes of the patients, we equally attempted to identify the prognosticators of progression-free survival (PFS) and overall survival (OS). To our knowledge, no other study has analyzed those factors. We equally analyzed the associated learning process and delineated the value of using this technique between the two different groups: HGG and LGG. To our knowledge, this represents the largest case series reported to date and provides further insights into the potential benefits and pitfalls of this combined approach.

3.1.2 Methods

Patient Selection

All cases of cerebral supratentorial glioma resections performed in the iMRI suite between January 2011 and June 2015 were identified from our prospectively maintained database. From those, patients who underwent an awake craniotomy with iMRI guidance were included in our study. In all of the awake cases, the tumor location was in proximity to eloquent brain regions such as receptive and expressive speech areas in the dominant hemisphere and motor or sensory cortex. The study was approved by Huashan Hospital's institutional review board.

Preoperative Evaluation

Clinical variables included age, sex, symptoms, and signs at initial presentation and Karnofsky Performance Scale score. Prior to each procedure, the attending surgeon performed a complete neurological assessment and the language function was assessed in detail, as described in a previous study performed at our center (J. Lu et al. 2013). The language function was assessed using the Aphasia Quotient score (spontaneous speech, comprehension, repetition, and naming).

Radiological data included the size and location of the tumor and the hemisphere involved (dichotomized into dominant and nondominant). The tumor location was classified into frontal, temporal, parietal, and insular lobes. Tumor infiltration and/or proximity of presumed eloquent cortex was also recorded and was classified in motor and language areas (such as supplementary motor area, precentral gyrus/central gyrus/internal capsule, and dominant hemisphere perisylvian language areas [superior temporal, inferior frontal, and inferior parietal areas]). All preoperative brain images were obtained in the diagnostic room of an iMRI-integrated neurosurgical suite using a 3-T scanner (MAGNETOM Verio 3.0 T, Siemens AG) one day prior to the procedure. The imaging protocol included a FLAIR sequence or contrast-enhanced 3D magnetization-prepared rapid-gradient echo sequence as well as diffusion tensor imaging (DTI). A blood oxygen level-dependent (BOLD) functional MRI was also performed preoperatively for all patients. A postprocessing workstation (Syngo MultiModality Workplace, Siemens AG) was used to reconstruct and merge the motor and language pathways and activation areas. The 3D reconstructed series were used intraoperatively.

Postoperative Neurological Evaluation

The clinical outcome was recorded prospectively by a certified senior neurosurgeon (J-S. W.). Serial clinical evaluations were performed at 1 month, 3 months, and 6 months after the initial surgery and thereafter on a yearly basis. Standard adjuvant treatment consisting of radiotherapy and/or chemotherapy was prescribed in all patients with HGG and inpatients with LGG with no total resection or with risk factors according to published clinical guidelines (Brem et al. 2005, 2011).

To analyze the neurological morbidity and functional outcome, we defined a short-term worsening language and motor neurological function as any new-onset or worsening function occurring within one month after the surgical intervention. Given the retrospective nature of the study, we attempted to distinguish the deficits directly related to the surgical intervention versus those that were related to tumor recurrence or treatment side effects. As such, we classified the patients as having a permanent deficit if their best neurological function recorded during the period between the third postoperative month and the end of the follow-up was worse than preoperatively. The percentage of worsening language and motor function was calculated from the total number of patients tested for that specific function.

Volumetric Analysis

Board-certified neuroradiologists blinded to the patient outcome performed the pre-, intra-, and postoperative volumetric assessment for all patients, using 3D magnetization-prepared rapid-gradient echo images with gadolinium for contrast-enhancing gliomas and T2-weighted FLAIR images for non-enhancing gliomas. For all cases, the final iMRI scan was either obtained immediately in the iMRI suite while the patient was still under anesthesia or within 72 hours after the procedure if further resections were performed with no iMRI confirmation (Figure 3.1). All pre-, intra-, and postoperative tumor segmentations were performed manually on a Macintosh platform using the OsiriX image processing software (Pixmeo) (Rosset, Spadola, and Ratib 2004). The volumes of original lesions or subsequent remnants were measured (Figures 3.2 and 3.3). The EOR was calculated as follows: (preoperative tumor volume – postoperative tumor volume)/preoperative tumor volume (Senft et al. 2010).

End Points

The primary end point was the EOR, and the secondary endpoints were PFS, OS, and surgery-related morbidity. Gross-total resection (GTR) was defined as the complete disappearance of all enhancing lesions (T1-weighted) and the complete disappearance of all T2-weighted FLAIR abnormalities for nonenhancing lesions. The interval between the date of surgery and the date of death or the date of last follow-up was used as the follow-up period for the OS analysis. Similarly, the follow-up duration for the PFS analysis was defined as the interval between the date of surgery and the date on which the first evidence of radiological progression was detected (defined as an increase in FLAIR/T2 abnormality or new contrast enhancement) or the date of the last known MR image without evidence of disease progression. A subgroup analysis was also performed according to the LGG (WHO Grade II) and HGG (WHO Grades III and IV) subgroups (Louis et al. 2007).

The findings of positive intraoperative functional cortical mapping were also recorded and classified as motor, verbal, or both. Because the results of subcortical mapping were not consistently recorded, they were not analyzed in this study. The learning curve was assessed after dividing the cohort into Group A (first 27 months) and Group B (last 26 months).

Surgical Intervention and iMRI

All procedures using iMRI were performed according to the technique described in a previously published study performed at our institution (J. Lu et al. 2013). This entails a novel minimally draping technique combined with a monitored anesthesia care approach. The detailed anesthesia technique was described in a previous publication and involves initially achieving a moderate sedation with boluses of intravenous propofol followed by continuous administration of a low dose of remifentanyl (0.01 µg/kg/min) or dexmedetomidine (0.1 µg/kg/hr) during mapping (J. Lu et al. 2013). Throughout the operation, supplemental inspired oxygen was delivered by nasal cannula or facemask, and no laryngeal mask airway or endotracheal tubing was applied during the iMRI acquisition phase. The iMRI-integrated neurosurgical suite included an operating room and a diagnostic room that had a movable 3-T magnet with a 70-cm working aperture. If a residual tumor that was amenable to further resection was identified according to the mapping and electrophysiological result, the navigational images were updated for further resection guidance. Lu et al. have given a detailed description of the technique used (J. Lu et al.

2013). During the operation, the communication channel between surgeons and patients used a brain function stimulation system that was designed in 2012 by our center and Sinorad Corporation.

Brain Function Stimulation System

During the process of accumulating experiences with awake surgery in the iMRI suite, we developed the brain function stimulation system (Chinese patent no. ZL 1220303939.1). The system can easily give stimuli signals (including images, text, sounds, and music) to the patient and simultaneously send the response messages of the patients (presented via language, limb motor, and finger touch) to the surgeons.

As illustrated in Figure 3.1, this system is composed of 3 parts including the patient interface, the operator interface, and the relative application software. The patient interface consists of the following parts: a light-emitting diode screen to project the stimuli, a camera to monitor the face and body of the patient, and a microphone to receive feedback from the patient. The operator interface includes the computer workstation that produces stimuli signals and the monitor display that allows the surgeon to observe the patient's responses. The software is specifically designed to define the stimulating mode preoperatively, thus facilitating the intraoperative maneuvers consisting of projecting and receiving the stimulation signal and response, for both the surgeon and the patient, respectively. This system facilitates the process of functional mapping, guarantees the safety of the patients, and provides an effective communication channel between the medical staff and the patients in the intraoperative environment.

Intraoperative Mapping

Intraoperative functional mapping was performed using cortico-subcortical electrical stimulation. A 5-mm wide bipolar electrode with a pulse frequency of 60 Hz and an amplitude of 2–10 mA was used. Subcortical stimulation was performed using a biphasic square-wave pulse delivered at 60 Hz with a current amplitude ranging from 2 to 20 mA. The somatosensory evoked potential was recorded with a 6-contact subdural strip electrode. If after-discharge activity indicated that the stimulation current was too high, the current amplitude was decreased by 0.5–1 mA. The central sulcus was identified by an N20 to P20 phase reversal. Intraoperative continuous transcortical motor evoked potential (MEP), followed by confirmatory subcortical stimulation, was performed when the resection was in proximity to the motor areas. All of the

positive sites were marked on the surface of the cortex with sterile tags, and the resection margin was taken within 0.5–1 cm of eloquent cortical areas. After defining both the functional mapping and anatomical landmarks, tumor resection was conducted with guidance from structural (T2-weighted FLAIR or T1-weighted contrast images) and functional (BOLD or DTI tractography images) navigation. The DTI tractography–based navigation, combined with continuous transcortical MEP monitoring and subcortical stimulation, was performed to localize the adjacent pyramidal tracts and to monitor the subcortical motor pathway. The resection was not continued when the tumor was found to be close to an eloquent area (the safe distance was considered to be approximately 8 mm), as evidenced by the occurrence of 1 or more of the following conditions: 1) obvious deterioration of language function (i.e., speech arrest without oropharyngeal movement) or deterioration of motor without oropharyngeal movement or deterioration of motor movement; 2) more than a 50% decline of compound muscle action potential in either extremity, elicited by subdural strip electrode during continuous transcortical MEP monitoring; and 3) the radiological margin of the tumor was reached in accordance with the intraoperative updated real-time MRI guidance. An iMRI was performed when the attending surgeon deemed that the resection was complete or when the functional mapping was positive on the surgical margin (Figures 3.2 and 3.3).

Statistical Analysis

Descriptive results are summarized as the mean, SD, median, and ranges for continuous variables, and as proportions for categorical variables. The EOR and clinical outcome were compared via the Pearson chi-square test or Fisher exact test. When assessing the impact of EOR, GTR was defined as 100% resection. For Kaplan-Meier curves plotting the EOR and OS or PFS, we divided EOR into $\geq 90\%$ and $< 90\%$ categories for LGG, because this is a common cutoff point for volumetric assessment of the impact of EOR (Sanai, Polley, and Berger 2010). Kaplan-Meier methods were used for time-to-event measures, log-rank tests were used to make comparisons, and Cox regression models were used to analyze the potential prognostic factors for the main outcome measures. All analyses were conducted using SPSS software version 19.0.0 (IBM, Inc.). A p value < 0.05 was considered statistically significant.

3.1.3 Results

Between January 2011 and June 2015, resections were performed in 672 patients with supratentorial gliomas in the 3-T iMRI integrated surgical suite. Of those, 139 (20.7%) patients underwent an awake craniotomy, among whom 106 (15.8%) had an iMRI scan (Figure 3.4). Patients' ages ranged from 18 to 76 years (mean 41.7 years). Seventy-four (69.8%) patients were men and 94 (88.7%) tumors involved the dominant side. A seizure was the presenting symptom in 56 (52.8%) patients. The demographic and radiological characteristics of patients are depicted in Table 3.1.

Extent of Resection, OS, and PFS

The number of iMRI evaluations ranged from 1 to 2 scans. The median and mean EOR were 100% and 91.97%, respectively. A GTR (complete radiographic resection) was achieved in 64 (60.4%) of patients.

The rate of GTR in patients with an LGG ($89.06\% \pm 19.6\%$ [mean $\pm$ SD]) was significantly lower than that in patients with HGG ($96.4\% \pm 9.1\%$) ($p = 0.026$). For the HGG group, the GTR ($p = 0.033$), the presence of short-term motor deficit ($p = 0.027$), and the WHO grade ($p = 0.005$) were independent prognostic factors of OS (Figure 3.5). Further analysis of PFS, performed for patients with LGG, demonstrated a survival benefit trend for patients who underwent a GTR ($p = 0.05$) and those who underwent further resection after the iMRI ($p = 0.083$) (Figure 3.6). The rate of GTR achieved was not significantly associated with the rate of short- or long-term deficits ($p = 0.538$ and $p = 0.847$, respectively).

For 62 patients, the iMRI demonstrated residual tumor. Further tumor resection was continued on the basis of the iMRI dynamic control in 30 cases (28.3%), with a median increase of the EOR of 10.1% (mean 11.4%, range 1%–25.8%). A GTR was achieved in 20 (67%) of 30 patients, and only four patients had less than 90% residual after the second resection was performed. A log-rank test was run to determine if there were differences in the survival distribution of the 62 patients based on whether further resection was performed. The difference in survival distributions for the two groups was not statistically significant (chi-square = 0.440, $p < 0.507$). No surgery-related death occurred and there were no complications related to the iMRI nor any adverse events caused by the high-field MRI.

Eloquent Site Involvement and Identification

The preoperative MRI showed that the following functional areas were involved or were found to be close to the tumor: both the motor and speech areas in 80 patients, language cortex in 12 patients, and motor cortex in 14 patients. The positive functional mapping site was detected in 91 patients (85.8%) intraoperatively and determined the functional boundaries of the resection.

Intraoperative Seizures

Three patients (2.8%) had partial intraoperative seizures and one patient (0.9%) had a generalized seizure; all seizures developed after direct cortical stimulation of the primary motor cortex. The focal seizures were controlled with ice irrigation, and an intravenous antiepileptic agent was administered to the patient who had a generalized seizure. The occurrence of seizures in these patients did not affect additional tumor resection, because the patients did not experience prolonged postictal paresis and were able to cooperate for the rest of the surgery.

Functional Outcomes

Over an average follow-up of 24.8 months, two patients were excluded when assessing neurological condition because of lack of functional follow-up data. At one month follow-up, 34 (37.4%) patients were found to have a worsening language function and 7 (7.7%) had a permanent language deficit (follow-up data were not available for 1 patient). In terms of the motor function, among the 94 patients in whom the motor area was involved, 19 (20.7%) patients had a worse motor function at the 1-month follow-up and 4 (4.3%) still had lower motor function at subsequent follow-up (data not available for 3 patients). As for the overall neurological deficit (involving language and/or motor deficit), after excluding two patients in whom no clinical follow-up data were available, the short-term neurological deficit rate was 46.2%, and the long-term neurological deficit rate was 8.7%.

Reason for Subtotal Resection

In our cohort, 30 (28.3%) patients underwent further resection after the iMRI showed residual tumor, and 4 (13.3%) of those 30 patients had an EOR < 90%. In those cases, further resection was precluded by functional mapping results when the tumor was infiltrating eloquent cortical or subcortical structures.

Learning Curve

The learning curve for using the combined technique was assessed for the neurological and radiological outcomes according to the period when the surgery was performed in the entire cohort. This was stratified as the first 27 months (Group A) and the last 26 months (Group B). In the first group, 34 (59.6%) of the 57 patients had an LGG, whereas in the second group, 30 (61.2%) of the 49 patients had an LGG (Figure 3.7).

Although it did not reach statistical significance, the cumulative experience gained by using iMRI seemed to impact the surgeon's ability to estimate the EOR, because the rate of GTR increased by 1.6% with time ($p = 0.869$). Also, the rate of performing further resection after an iMRI decreased significantly in the second group by 26.1% ($p = 0.005$). The improved ability to perform a maximal safe resection also manifested a decreased rate of postoperative short-term ($p = 0.101$) and long-term ($p = 0.132$) neurological deficits.

3.1.4 Discussion

Mounting evidence supports an aggressive EOR, because it might increase the OS of patients. In the review performed by Sanai and Berger (Sanai and Berger 2008), GTR (defined as a complete radiographic resection) improved the mean survival time for patients with all WHO grades. Some surgeons also advocate for early resection of LGG, because it is associated with increased survival and has the potential to decrease the chance of malignant progression (Hugues Duffau 2016). Nonetheless, for both low- and high-grade tumors, the invasion of eloquent areas and the difficulty in distinguishing glioma infiltration from the normal brain significantly impact the extent of GTR performed. The combination of iMRI and awake craniotomy is demanding but well tolerated by patients. Careful preoperative preparation for both the iMRI process and the cortical stimulation is essential to ensure patient compliance. Assuring adequate cooperation between intraoperative personnel and the patient also has significant impacts on the flow and success of the surgery. None of our patients had adverse events during the scanning period and all images yielded a good diagnostic quality.

In this study, we present our clinical experience with 106 patients with gliomas who underwent combined awake functional mapping and iMRI guidance. Clinical and radiographic data were collected and analyzed to identify preoperative predictors of OS, PFS, and EOR. To

our knowledge, this is the first study that assessed the impact of combined awake craniotomies with iMRI-guided resection for patients suffering from gliomas located in the vicinity of the eloquent cortex.

One hypothesis is that with accurate identification of functional tissue, supramaximal resection could be performed despite the occurrence of a possible transient neurological deficit, because tissue plasticity and reorganization could allow subsequent recovery. This aggressive approach could provide a survival benefit; however, individualized factors should be taken into account. For instance, given the dismissal prognosis of patients with HGG, the impact of immediate/short-term neurological deficits on QOL should be balanced with the survival benefit of such an aggressive approach.

In our study, the utility of iMRI to identify residual glioma was 58.5%, which is in accordance with previous reports. However, additional further resection was feasible in only 30 of 62 patients (48.4%), given the fact that tumor proximity to eloquent areas precluded further resection. Although functional imaging, such as DTI and functional MRI, is used preoperatively to plan the tumor resection, the brain shift that occurs as the resection proceeds makes their use less reliable. Intraoperative real-time DTI tractography was performed in most patients; however, due to the retrospective nature of the study, we were unable to compare the results obtained by this technique with the subcortical stimulation (Zhu et al. 2012). The other alternative would be to perform intraoperative BOLD functional MRI (J. Lu et al. 2013) however the benefit of prolonging the amount of time required for an awake case to perform functional imaging instead of direct brain mapping is not clear. Further prospective studies should investigate how closely these modalities replicate the accepted gold standard of electrophysiological monitoring.

Table 3.2 compares the studies investigating the benefit of iMRI to guide further resection in awake craniotomies for supratentorial glioma. As depicted, just two other studies performed a volumetric analysis of the resection rate. The rate of GTR achieved in our study is similar to the rates reported previously (J. Lu et al. 2013; Leuthardt et al. 2011; Maldaun et al. 2014). Although OS and PFS were different between the LGG and HGG groups, the relatively short observational period (a mean of 24.8 months) prevents us from making any definitive conclusion for the LGG group based on these results. Nonetheless, to our knowledge this is the first study to analyze the clinical outcomes and the follow-up period is considerably longer than in the previous reports.

Impact on Neurological Outcome

Cortical and subcortical stimulation represents an established technique used to identify functional cortical areas or connective fiber tracts. This technique is essential for gliomas located near the eloquent cortex because if cortical stimulation results in reversible deficits, this area cannot be surgically removed, even if tumor infiltration is present. In a previous study, a presumed eloquent LGG location was found to be associated with both a lower PFS and shorter OS (Chang, Smith, et al. 2008). The eloquent brain involvement was found to be a strong predictor of subtotal resection, and incomplete debulking of the tumor burden directly affected the PFS and long-term OS. Also, Haglund et al. reported that the risk of transient language deficit is 36% and that of permanent deficit is 0% when resection is limited to a distance of > 1 cm from the nearest language site; whereas with a margin < 0.7 cm, the risks were 100% and 43%, respectively (Haglund et al. 1994). In our study, the rate of neurological deficits was higher than in previous reports and this is probably accounted for by the more aggressive EOR performed. Although the presence of neurological deficits was not found to affect OS and PFS, a longer observational period with prospective clinical follow-up, including QOL measures, should be undertaken to assess the impact on long-term survival. We nonetheless observed a significant association between worsening neurological function in patients with HGG and OS. This should raise awareness in terms of the impact of performing an aggressive resection that causes a subsequent deficit in patients who have a poor prognosis.

Given the ability of iMRI to improve EOR, patients treated with iMRI guidance would be expected to have a survival advantage compared with patients for whom intraoperative assessment of residual tumor is performed using conventional measures. In our study, obtaining GTR ($p = 0.033$) was an independent prognostic factor of OS in the patients with HGG who had an iMRI. Similarly, a possible survival benefit trend for the patients who underwent a GTR ($p = 0.05$) was noticed in the patients with LGG. No definitive conclusions can be drawn in terms of the impact of iMRI-guided resection on survival time, given the retrospective nature of this study and the lack of a control group. However, our results are in accordance with the previous literature that suggests a survival benefit with the use of iMRI.

Thus, combining methods that enable the neurosurgeon to appreciate the full extent of the glioma infiltration and to visually distinguish these areas from eloquent brain regions represents

state-of-the-art surgery because it enables a safe radical resection according to individual cortico-subcortical functional boundaries(Tuominen et al. 2013; J.-F. Lu et al. 2013; Hugues Duffau 2012; De Witt Hamer et al. 2012). To unequivocally demonstrate the value of iMRI, a prospective, randomized, controlled clinical trial is underway at our center.

Learning Curve

As outlined in Figure 3.7, some general trends were noticed when assessing the outcomes, according to the time period when the surgery was performed. Although no statistical significance was reached, the data depict the dilemma faced by surgeons when resecting tumors located in eloquent areas. Surgeons often perform a less aggressive approach to decrease the rate of postoperative neurological deficits, but this might result in a suboptimal tumor resection. Although the final EOR and GTR were relatively similar between the two groups, there was a significant decrease in the rate of further resection after the iMRI demonstrated residual tumor in the second group. This suggests that the ability to judge maximal safe resection of gliomas in eloquent areas improves with time, allowing the surgeon to reach the functional margins more accurately. Although the contribution of iMRI to improving that skill can play an important role, increased experience of the surgeon - independent of the surgical technique used - might also affect this result.

Strengths and Limitations

Independent clinical reviewers with standardized follow-up formats followed all cases, and only 6 patients were lost to follow-up. Although the outcomes are limited to two observers and we could not present reliability data, the staff performing the data collection were senior members with extensive experience (> 20 years) and were not primarily involved in care of the patients.

Furthermore, the retrospective nature of the study prevented us from performing a detailed assessment of the exact neurological function or assessing other factors that might have interfered with the results. This might have an impact on the ability to accurately measure OS and PFS. Also, given that we are reporting a single-center experience, extrapolation of our results should be performed carefully.

Another limitation is that we did not assess the impact of obtaining the MRI on the length of the surgery. Nonetheless, at our institution, we often perform additional sequences for research purposes, so the image scanning time might not reflect the actual time required for clinical data acquisition. Furthermore, because this is a retrospective study, the time spent positioning, draping the open skull, and transferring the patient into and out of the magnetic bore could not be assessed. However, compared with other studies, the use of the monitored anesthesia care technique should decrease the total time required for these preparations because no additional airway management was performed for our patients.

In summary, this retrospective study outlines the dichotomy presented by the synergistic use of combined intraoperative imaging and neurophysiological monitoring for gliomas located in proximity to eloquent areas. Dealing with these challenging tumors requires extensive knowledge of the natural history and anatomical peculiarities of the tumor. The use of iMRI allows the surgeon to achieve a more aggressive resection based on the intraoperative evaluation of the EOR. Nonetheless, as outlined above, to achieve a maximally safe tumor resection, protection of the functional areas is paramount and the localization of functional structures is a prerequisite for safe resection. Although intraoperative real-time DTI tractography can be realized based on iMR images acquired, in our center the use of intraoperative brain mapping is considered the gold standard for identification of eloquent cortex and subcortical functional pathways. Positive responses to white matter stimulation prompt the surgeon to change the surgical strategy because damage of the functional structures could cause irrevocable neurological deficits.

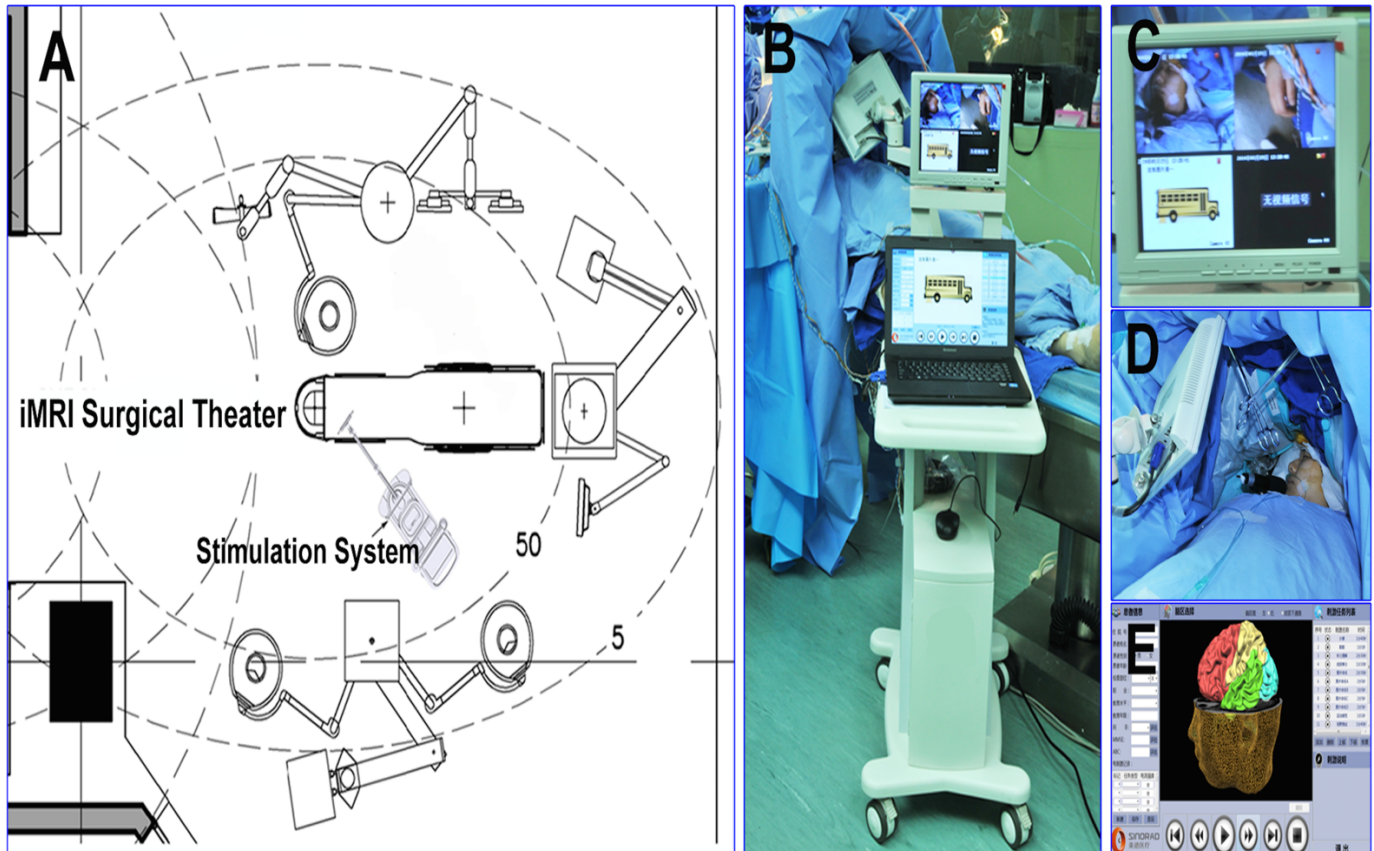
3.1.5 Conclusion

Combined awake craniotomy and iMRI is a safe and efficient technique, allowing maximal safe resection of lesions situated near eloquent cortex. This large case series adds to the existing literature that suggests a possible survival benefit derived from maximal resection for both LGG and HGG. Furthermore, a worsening short-term neurological function seems to affect the OS in HGG. The use of iMRI allows the surgeon to achieve a greater EOR, while the neurophysiological testing and functional mapping delineate the cortico-subcortical functional boundaries. Further well-designed prospective studies are required to validate these results.

Meanwhile, the decision between supramaximal resection and functional preservation should continue to be individualized and take into account the QOL of the patient.

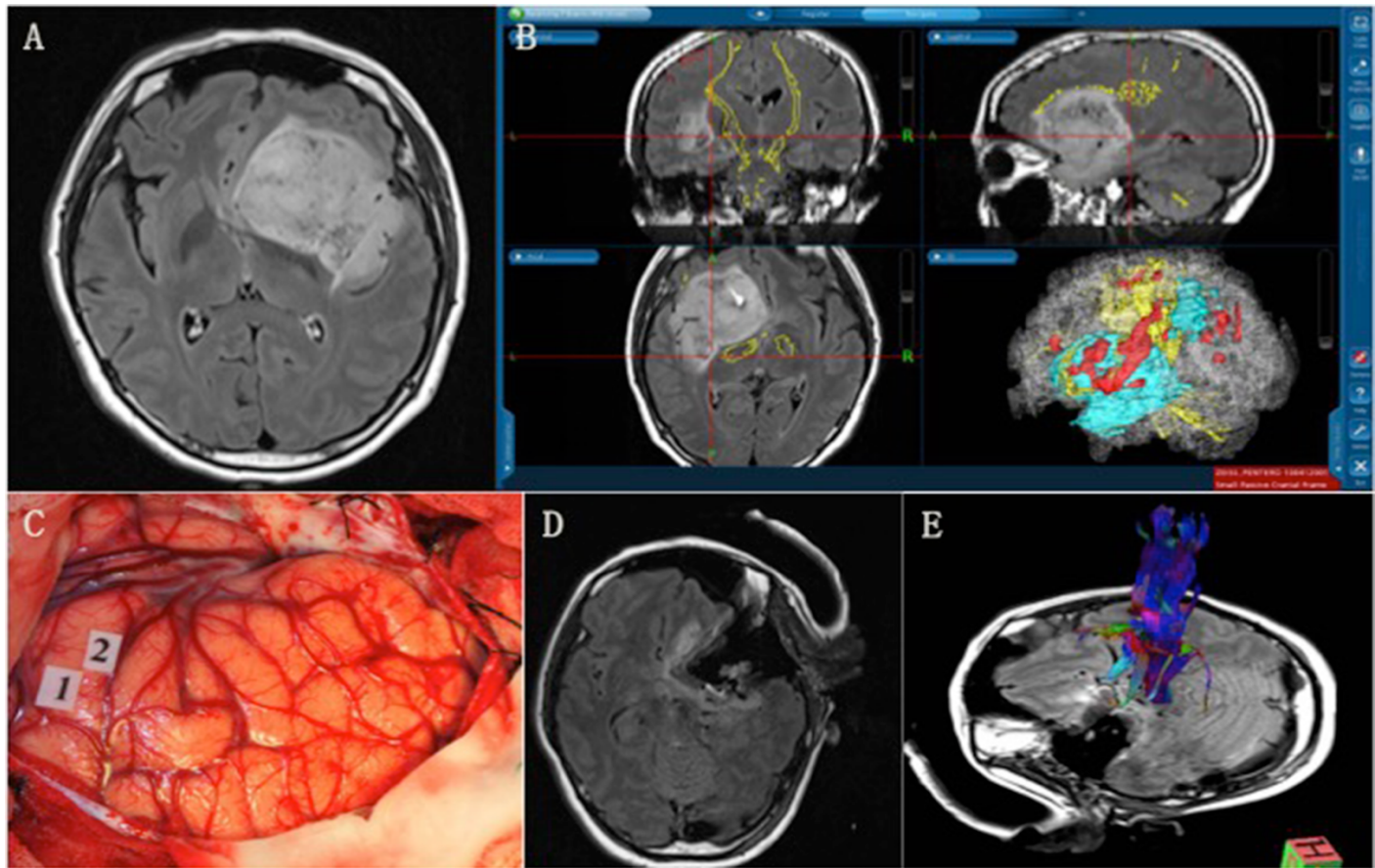
3.1.6 Figures and Legends

Figure 3.1 The location of the brain function stimulation system in the iMRI suite



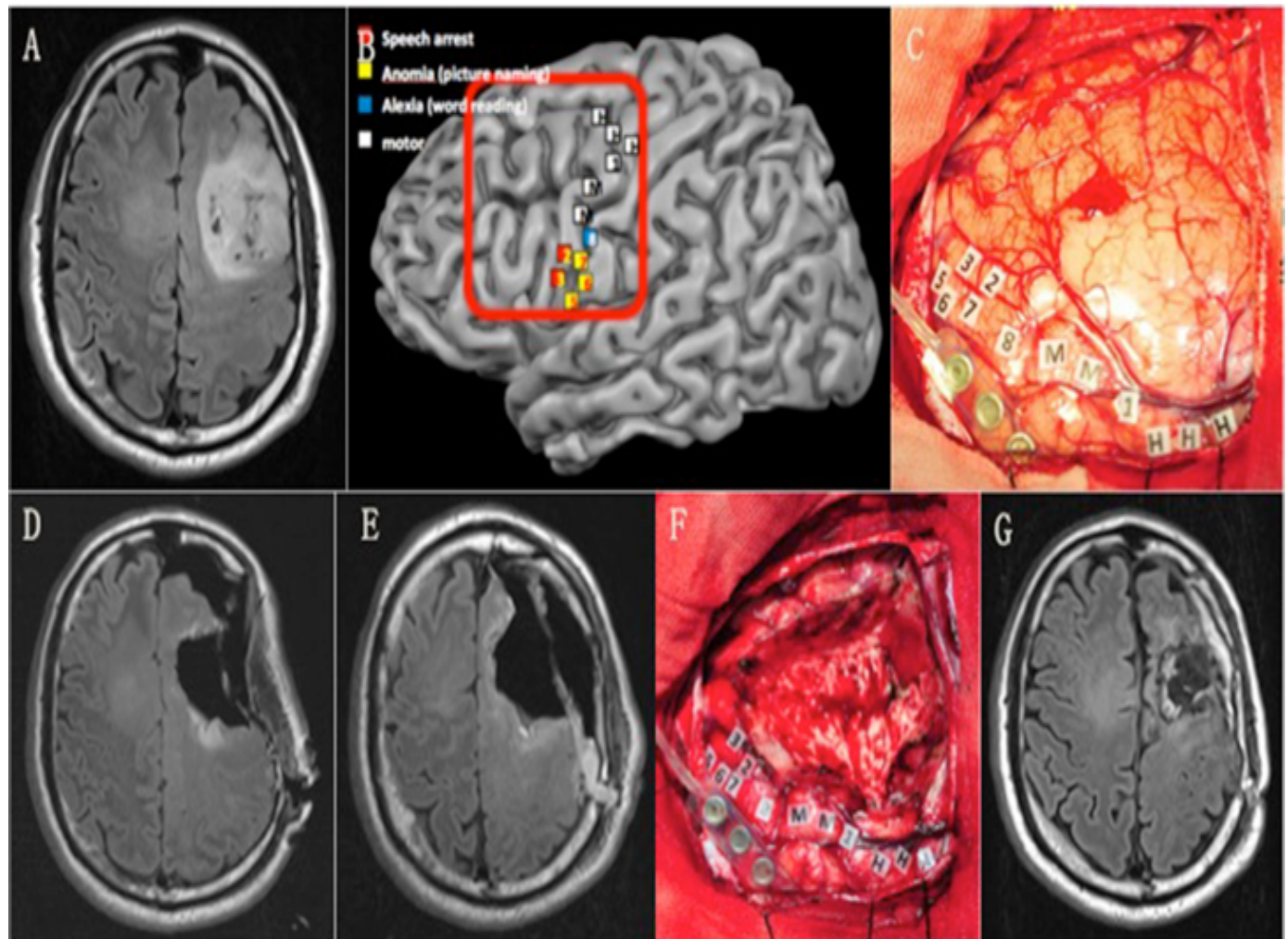
The brain function stimulation system in the iMRI suite is shown (A and B). The surgeon's interface screen shows the patient's response (C) to stimulus signals sent by the working station to the patient's interface (D). The stimulation tasks are automatically chosen according to the location of the tumor (E).

Figure 3.2 Illustrative Case 1



A 43-year-old woman with a 113.96 cm³ left frontotemporal astrocytoma (WHO Grade II). Preoperative structural and functional MR images (BOLD and DTI, respectively) show the relationship among the tumor, the language area (depicted in red), and the pyramid tract (depicted in yellow)(**A and B**). Intraoperative functional language mapping confirmed the preoperative functional MRI results (**C**). Resection of the tumor to the functional margin, as manifested by a decrease of the MEP potential, was performed. The intraoperative structural and DTI images (**D and E**) confirmed that maximal resection was obtained, with a residual tumor volume of 8.86 cm³. The patient experienced transient right limb weakness in the immediate postoperative period but was completely recovered 1 month after the operation.

Figure 3.3 Illustrative Case 2



A 48-year-old man with a left frontal lobe astrocytoma (WHO Grade II). Preoperative structural imaging showed the tumor was in proximity to the language and motor areas (A). Intraoperative mapping results confirmed that the language and motor areas (B and C) were at the posterior margin of the tumor. The first iMRI evaluation showed a residual at the posterior part of the tumor cavity (D). Because the electrophysiological result allowed further resection, that area was resected (E) while preserving the areas with positive functional mapping (F). The patient had mild language and motor deficits in the immediate postoperative period but was completely recovered 1 month after the operation (G).

Figure 3.4 Description of the cohort

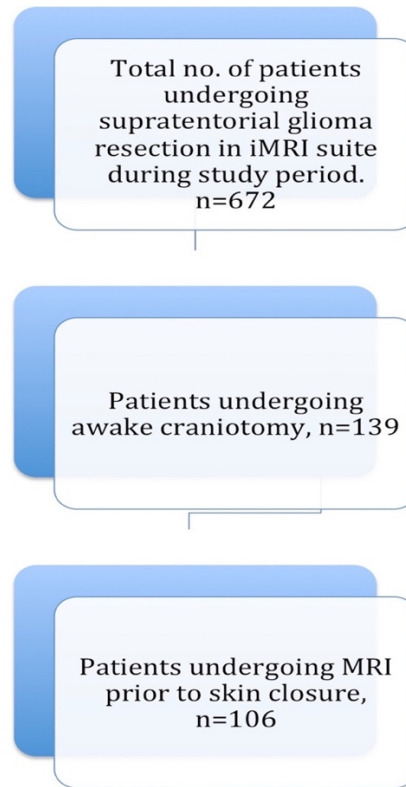


Figure 3.5 Kaplan-Meier curve of OS for the 42 patients with HGG, stratified by presence of short-term motor deficits (log-rank test, $p = 0.016$). Cum = cumulative.

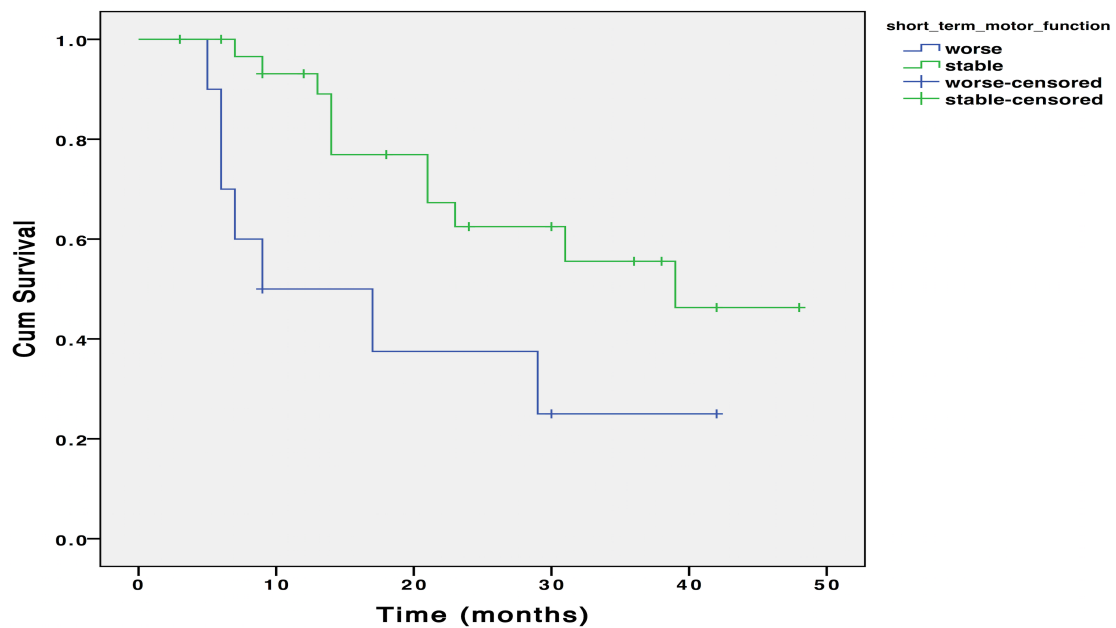


Figure 3.6 Kaplan-Meier curves showing PFS for the 64 patients with LGG, stratified by GTR (log-rank test, $p = 0.050$) (left) and by whether further resection was performed ($p = 0.083$) (right).

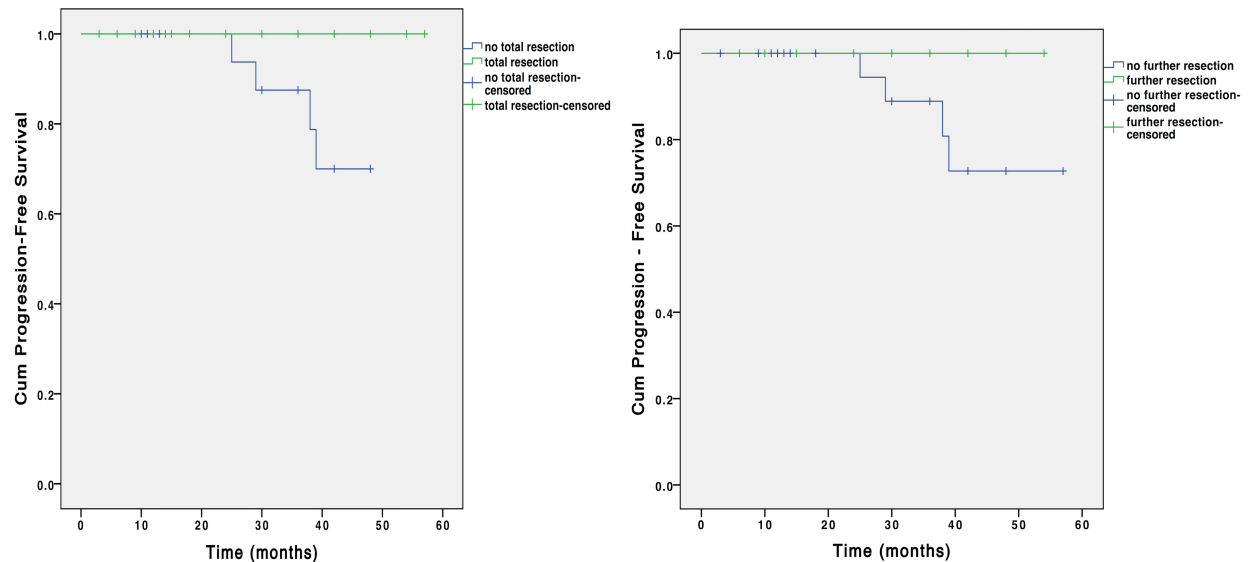


Figure 3.7 Neurological and radiological outcomes according to time period when procedure was performed.

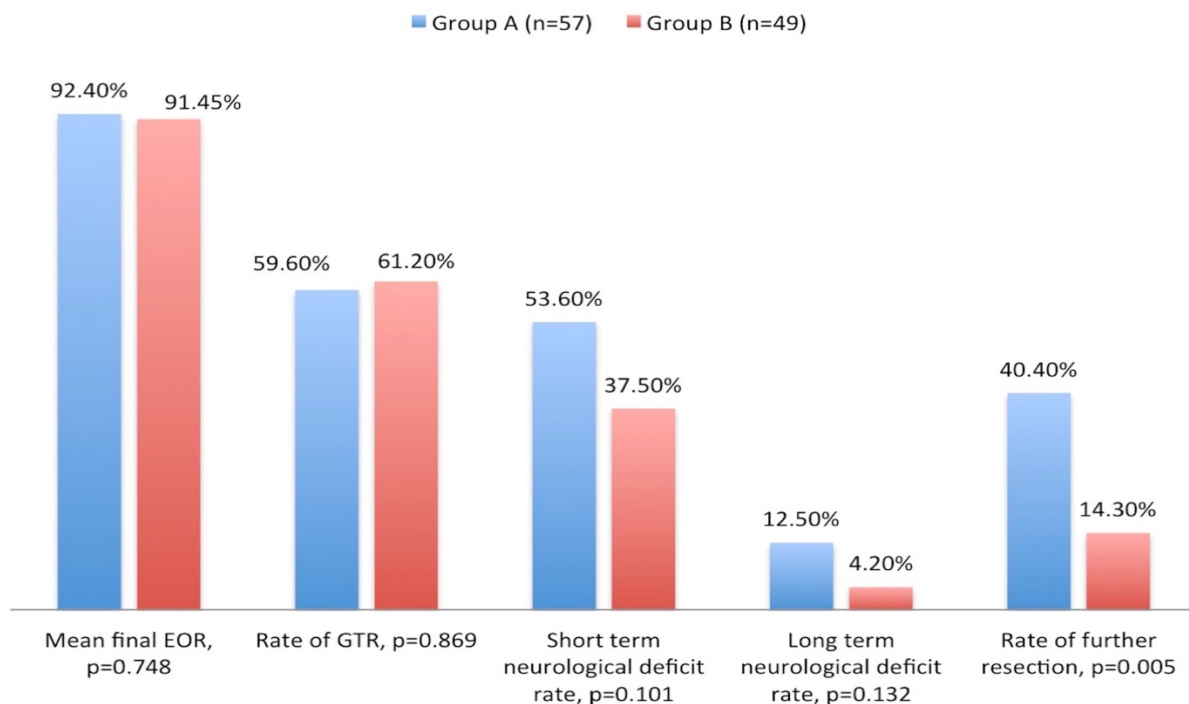


Table 3.1 Demographic, clinical, and neuroradiological characteristics of 106 patients undergoing combined awake craniotomy and iMRI for glioma resection

Characteristic	Value
Age at time of op, yrs	
Mean $\pm$ SD	41.7 $\pm$ 12.5
Range	18–76
Sex, n (%)	
M	74 (69.8)
F	32 (30.2)
Location of lobe, n (%)	
Frontal	48 (45.3)
Parietal	9 (8.5)
Temporal	18 (17.0)
Insular	31 (29.2)
Seizure as presenting symptom, n (%)	56 (52.8)
Preop tumor vol, cm ³	
Mean $\pm$ SD	58.0 $\pm$ 37.9
Range	3.5–181.3
Follow-up, mos	
Mean $\pm$ SD	24.8 $\pm$ 15.1
Range	3–57
Adjuvant therapy, n (%)	
None	12 (11.3)
Radiotherapy	22 (20.8)
Chemotherapy	22 (20.8)
Radiochemotherapy	48 (45.3)
NA	2 (1.9)
Dominant hemisphere involvement, n (%)	94 (88.7)
WHO grade, n (%)	
II	64 (60.4)
III	17 (16.0)
IV	25 (23.6)

Table 3.2 Review of the literature on the radiological and clinical results obtained with combined high-field iMRI guidance and awake craniotomy

Authors & Year	No. of Procedures	Strength of iMRI	Volumetric Analysis	Follow-Up (mos)	Further Resection After iMRI	Outcome Measure	EOR Increase After Further Resection	GTR
Nabavi et al., 2009	38	1.5 T	No	0	NR	NR	NR	NR
Weingarten et al., 2009	10	1.5 T	No	0	7 of 10 cases	30% (3 of 10) transient short-term deficits	NR	70%
Goebel et al., 2010	25	1.5 T	No	NR	20 of 22	32% had a decline in neurological deficits that persisted at 5 days postop	NR	NR
Parney et al., 2010	1	1.5 T	No	NR	Not performed	No deficit	NR	NR
Leuthardt et al., 2011	12	1.5 T	No	1	6 of 12	50% (5 of 10) worse in the immediate postop period; 10% (1 of 10) persisted at 1-mo follow-up	NR	5 (41.7%)
Tuominen et al., 2013	20	0.23 T	No	NR	NR	Transient deficit (10%); permanent (5%)	ND	50%
Lu et al., 2013	30	3 T	Yes	6	11 of 30 (36.7%)	40% suffered from transient language deficits; 1 (3.3%) permanent	7.5%	18 (60%)
Maldaun et al., 2014	42	1.5 T	Yes	1	17 of 42 (40.5%)	11 (26.2%) in the postop period; 1 (2.3%) persisted at 1-mo follow-up	11%	17 (40.5%)

ND = not determined; NR = not recorded.

Article 3. Contribution of combined intraoperative electrophysiological investigation with 3-T intraoperative MRI for awake cerebral glioma surgery: comprehensive review of the clinical implications and radiological outcomes

3.2 State of art awake craniotomy patient - centered mapping system development

High-grade gliomas represent the most lethal primary brain tumour with less than 5% of patients surviving at 5 years despite optimal treatment (Sanai and Berger 2008). Conversely, the diffuse low-grade gliomas are thought to represent a chronic invasive lesion that eventually undergoes malignant transformation leading ultimately to death (Hugues Duffau and Taillandier 2015; Ostrom et al. 2014). Substantial evidence suggests that a more extensive surgical resection is associated with longer life expectancy (Sanai and Berger 2008; Tarapore, Chang, and Berger 2011) and awake functional mapping allows surgeons to achieve that while minimizing neurological deficits, thus improving patient's survival (Sun et al. 2011; Nimsy et al. 2005; Spina et al. 2017; Nakaji et al. 2014; D. Ghinda et al. 2016). Furthermore, the concept of eloquent brain function was refined to include higher executive and neurocognitive functions that need to be taken into account and protected during the management of those patients to increase the quality of life of the patients (Dziedzic and Bernstein 2014; Serletis and Bernstein 2007; Kirsch and Bernstein 2011; Racine et

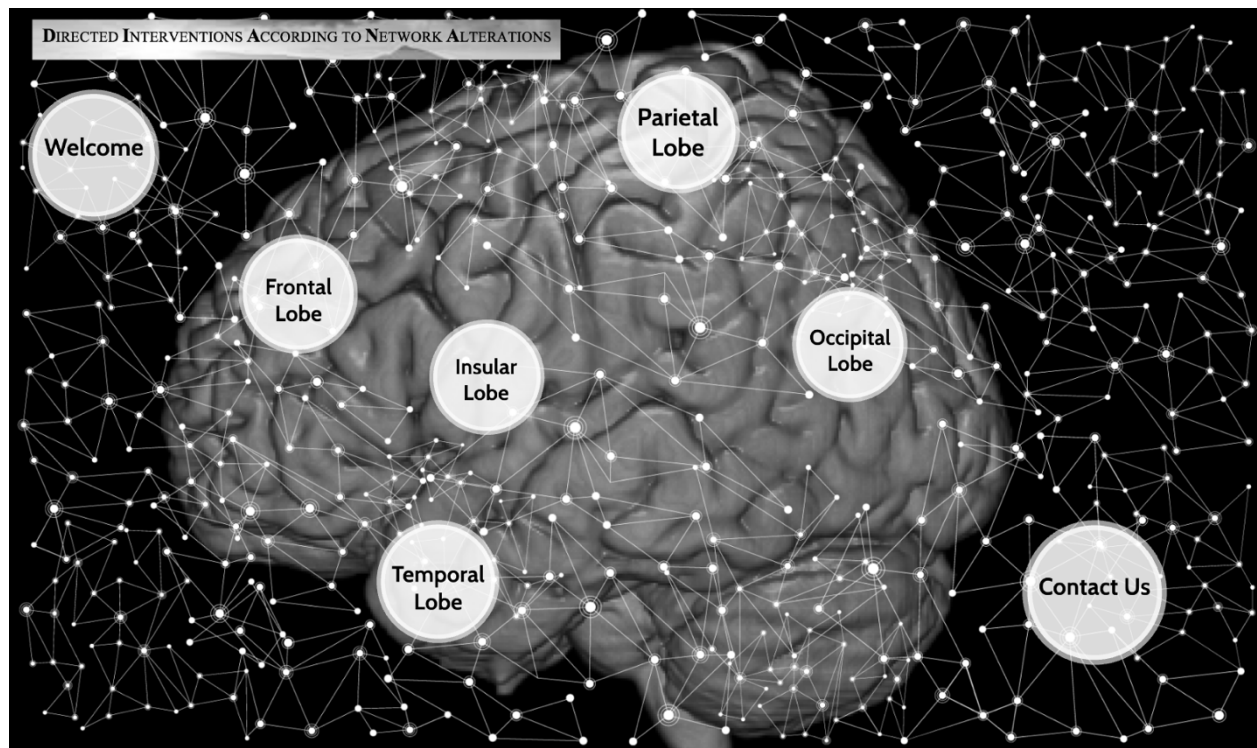
al. 2015; Yordanova, Moritz-Gasser, and Duffau 2011). A comprehensive pre-operative preparation and intra-operative assessment of patients treated with this approach demonstrated an improvement in the quality of life and survival of patients (Figure 3.1; De Witt Hamer et al., 2012; Ghinda and Duffau, 2017; Robles et al., 2008; Ruis et al., 2014). Moreover, the need of an interdisciplinary team to ensure a successful approach was emphasized in multiple studies (Meng et al., 2015; Saito et al., 2009; Ruis et al., 2014).

At our center, there is currently no formal pre-/intra-operative assessment method which can impact the patient's performance and cooperation during the procedure and ultimately the outcome of the patients. The purpose of awake craniotomy is to allow neurosurgeons to perform intra-operative mapping of the functional brain areas in order to increase the extent of tumour resection while protecting neurological function, thus improving patient's survival and quality of life. Although patient's performance and cooperation during the procedure is essential for an accurate decision of the surgical strategy, many patients are anxious and not familiar with the intra-operative mapping procedure. Furthermore, the patient's positioning intra-operatively and the noisy operative environment often prevent the neurosurgeon to monitor the patient's responses. This can result in the need to perform repetitive tests that increase the patient's fatigue. These factors directly impact the neurosurgeons' ability to perform an optimal surgical intervention while also negatively impacting the patient's experience of the treatment received.

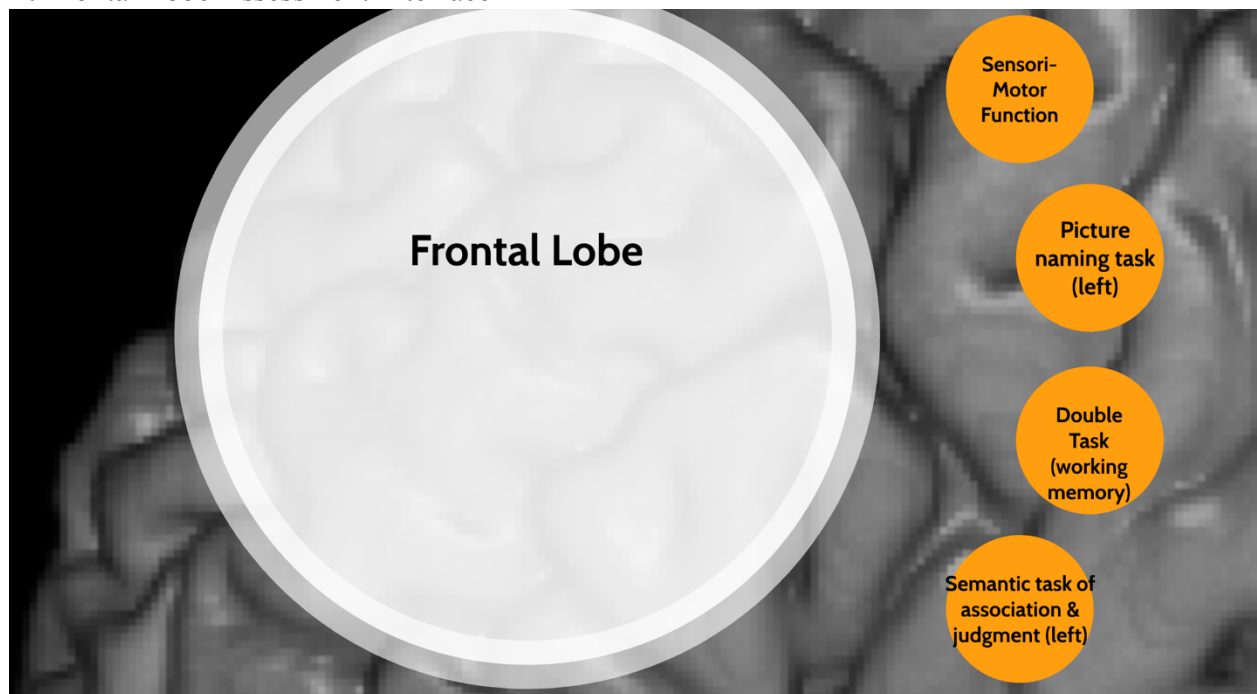
The developed on-line brain mapping aid (Figure 3.8) was designed to improve the quality of care provided to patients by 1) allowing patients to get accustomed with the tests used intra-operatively prior to the surgery, thus decreasing their anxiety and increasing their cooperation; 2) provide a tool allowing physicians to evaluate in a timely and objective manner the results of the intra-operative functional mapping. We expect that the implementation of such program will improve the patient's experience and long-term functional outcomes.

Figure 3.8 Electronic brain function assessment system facilitating the process of functional mapping (available on line upon request).

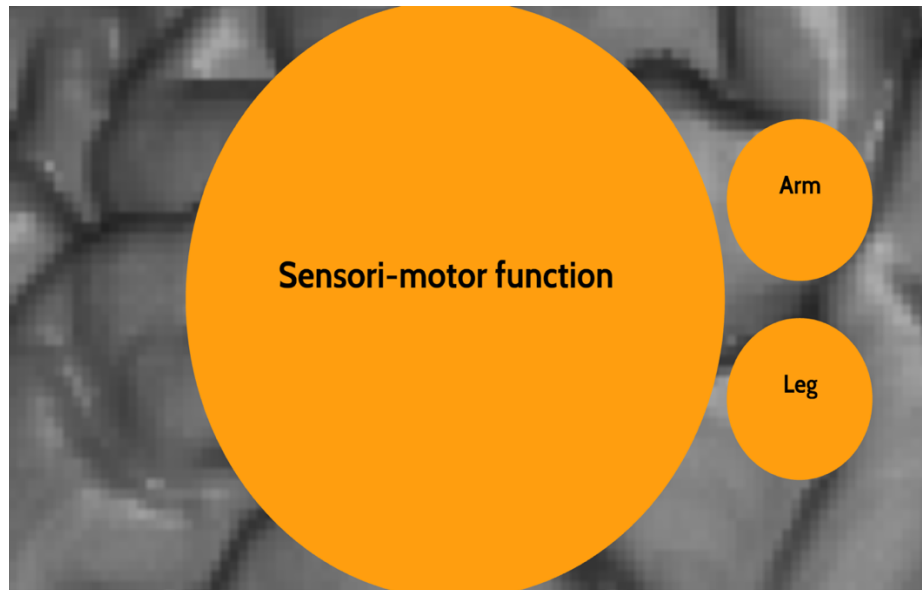
A. Main User Interface



B. Frontal Lobe Assessment Interface



C. Sensori-Motor Function Assessment Interface



Open and close your hand



Move your foot up and down



D. Picture Naming Test Assessment Interface

Open and close your hand

AND

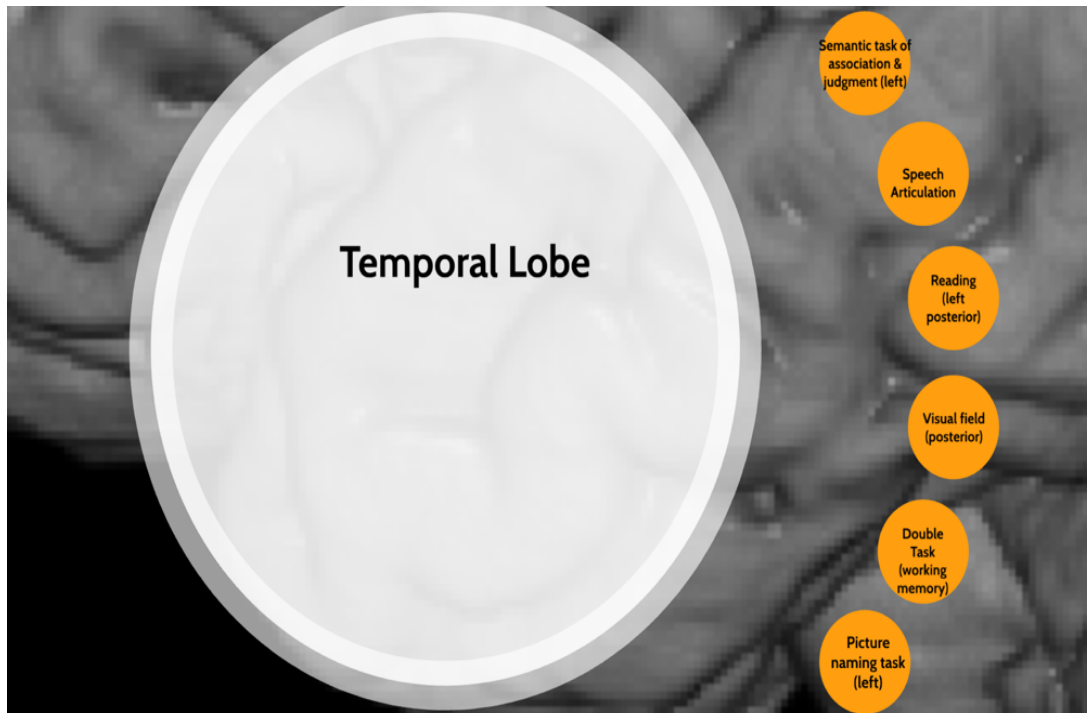
read aloud: "This is a ..."
and name the object shown in the picture.



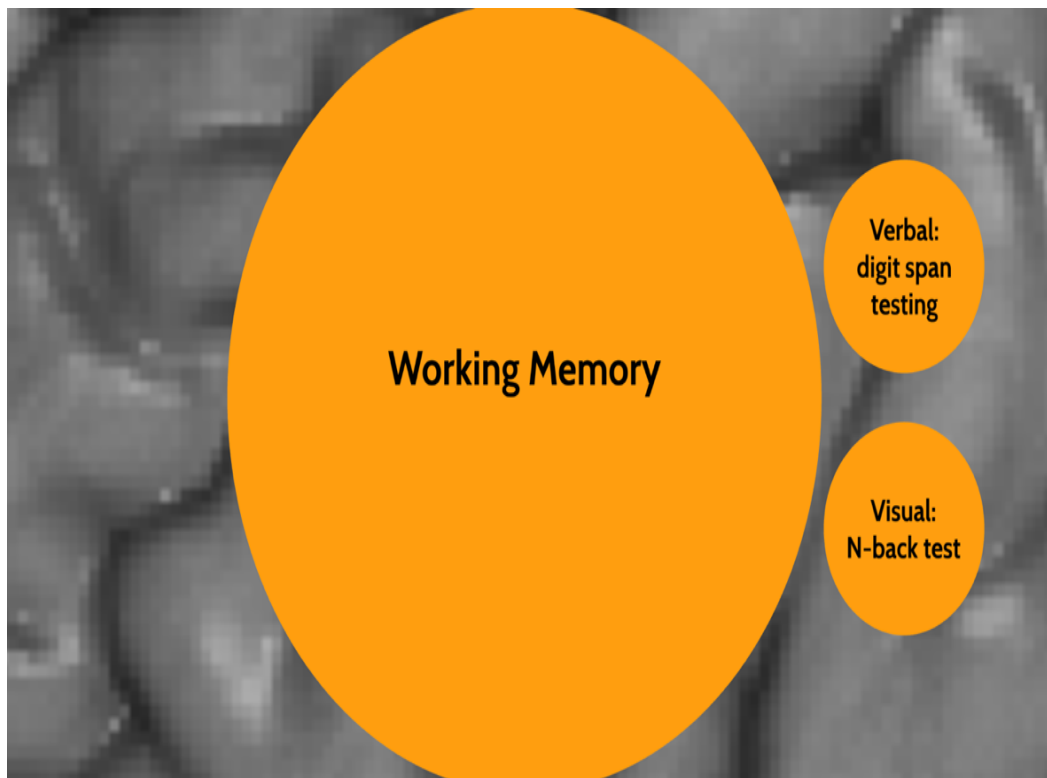
This is a



E. Temporal Lobe Assessment Interface



F. Working Memory Assessment Interface



N-Back working memory task

In this test, you will see a sequence of letters. Each letter will be shown for a few seconds and you need to decide if you saw this letter 3 trials ago. If you saw the same letter 3 trials ago, press the right (**R**) click of the mouse. If it was NOT shown 3 trials ago, press the left (**L**) click of the mouse.

For example, if you get the letters bellow, press:

ACJHCUFBU

 Mouse click -> **L L L R L L L R**

 L - Left R- Right

G. Parietal Lobe Assessment Interface

Parietal Lobe

Select tests:

- Sensori-motor function
- Sensori-Motor Function
- Double Task (working memory)
- Picture naming task (left)
- Calculation (left)
- Line bisection task (right)

E. Line Bisection Task Assessment Interface

Touch the mid-point of the line below:

Chapter 4 -ELECTROPHYSIOLOGICAL CHANGES IN ECOG

In this chapter, the distinct electrophysiological changes observed in the tumoral and surrounding tissue are described. The first part of the chapter investigates the electrophysiological signature of tumoral tissue from scale-free analysis of intraoperative ECoG during awake craniotomy for glioma-related epilepsy. This study portrays for the first time, to our knowledge, distinct power law exponent features in the tumoral tissue which could provide a potential novel electrophysiological marker in the future. The distinct features seen in the peritumoral tissue of gliomas seem to indicate the area where both the onset of epileptiform activity and the tumor infiltration take place. Subsequently, we also analyzed how the spontaneous activity of the brain interacts with stimulus-induced activity. The electrodes overlying the tumoral tissue revealed lower power in faster frequencies across tumoral area compared to the surrounding tissues and the spectrum power difference between healthy and tumoral tissue within pre-stimulus time period indicated significant ($p < 0.0054$) alpha band power difference between healthy and tumoral tissue. Furthermore, we showed differences between peritumoral electrodes in trial-to-trial variability (TTV) reduction which could be considered as an index of the presence (no TTV reduction) or absence (TTV reduction) of infiltration of tumor cells into peritumoral tissue. In order to quantify the brain shift influence for assessing the accuracy of our method, we equally calculated the extent of brain shift immediately after ECoG recoding by using the intra-operative MRI. This approach was not done previously to our knowledge.

4.1 Scale Free Electrophysiological Signature in Glioma Related Epilepsy

Electrocorticography (ECoG) has been utilized in many epilepsy cases however the use of this technique for evaluating electrophysiological changes within tumoral zones is sparse.

Nonetheless, epileptic activities seem to arise from the tissue surrounding gliomas suggesting a link between epileptogenesis and glioma cell infiltration in the peritumoral area. The purpose of this study was to implement novel scale free measures to assess how cortical physiology is altered by the presence of an invasive brain tumor.

4.1.1 Introduction

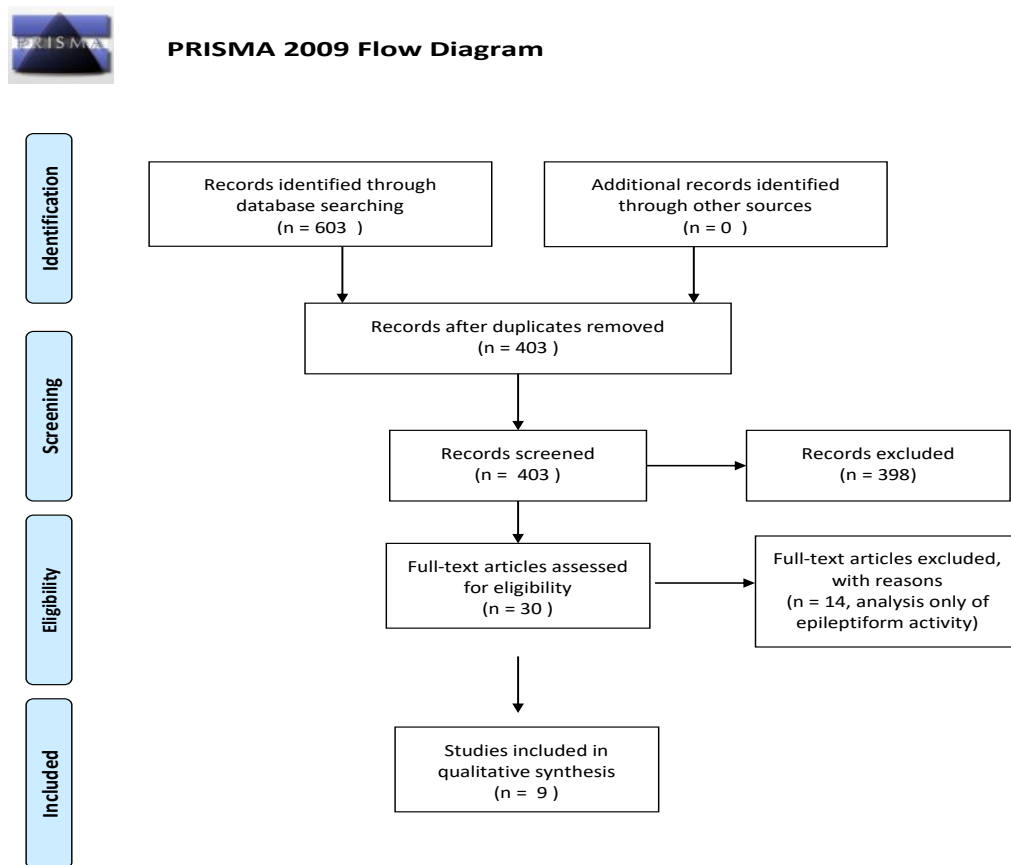
Although the main presenting symptom of low-grade gliomas are seizures, the pathophysiological mechanisms and related structural-functional abnormalities underlying the epileptogenesis in patients with these tumors remain unclear (Pallud et al. 2014; Pallud, Capelle, and Huberfeld 2013; M. De Groot et al. 2012).

The recording of electrical changes in the brain by electrodes placed directly on the cerebral cortex, electrocorticography (ECoG), has previously been used for delineation of tumours when the non-invasive imaging techniques were not yet available. For instance, the electroencephalogram (EEG) had localizing value in cases of brain tumour and epilepsy and the occurrence of delta waves was thought to be a better guide than areas of electrical silence (Van Der Horst 1947). Similarly, subcortical tumors have been localized by the presence of slow wave activity over the tumour while the epileptogenic focus could be determined by focal spontaneous spiking, induction of seizures by electrical stimulation and production of long-lasting after-discharges (Marshall and Earl 1949). The use of ECoG for cerebral tumours revealed distinct changes with infiltrative tumours (Ectors and Achslogh 1950), for instance slow delta-waves were found in the surrounding edema (Jung, Riechert, and Heines 1951). The origin of these slow waves was assumed to be related to intracranial hypertension however, another study analysing the morphological and pathophysiological features of the peritumoral area concluded that those EEG changes result from direct influence of the tumor on brain parenchyma, rather than from peritumoral edema or intracranial hypertension (Hossmann, Wechsler, and Wilmes 1979). As such, historically, ECoG was not only of scientific interest but was also of great practical value for neurosurgery.

Besides delineating the eloquent areas that need to be preserved during the surgical resection, ECoG use for assessment of electrophysiological changes occurring in the setting of glioma is currently a relatively non-explored domain. It is postulated that the peritumoral neocortex around gliomas represents a pivotal structure both for the genesis of glioma related epilepsy (GRE) and for infiltration by glioma cells, which has significant implications in terms of the treatment recommended to the patients and their oncological prognosis (M. De Groot et al. 2012; Englot et al. 2012; Huberfeld and Vecht 2016; Cowie and Cunningham 2014; Shamji, Fric-Shamji, and

Benoit 2009). In a systematic review performed on this subject, using medical subject headings and text words related to ECoG, epilepsy and gliomas in MEDLINE (OVID interface, 1946 onwards), EMBASE (OVID interface, 1947 onwards), and the Cochrane Central Register of Controlled Trials, we found only nine articles using EEG or ECoG for assessment of glioma - related electrophysiological changes (Figure 4.1). In this paper, we outline a novel in vivo electrophysiological investigation of tumoral tissue in patients undergoing awake craniotomy for tumor resection. Subsequently, we discuss our findings in the context of the available literature.

Figure 4.1 PRISMA Flow Diagram



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit www.prisma-statement.org.

4.1.2 Methods

Subjects and Recordings

Twelve patients undergoing awake craniotomy for a newly diagnosed intracranial glioma were enrolled. Six of the patients presented with multiple seizures. A monitored anesthesia care approach was adopted for all patients as per previous publications (D. Ghinda et al. 2016). After the anesthesiologists administered premedication by infusing 0.02–0.03 mg/kg midazolam and 5 mg tropisetron, intravenous lines, a central venous catheter, an arterial line, and an urethral catheter were inserted. The supraorbital, supratrochlear, zygomaticotemporal, auriculotemporal, greater occipital, and lesser occipital nerves were blocked bilaterally using a mixture of lidocaine (0.67%) and ropivacaine (0.5%). Once the patient was brought into moderate sedation with boluses of intravenous propofol, the head was fixed into its fitted position using a custom-designed high-field MRI-safe head holder (DORO Radiolucent Headrest System, Pro Med Instruments GmbH, Freiburg, Germany), which was integrated with an IMRIS operating room table and head coils (IMRIS). Once the scalp was prepared and draped, remifentanyl (0.01–0.03 lg/Kg/min) or dexmedetomidine (0.1–0.7 lg/Kg/h) were administered for analgesia. To minimize brain swelling, mannitol (1 g/kg) was infused intravenously before the dura opening. Because of the minimal draping, no laryngeal mask airway or endotracheal tubing was applied.

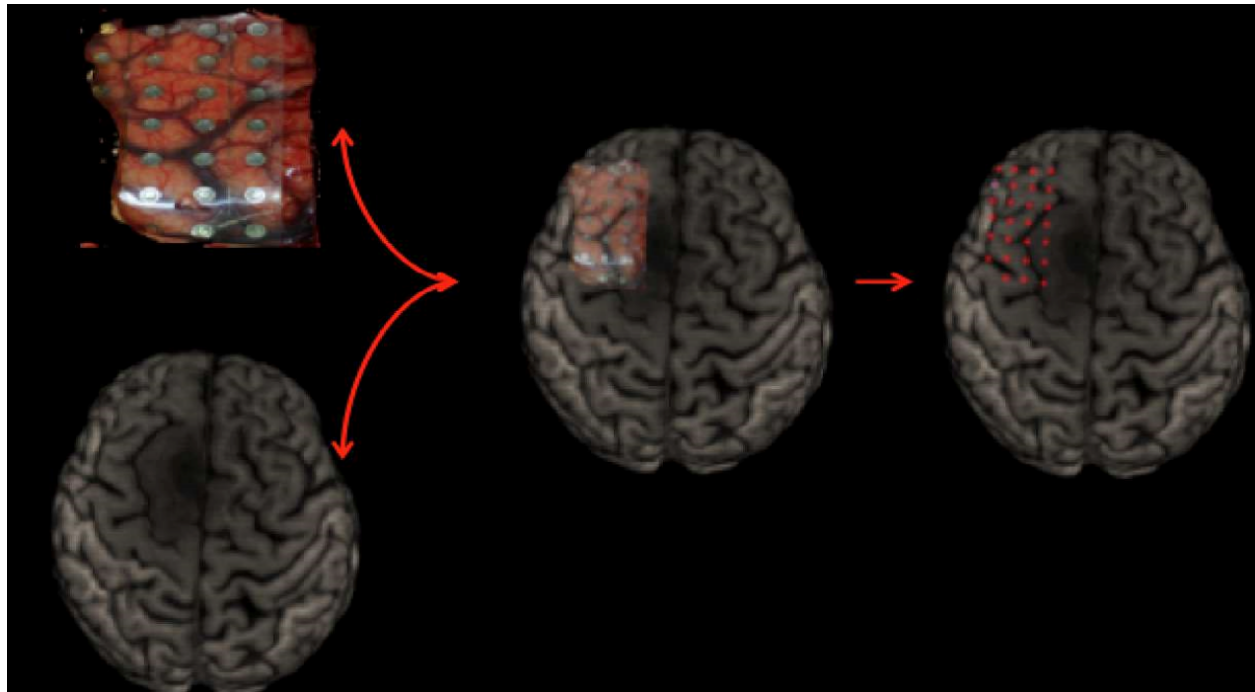
The ECoG grid was placed over the exposed brain surface as indicated by the needs of the patient. ECoG signals were recorded with a 20, 32 or 48-channel grid of 4.0 mm diameter electrodes with 2.3 mm exposure (Ad-tech, Germany) connected to a BP amplifier (Brain Product, Germany). The reference electrode was placed on the contralateral earlobe to avoid any potential increase in coherence due to the reference electrode location.

Five min of recording in the awake state were acquired prior to the tumor resection. For the six patients presenting with seizures, the ECoG signal was also acquired in the anesthetic state. The signals were sampled at 1000 Hz with a hardware filter between 0.01 Hz and 1000 Hz. The ECoG electrodes for each patient were classified in TT (tumoral tissue), HT (macroscopically healthy-tissue) and PT (peritumoral tissue) according to the radiological defined boundaries as per the conventional structural MRI: FLAIR for non-enhancing diffuse low-grade gliomas and the T1 MPRAGE with contrast for contrast enhancing tumors (Ellingson, Wen, and Cloughesy 2017) (Figure 4.2B).

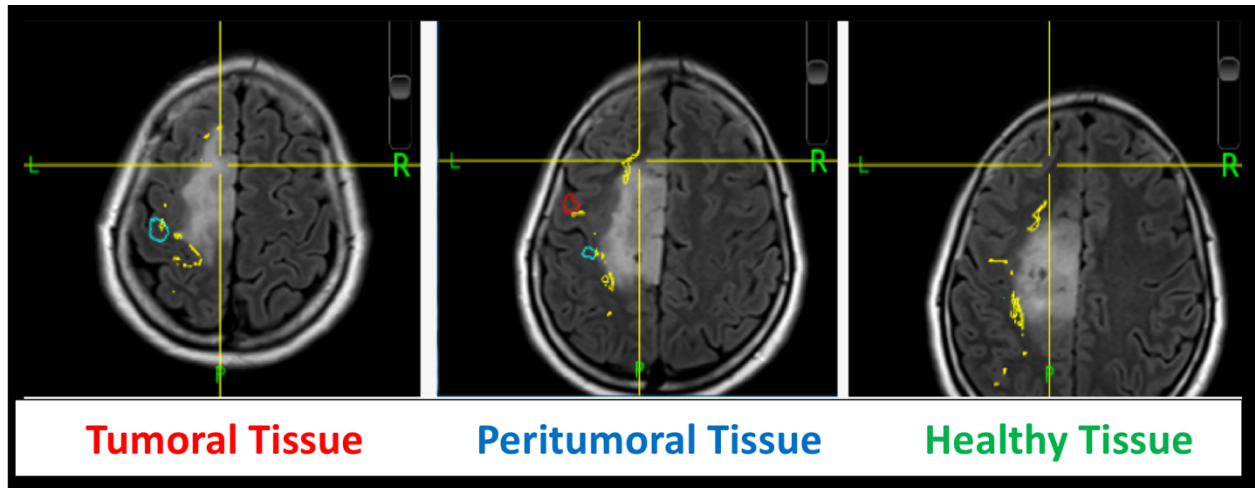
ECoG localization method via intra-operative photography

An intra-operative picture was taken after the electrode grid was placed over the exposed brain. Intra-operative location of each electrode were recorded with the neuro-navigation system (Medtronic, Inc., Minneapolis, MN, USA). The exact location of the electrodes in relation to the tumor was then recorded after the electrodes were placed on the cortical surface segmented from the pre-operative MRI (Figure 4.2A). We confirmed the electrode locations using the neuronavigation images thus reducing the risk of error in electrode localisation.

Figure 4.2 Projection of ECoG grid on the anatomical scan and anatomical localization



A. Intra-operative photograph merged with the cortical surface segmented from the pre-operative MRI. On the upper left, we can see the ECoG grid with the 32 electrodes projected onto the brain as based on an intra-operative photography. On the lower left, we can see the anatomical picture (pre-operative MRI). In the middle, we can see the merging between anatomical scan (lower left) and ECoG grid (upper left), while on the right, we show the single electrodes' localization covering the border territory between tumor tissue, peritumoral tissue, and healthy tissue.



B. Intra-operative neuronavigation method showing different tissue types.

ECoG Signal Analysis

ECoG signals were manually inspected for quality using EEGLAB for full-length data visualization, as well as an in-house MATLAB script for event-related potential (ERP) and trial-to-trial variability (TTV) analysis. Data for electrodes, or channels, were deemed to be of untrustworthy quality if obvious artifacts were present in the averaged ERP trials, TTV trials, or in at least $\frac{1}{4}$ of the full length of channel data. The entire set of data for these bad channels was removed from further analysis. An uninterrupted 5 minutes resting-state signal was used for further analysis. We calculated the power law exponent (PLE) using an in-house MATLAB script as per previous publications (Wolff, Gomez-Pilar, et al. 2019; Wolff, Di Giovanni, et al. 2019; Z. Huang et al. 2014; He 2011, 2014). Coarse-graining spectral analysis (Yamamoto and Hughson 1991) was applied to the uninterrupted 5 min resting-state data to separate harmonic activity from the non-harmonic or scale-free activity of interest. The power spectral density of the uninterrupted 5 min resting-state data was then calculated using Welch's averaged, modified periodogram method of spectral estimation (Welch 1967) with 50% windowed segment overlap, eight window segments, and a Hamming window type. Subsequently, power spectrums of each channel were then averaged for each tissue type and this averaged spectrum was log-log transformed according to previous studies (Bullmore and Sporns 2009; He et al. 2010; Z. Huang et al. 2014). A notch filter was applied in software to the 45-55 Hz band to eliminate mains electricity noise and for

PLE-fitting continuity, a linear interpolation replaced the spectrum across this bandwidth. MATLAB's fit function was then used to fit a linear polynomial curve to log-log spectrum, and the slope of this line was extracted as the PLE value. One PLE value was extracted for each tissue type, i.e. tumoral, peritumoral and respectively tumoral, and all PLEs were compared using a paired T-test. A p value < 0.05 was considered statistically significant.

4.1.3 Results

When assessing scale free measures, we noticed that the electrodes over the tumoral tissue were found to have a higher PLE value than the surrounding electrodes for the twelve patients (Figure 4.3). This can entail that the tumoral tissue displays a higher level of entropy than the surrounding tissue.

For the six patients presenting with multiple seizures for which ECoG recordings were obtained in the two anesthetic states (i.e. awake and anesthesia), we observed that the electrodes above the peritumoral tissue displayed smaller changes between the anesthesia and awake state than the changes observed across the adjacent tissues, respectively over the HT and TT (Figure 4.4). Furthermore, the power spectrum results showed, for most of the patients, an intermediate feature of the PT's power spectrum when comparing with the results of the HT and TT as showed in the individual results (Figure 4.5 and Appendix 1).

ECoG equally allowed us to investigate scale free related changes in the resting state activity of tumor tissue and its reactivity to anesthesia: we observed an increased PLE in the anesthetic state for all types of tissues (with a larger extent within the tumoral tissue) and additionally, a different behaviour of the PT when comparing PLE in the two anesthetic states (smaller PLE changes than in the adjacent tissues). We thus noticed a lack of reaction to anesthesia in the peritumoral tissue and hypothesized that it is related to a different tissue type which maintain the already high PLE values.

Figure 4.3 Power Law Exponent in the awake resting-state activity for distinguishing healthy, tumoral and peri-tumoral tissue (* significant at $p<0.05$).

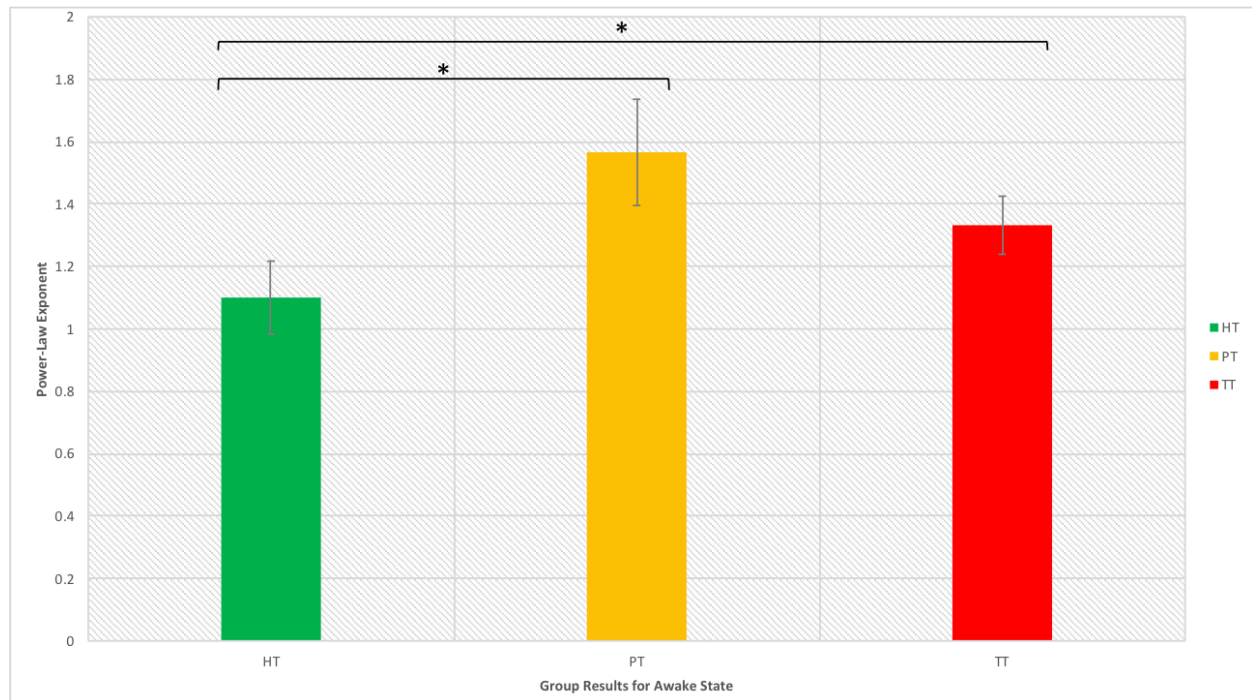


Figure 4.4 Power Law Exponent results in the anesthetic and awake states (* significant at $p<0.05$).

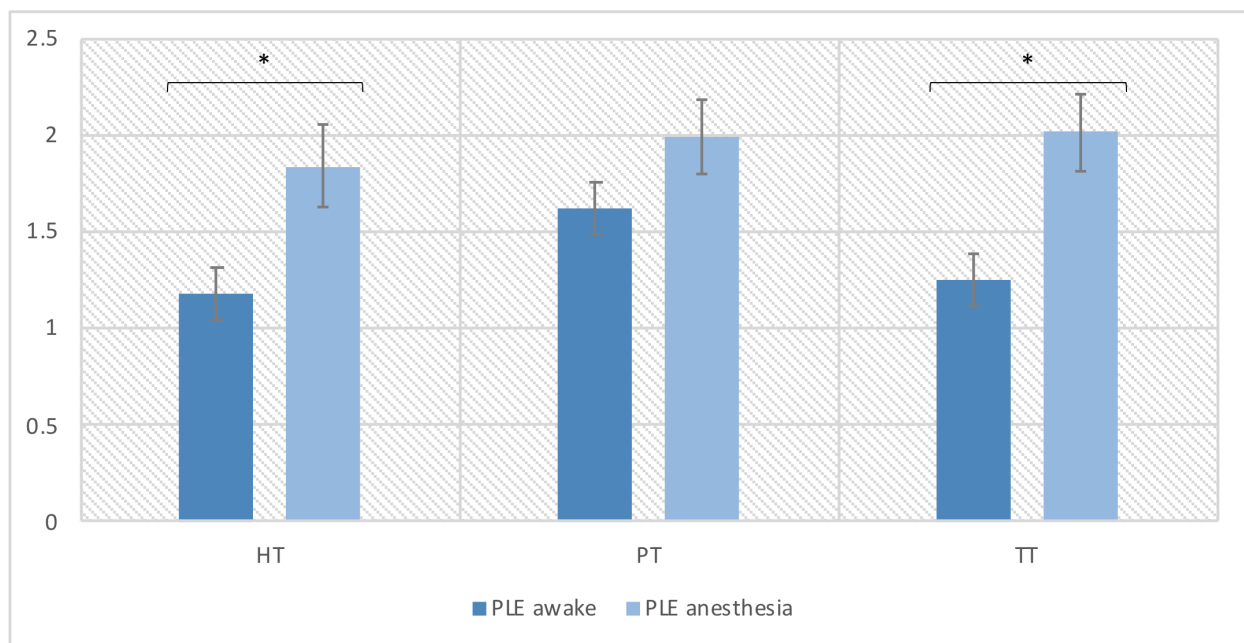
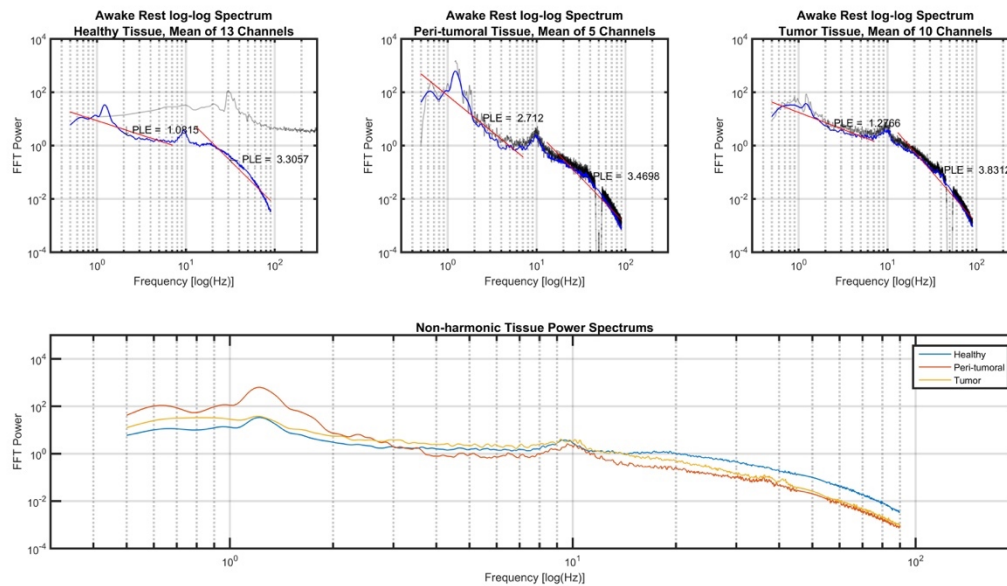
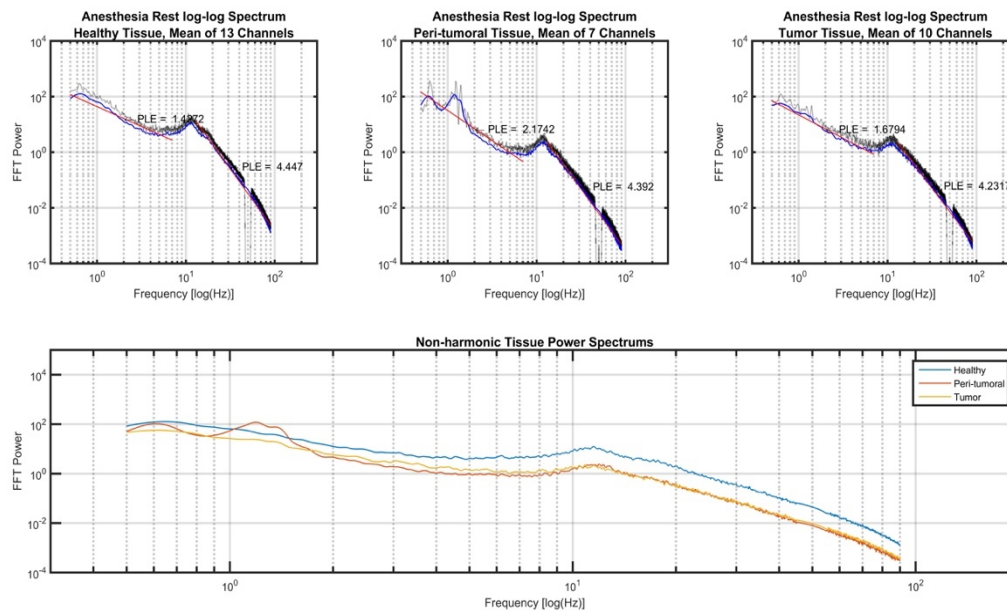


Figure 4.5 Power Law Exponent results in individual cases.

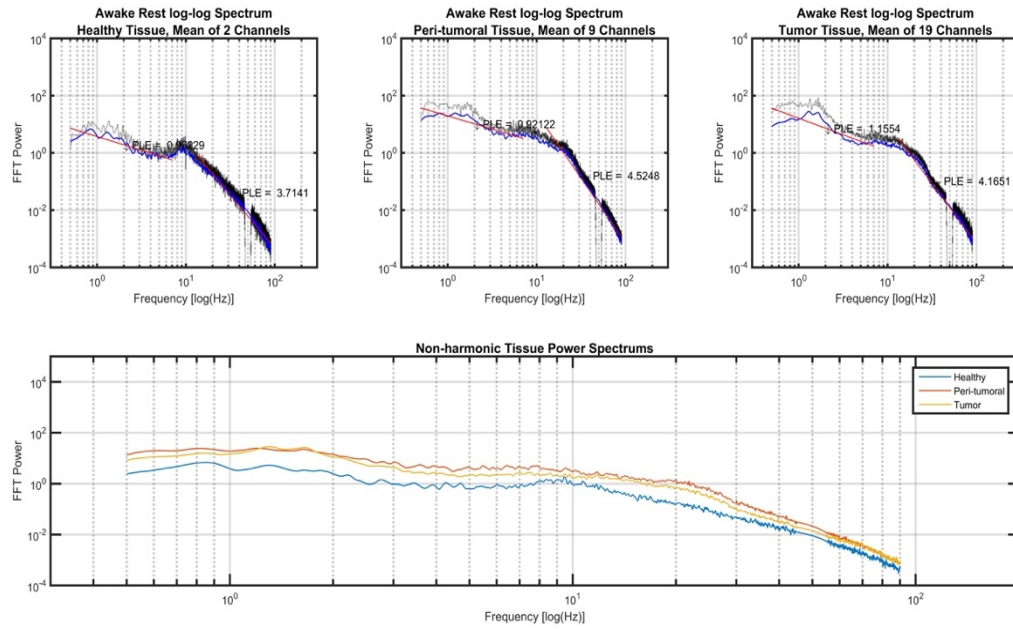
1. A Subject 1 in awake state



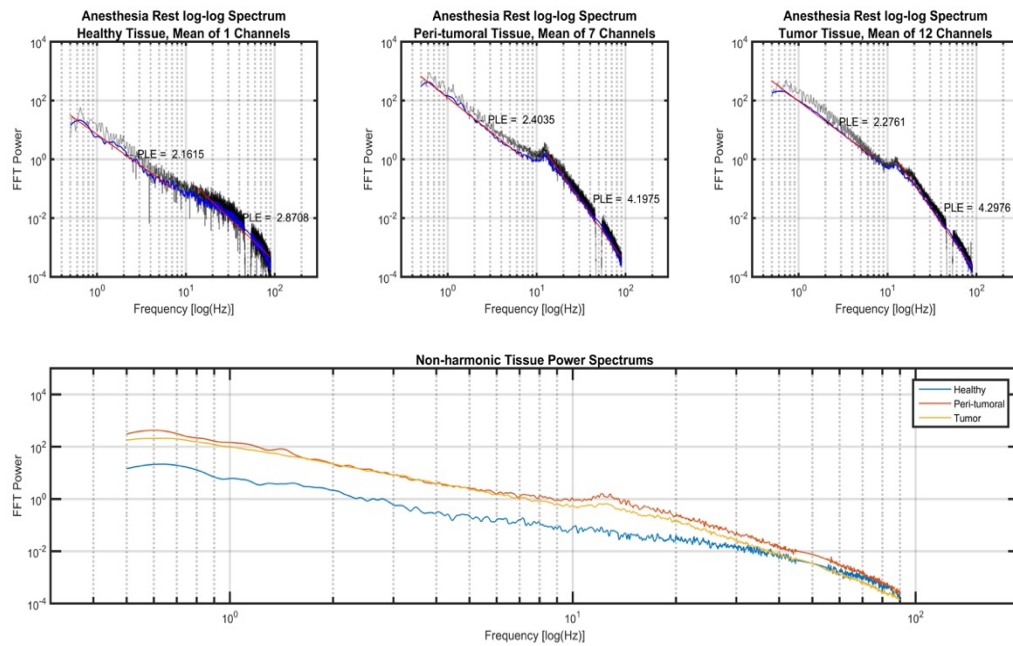
1. B Subject 1 in anesthetic state



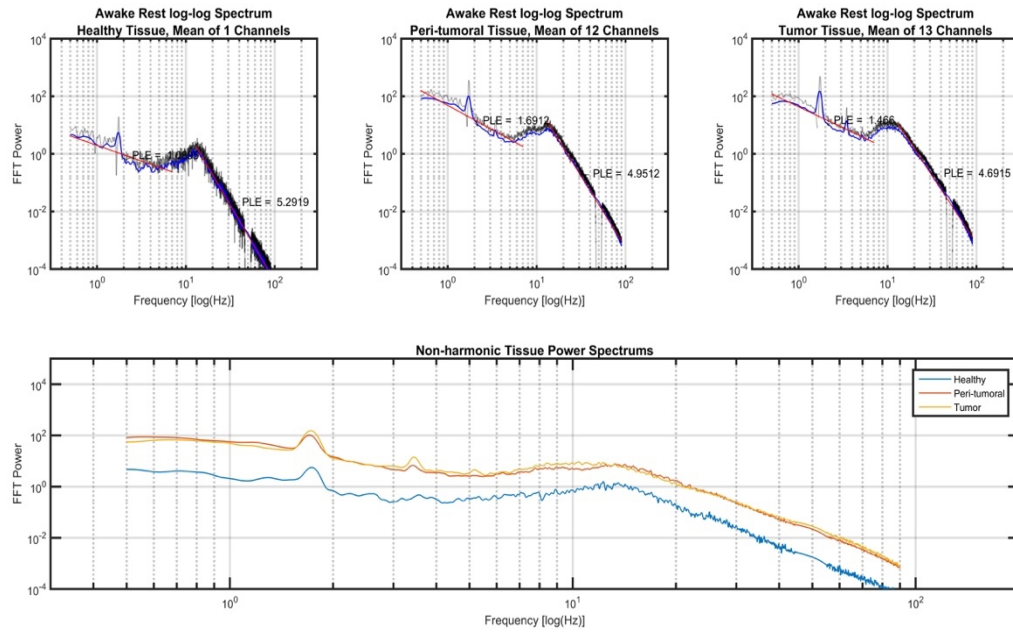
2. A Subject 2 in awake state



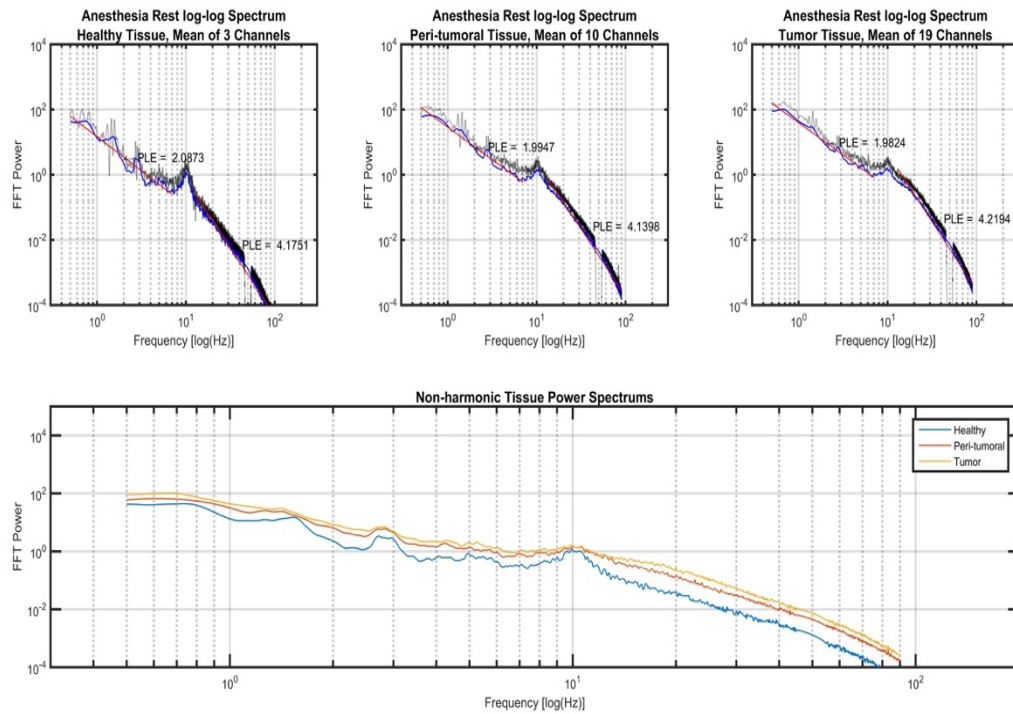
2. B Subject 2 in anesthetic state



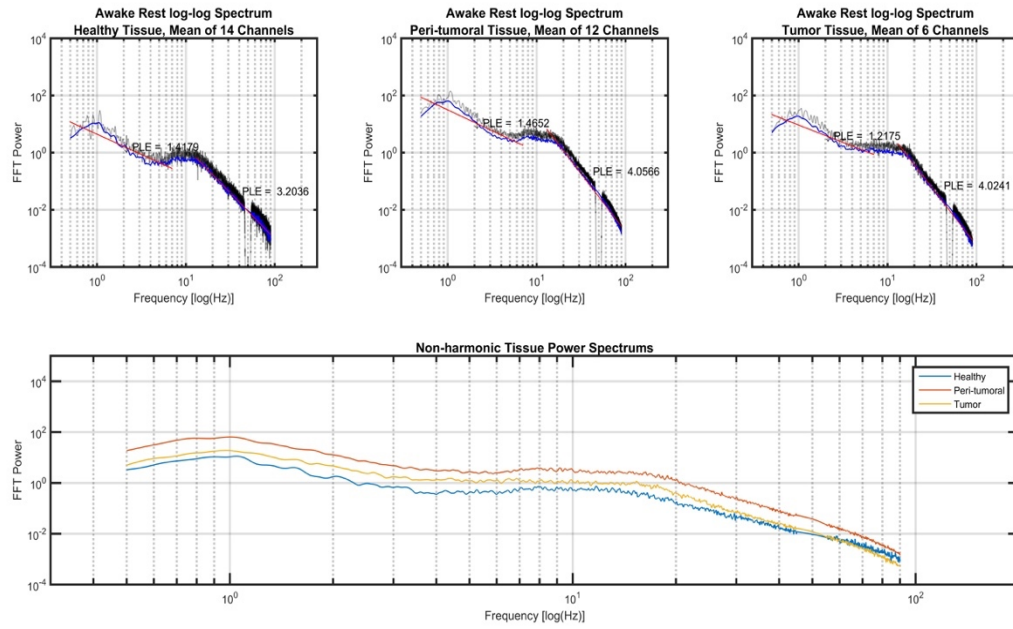
3. A Subject 3 in awake state



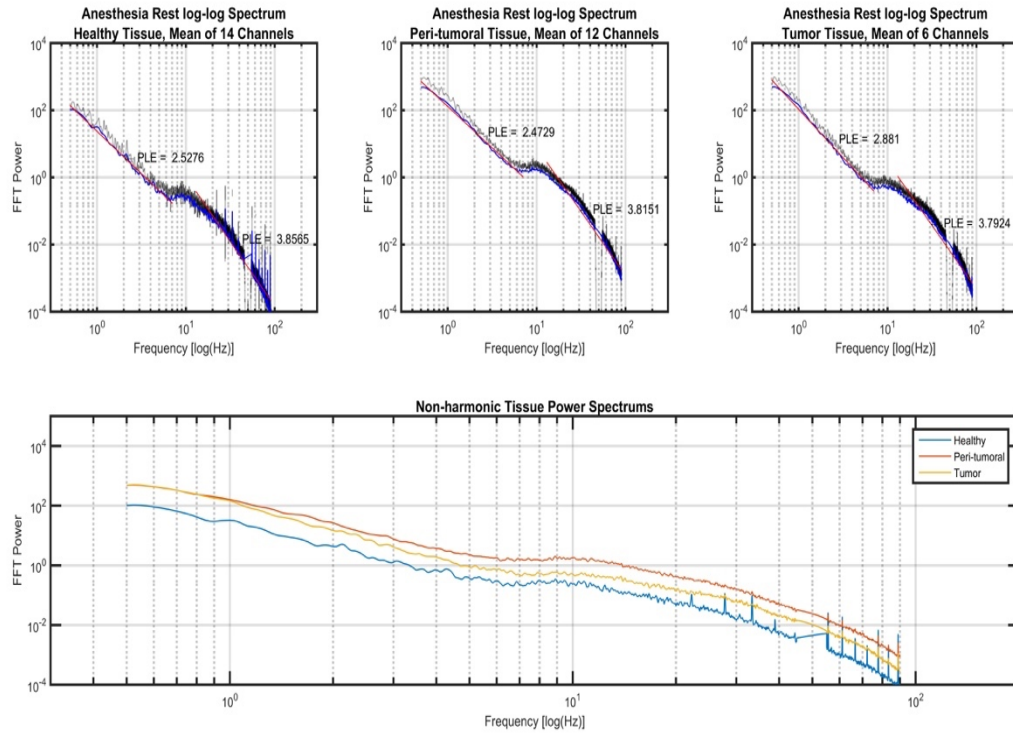
3. B Subject 3 in anesthetic state



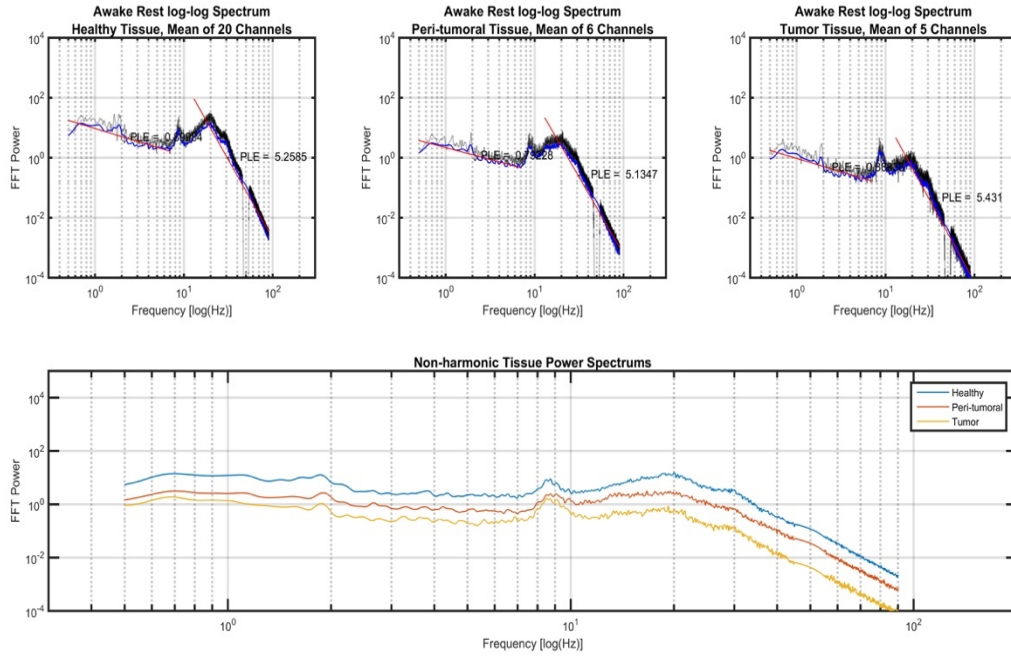
4. A Subject 4 in awake state



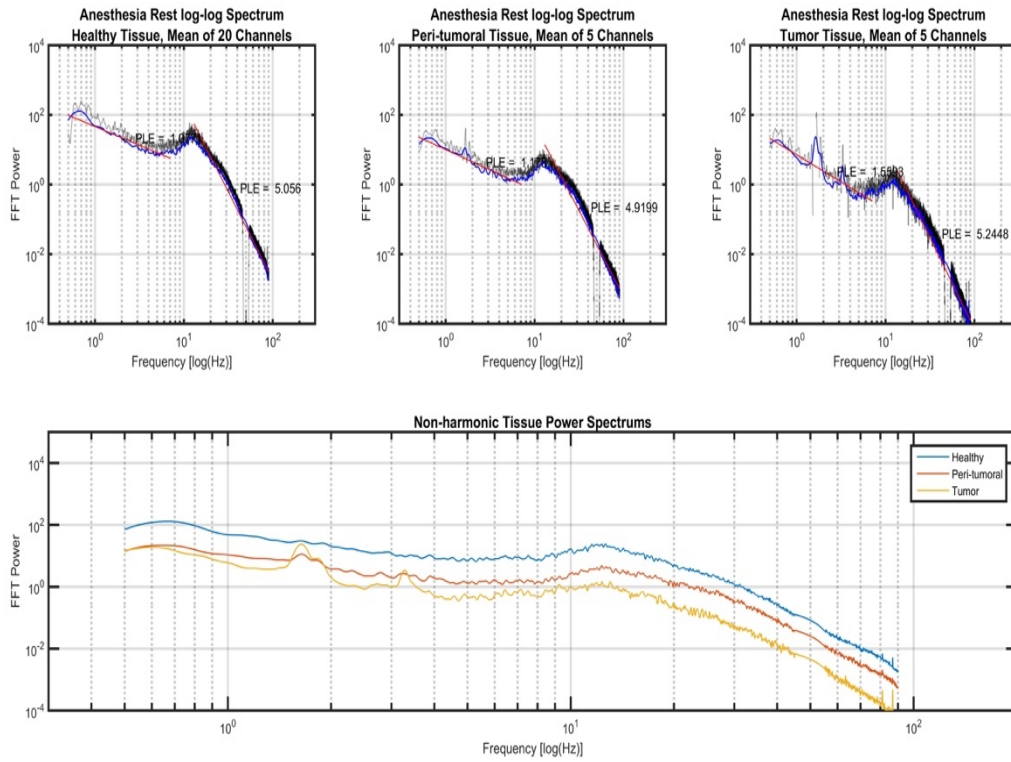
4. B Subject 4 in anesthetic state



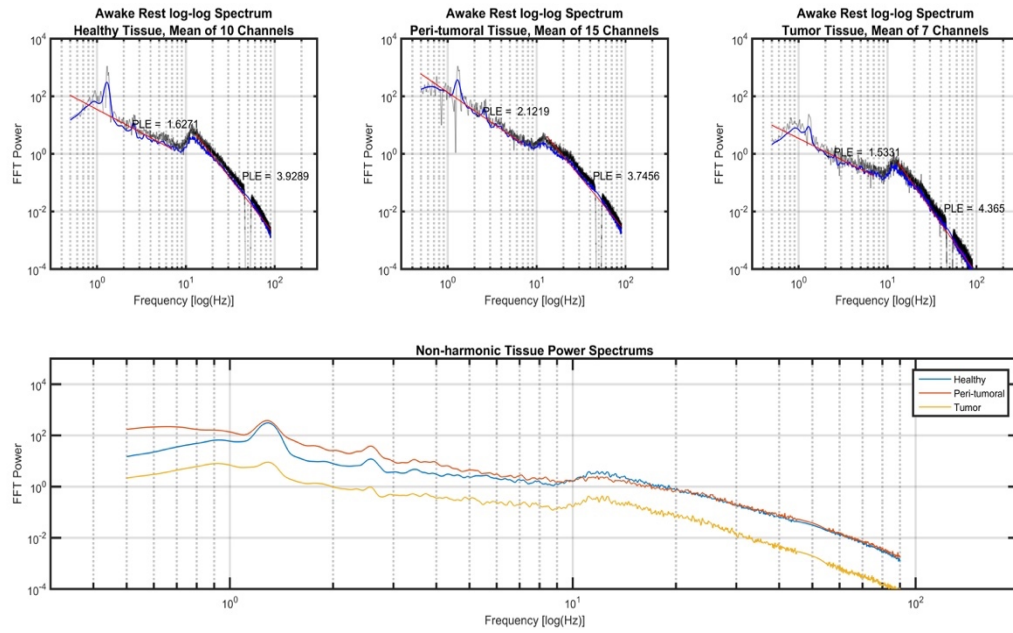
5. A Subject 5 in awake state



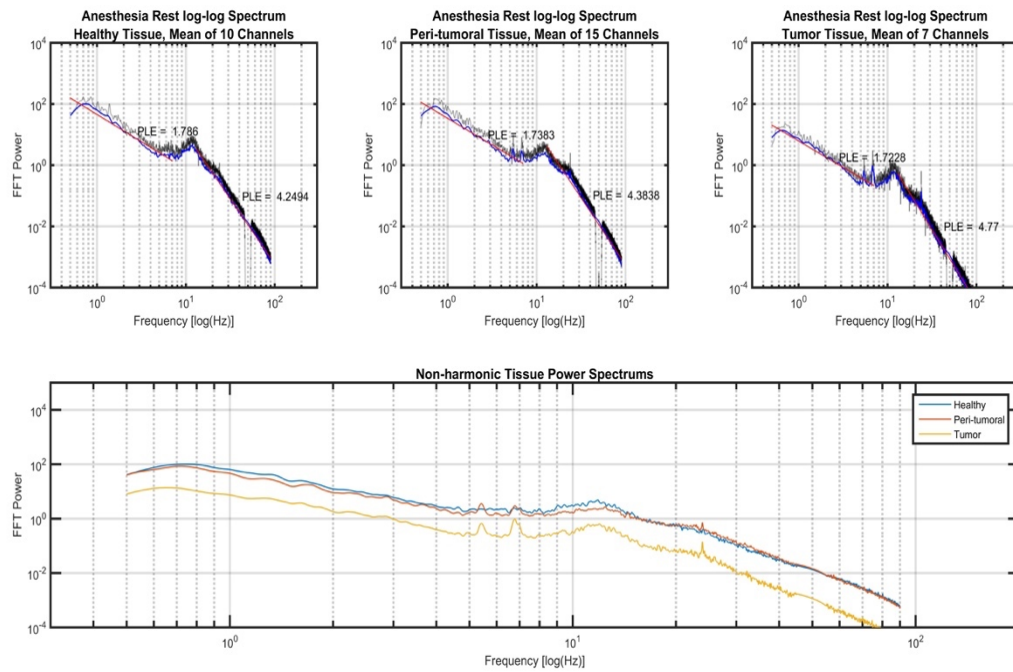
5. B Subject 5 in anesthetic state.



6. A Subject 6 in awake state.



6. B Subject 6 in anesthetic state.



4.1.4 Discussion

Extrapolating our group's previous work in functional MRI, for the first time to our knowledge, the analysis of the signal from ECoG electrodes in this study concentrated on power law exponent. Power law distributions have been reported in many distinct scientific, geographical and social phenomena and have been associated with long-term memory behavior, self-similarity and fractal structures (Tenreiro Machado, Pinto, and Lopes 2013). Power law was initially utilized to characterize the distribution of wealth in a society being known as the Pareto principle, where a small percentage of the population (20%) has a large proportion of wealth (80%), also referred as the 80-20 rule. Similarly, our investigation of the cortical electrophysiological changes of twelve patients undergoing glioma resection revealed distinct electrophysiological features across the tumoral area compared to the surrounding tissues, namely a higher PLE value over the tumoral tissue than for the surrounding areas. The tumoral tissue difference between the two anesthetic states resembled to the one observed in macroscopically healthy tissue which could imply a functionally independent state of the tumoral tissue. This has been previously suggested in recent articles, which demonstrated that gliomas develop functional multicellular network structures where tumour cells “communicate and cooperate with each other in a complex but ordered manner that is by itself reminiscent of a functional organ” (Osswald et al. 2015, 2016). Therefore, as a correlate, the tumor could be seen like the tall trees that continue to grow using more resources from the ecosystem on the expense of smaller trees (Scanlon et al. 2007), for instance surrounding cells, that don't have access anymore to the scarce resources.

Furthermore, the tumour's surrounding tissue showed specific features. As depicted in Figure 4.4 and Figure 4.5, the PT tissue displays an intermediate feature between the TT or HT-electrodes as well as a smaller PLE change when investigating the difference between the two anesthetic states. Although, no ictal or inter-ictal discharges were recorded, we suspect that these features are related to tumor cell infiltration and further studies with prolonged recordings and combined genetic analysis could test our hypothesis. The loss of PLE change in the PT between the two anesthetic states, i.e. awake and anesthesia, in patients presenting with seizures could thus suggest a distinctive state of the peritumoral area which could thus represent an interlaid area

between the brain and the functional tumor with specific characteristics favoring the generation and propagation of seizures.

The origins and mechanisms of GRE are multifactorial (M. De Groot et al. 2012) and the current evidence suggests that there is a link between epileptogenesis and glioma cell infiltration in the peritumoral area since epileptic activities seem to arise from the neocortex surrounding the gliomas. Also, glioma cell infiltration seems to promote growth and recurrence of tumors at sites around their core. In a recent study performed by Pallud et al, it was demonstrated that in slices of human tissue, the peritumoral neocortex infiltrated by glioma cells generates spontaneous interictal discharges that depend on both glutamatergic and GABAergic signaling. This link may explain both the anti-epileptic effects of oncological treatments (Koekkoek, Kerkhof, et al. 2015; Koekkoek, Dirven, et al. 2015) and the increase in seizure frequency as tumors progress (Englot et al. 2012, 2011; Chang, Potts, et al. 2008).

Early operative intervention and gross-total resection contribute strongly to seizure freedom and improving quality-of-life (Pallud et al. 2014; Breemen, Wilms, and Vecht 2007; Jongeling et al. 2015). Emerging evidence advocates an aggressive and early surgical approach and suggests that the opportune time to perform epilepsy surgery may be when the patient is already undergoing oncosurgery in the same area (Huberfeld and Vecht 2016). As such, proper seizure diagnosis and therapy requires accurate identification and management of the infiltrated peritumoral tissue.

Electrocorticography plays an important role in the localization of epileptogenic foci and for the evaluation of the effects of microsurgical procedures intraoperatively. An accurate estimation of the epileptogenic cortex and its removal requires the estimation of spatial spread of ECoG. Recent studies estimating the spatial spread of ECoG suggested that although brain's signals capture different features of the neural network, ECoG is a local signal (diameter of ~3 mm) confirming the precision required for its use in determining the epileptogenic focus (Dubey and Ray 2019). Berger and colleagues previously proposed that electrocorticography-guided epilepsy surgery for pediatric patients with brain tumors is highly effective (M S Berger et al. 1993). Given that some patient populations cannot participate during awake craniotomies (i.e. age, severe neurological deficits), several investigators have utilized high-gamma (hg) ECoG changes

during language and motor tasks to localize eloquent cortex and have reported good concordance with CSM (Sinai et al. 2005; Qiu et al. 2014; Qian et al. 2011; Miller, Weaver, and Ojemann 2009; Genetti et al. 2013). High gamma activity (HGA) between 80 and 140 Hz on electrocorticography is assumed to reflect localized cortical processing, whereas the cortico-cortical evoked potential (CCEP) can reflect bidirectional responses evoked by monophasic pulse stimuli to the language cortices when there is no patient cooperation. The use of "passive" mapping was thus proposed by combining HGA mapping and CCEP recording without active tasks during resections of brain tumors in the awake state (Y. Tamura et al. 2016).

In tumoral epilepsy surgery, the first aim is rather to maximize the extent of tumor resection while minimizing postsurgical morbidity, in order to increase the median survival as well as to preserve the quality of life (Hugues Duffau 2013b). Gross-total resection of tumors is also a critical factor in achieving seizure freedom (Englot et al. 2011) in infiltrating tumors such as gliomas, where these lesions migrate along white matter tracts. A significantly higher resection volume was associated with a higher chance of favorable seizure outcome in multiple studies, especially in lesional epilepsy involving long-term epilepsy-associated diffuse glioma (Roessler et al. 2016). As such, extraoperative mapping by strips/grids is often not sufficient in tumoral surgery, since in essence, it allows study of the cortex but cannot map subcortical pathways (Hugues Duffau 2013b). Although, recent research demonstrated that awake craniotomy can be performed safely without ECoG, even in patients with preoperative intractable epilepsy (Boetto et al. 2015), some studies showed that when complete ECoG-guided resection is performed there is a greater likelihood of long-term seizure freedom (Fallah et al. 2015).

The concept of individualized surgery for managing glial tumors is based on the goal of achieving a maximal tumor resection and seizure freedom without inducing new neurological deficits (Robles et al. 2008; H Duffau 2015; Mandonnet and Duffau 2014; Hugues Duffau 2012). We already dispose of ample information in terms of the clinical and biological behavior of gliomas. Nonetheless, the persistent challenge remains integrating the information obtained from various analyses in a way that provides a comprehensive view of the alterations underlying the interplay between epileptogenesis and oncology. Scale free measures such as PLE are ubiquitously present in many scientific topics and this research represents the first confirmation

of its relevance to the tumoral and peri-tumoral tissue. Further research including in-vivo neural recordings could help improve our insight into latent sources of PLE changes.

4.1.6 Genetic correlates of GRE

Several studies suggested that tumor invasion may be linked to epileptogenesis since epileptic activities seem to arise from the neocortex surrounding the gliomas (Haglund et al., 1992; Pallud et al., 2014). The perilesional cortex resected from patients with epilepsy associated with different types of focal brain lesions, including glial tumours, revealed different cytoarchitectural and neurochemical changes (Goel et al., 2003; Shamji et al., 2009). Previous research showed that peritumoral neurons show downregulation of inhibitory synapses and upregulation of excitatory synapses (McNamara, 1999) with abnormal electrophysiological properties (Steriade and Amzica, 1999).

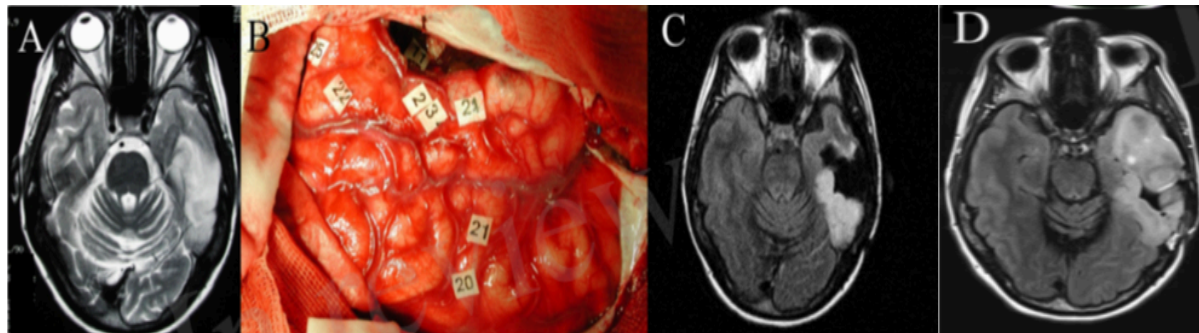
Increasing malignancy of gliomas was previously reported to correlate with a decrease in the density of γ -aminobutyric acid (GABA) binding sites (Jussofie et al., 1994), suggesting an association of the proliferative activity and the expression of GABA receptors. Evidence that GABA and GABA_A receptors play an active role in inhibiting proliferation was also obtained for non-malignant but proliferating cells, the cortical progenitor neurons (LoTurco et al., 1995). The expression of GABA receptors was also linked with growth of glioma cells as the disappearance of functional GABA_A receptors parallels unlimited growth typical for malignant tumours lines (Young et al., 2009). Smits et al. compared mRNA levels of all nineteen GABA-A channel subunits in gliomas of different malignancy grade and histological subtype and demonstrated functional binding sites for the GABA-A channel receptor in low-grade and anaplastic gliomas but not in glioblastomas (Smits et al., 2012). This finding was consistent with other reports and it was postulated that tumor cells equipped with functional GABA-A channels may be able to maintain low or intermediate proliferative activity with physiological levels of GABA in the extracellular fluid (Hu et al., 2015; Olsen et al., 2008).

Previous work published by our team in this field postulates that GABA-A receptor density is a marker of structural (and functional) integrity of tissue (Qin et al., 2015, Duncan et al. 2013, Duncan et al., 2012, Fourget et al., 2011). Moreover, our team demonstrated that structural brain

damage can be reflected in cortical density of the benzodiazepine receptor, which is a part of the GABA receptor complex (Wu et al., 2015). The distribution of the benzodiazepine receptor represents a highly sensitive indicator of even subtle structural damage and it can be assessed by the ligand 11 C-flumazenil. We therefore hypothesized that an unbalance between GABAergic inhibitory signals and the excitatory pathways is present in the already infiltrated but not macroscopically-altered transitory zone and the non-infiltrated tissue. Additionally, this hypothesis is grounded on the fact that seizures in general, as the predominant clinical symptom in patients with gliomas, are closely linked to abnormal levels of GABAergic mediated neural inhibition. As shown in the Supplementary Appendix from Chapter 5 (Figure S6), we found distinct GABA alterations in tumoral and peritumoral tissue of patients with glioma.

In summary, the origins and mechanisms of GRE are multifactorial however the current evidence suggests that there is a link between glioma cell infiltration into the cortical tissue and the abnormal GABA presence in the peritumoral area which may explain the increase in seizure frequency as tumors progress (Figure 4.6). Two clinical studies that we performed to assess the relationship between tumor recurrence and seizures confirmed this finding (Poster 1 and Poster 2 in Appendix). Thus, the peritumoral neocortex around gliomas seems to represent a pivotal structure both for the genesis of epileptic activity and for infiltration by glioma cells, which has significant implications in terms of the treatment recommended to the patients and their oncological prognosis (Englot et al., 2012; De Groot et al., 2012). As it is currently postulated that the peri-tumoral cortex and an imbalance between excitatory and inhibitory pathways may represent a key factor for the generation and propagation of seizure activity, a detailed analysis of the genetic and epigenetic alterations could clarify the tumor-related epileptogenesis.

Figure 4.6. Example of the epileptic seizure history for a patient with a left temporal diffuse low-grade glioma.



Axial FLAIR-weighted MRI (A) showing a left temporal DLGG in a 36-year-old patient who presented with isolated seizures and no neurological deficits. Intraoperative photograph during the first awake surgery (B), after resection was performed according to individual functional boundaries. Postoperative axial FLAIR-weighted MRI (C) revealing a partial resection, with a posterior residual tumor voluntarily left for functional reasons. Ten years later, epileptic seizures recurred concomitantly with an imaging progression as demonstrated on the axial FLAIR-weighted MRI (D) (from Ghinda and Duffau, 2017).

4.1.7 Magnetic Image Spectroscopy in Glioma

In order to investigate in a non-invasive fashion biochemical markers like glutamate and GABA to distinguish infiltrated and non-infiltrated tissue across the radiologically-defined macroscopic boundaries of TT and HT we attempted using multi-voxel magnetic resonance spectroscopy (MRS) to characterize different tissues during resting state (see Figure 4.7 and 4.8 for multi-voxel MRS in single subject with glioma). As genetic data show a potential important role of glutamate in glioma (J. de Groot and Sontheimer 2011; Simon and von Lehe 2011; Maus and Peters 2017) we hypothesized altered, i.e., increased glutamate levels, in both infiltrated PT and TT compared to non-infiltrated PT and HT in resting state multi-voxel MRS.

A pilot data was performed at the Ottawa Hospital and the Brain Imaging Center in Ottawa and we were able to show increased glutamate in TT of high-grade glioma, i.e., GBM, when compared to low-grade glioma (Figure 4.9 and 4.10). The same regions, i.e., PT, TT, and HT, were targeted as voxels in multi-voxel MRS to measure metabolic and cellular substances like NAA (N-Acetyl-aspartate), cholin, myositol, and lactate as well as biochemical measures like glutamate and GABA (Figure 4.9 and 4.10) (Z. Huang et al. 2017; Wiebking et al. 2014; Bogner et al. 2014). The results obtained during our investigation are showed below.

Figure 4.7 Multi-voxel MRS in a pilot subject with glioma

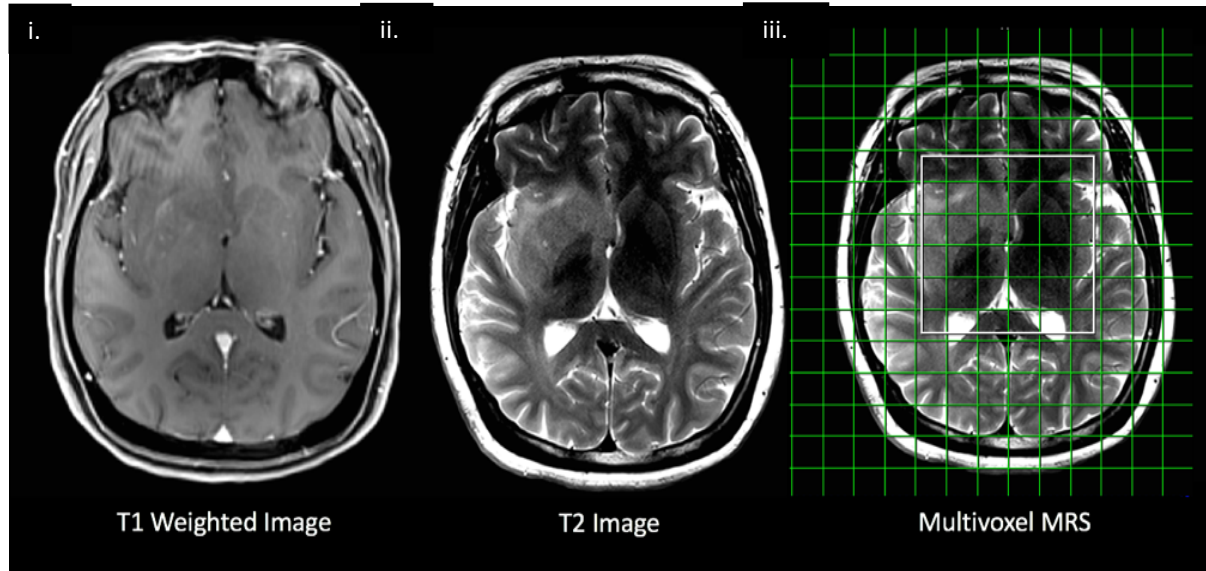


Figure 4.7.a The figure shows on the (i) anatomical images (T1WI), (ii) the anatomical T2 image; and (iii) the multi-voxel grid of MRS overlaid on T2 image by the of a patient with a right temporal glioma.

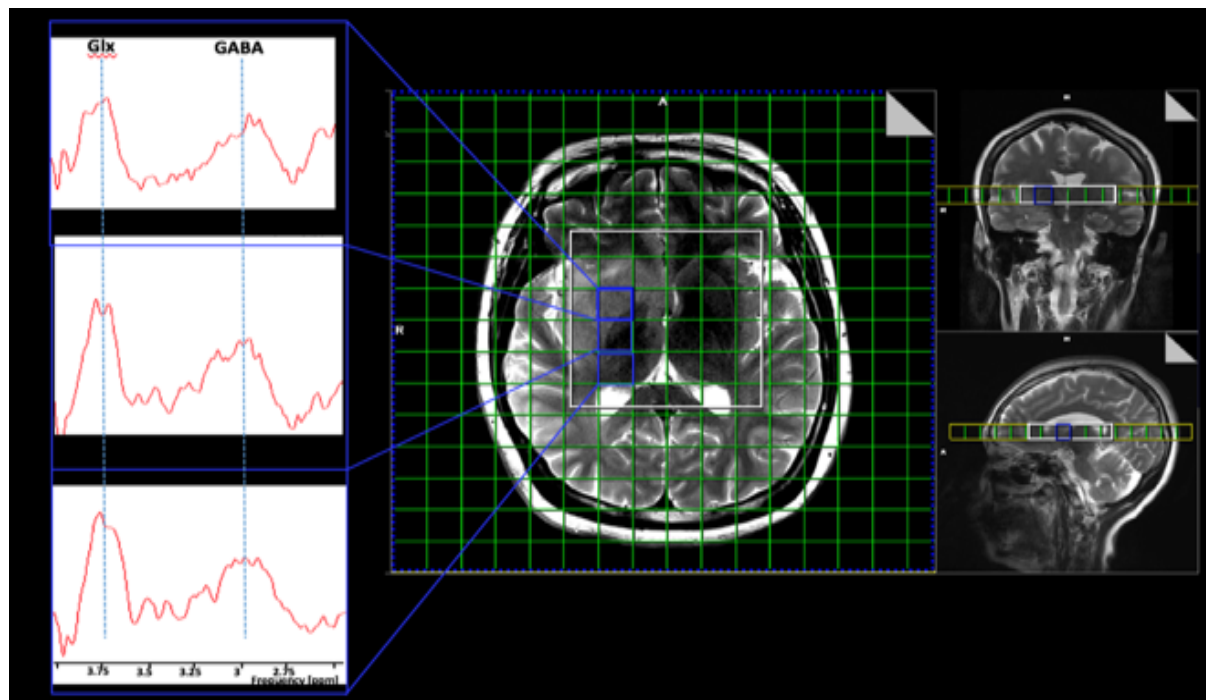


Figure 4.7.b: The figure shows the results of the above shown subject (see image in the middle of the panel) for the spectra of Glx (glutamate and glutamine) and GABA for three voxels GABA and Glx (glutamate and glutamine) in three different voxel of the multi-voxel grid (left and middle) on the lower level of the brain (see on the right) as acquired in multi-voxel MRS.

Figure 4.8 Orientation of the MRS slice.

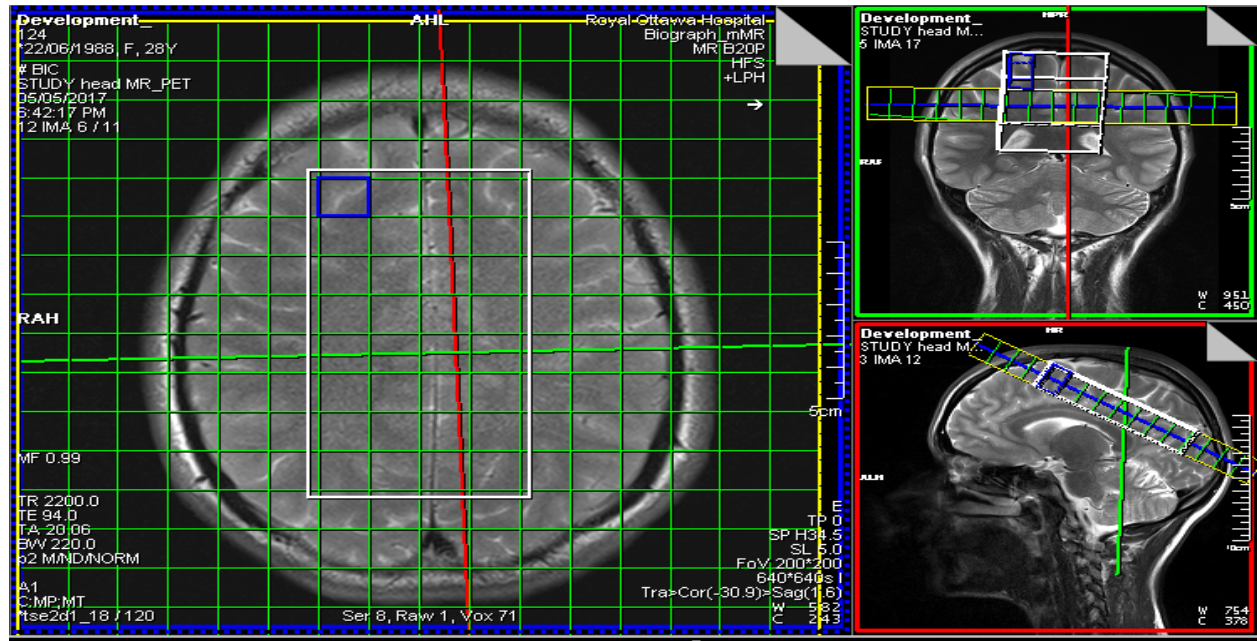
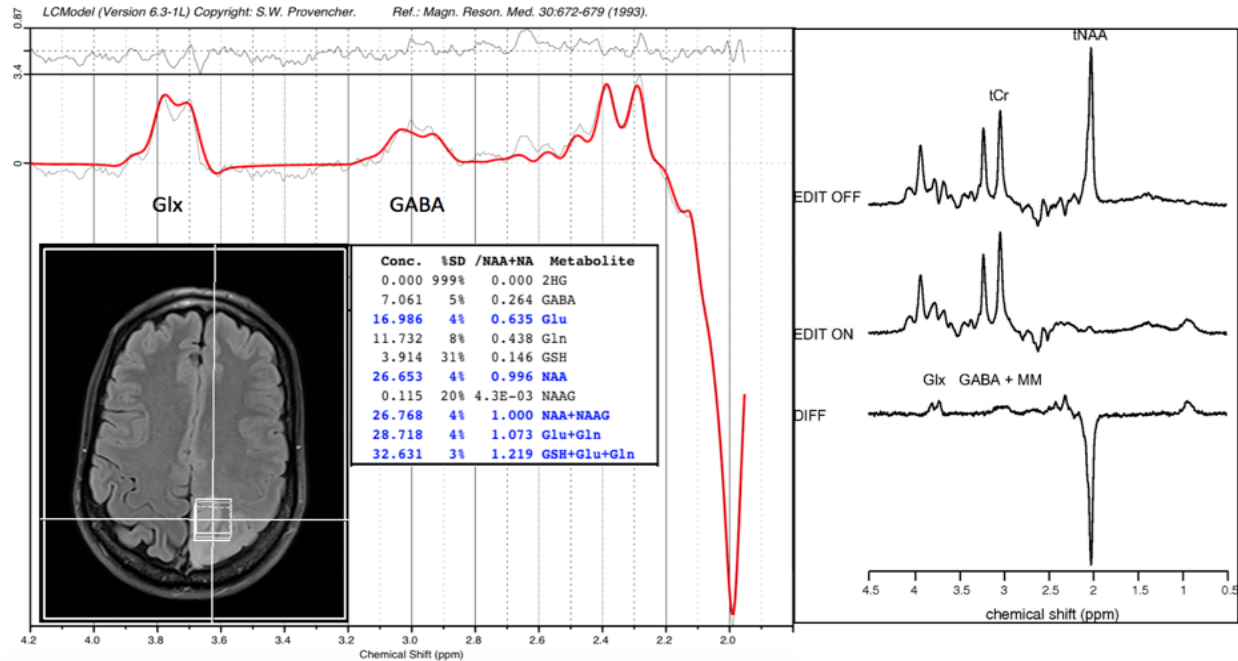


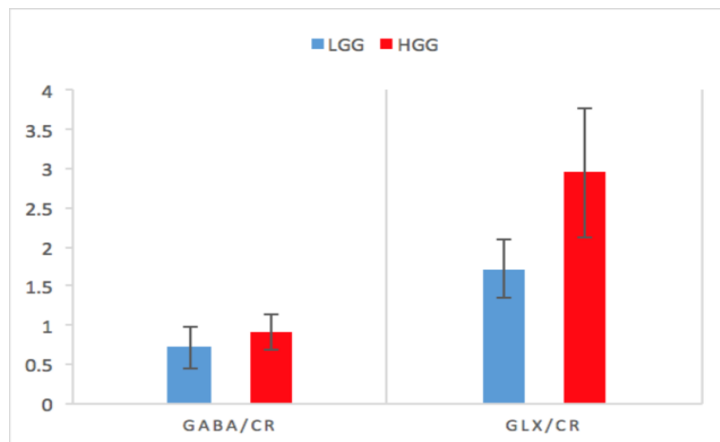
Figure 4.9. Single-voxel pilot MRS data from subjects with low- and high-grade glioma



The MRI image on the left shows the localization of the voxel for acquiring single-voxel spectra using the MEGA-PRESS sequence as acquired in Ottawa. While the rest of the figure shows the spectra as obtained in that particular voxel focusing on the glioma tumor tissue itself (white area in image). The spectra quality is good as the CRLB values for the main metabolites (like GABA, Glutamate, Glutamate and

Glutamine (Glx), NAA) in below 10%. Schema on the right represents the “EDIT OFF,” “EDIT ON,” and difference (DIFF) spectra as applied in our data (figure from Tremblay et al., 2013). The Glx (glutamate and glutamine) and GABA were obtained from the difference spectrum.

Figure 4.10 Single-voxel MRS data results from subjects with glioma



The figure shows the MRS data for concentrations of GABA (as against Creatine, GABA/Cr; left) and Glutamate/Glutamine (as against Creatine, Glx/Cr; right) for $n = 3$ subjects with low-grade glioma (LGG; blue bars) (i.e., grade II diffuse astrocytoma) and $n = 6$ subjects with high-grade glioma (HGG; red bars) (i.e., grade III and IV glioma) using single voxel spectra over the tumour area (as shown above in Figure 4.9). The main result here is that the concentration of Glx (including Glutamate and Glutamine) (panel on the right) is elevated in the high-grade glioma (red bar) when compared to the low-grade glioma (blue bar) whereas that is not the case for GABA (see panel on the left).

4.1.8 Conclusion

In summary, we present for the first time distinct power law exponent features in the tumoral and peritumoral tissue of gliomas that seems to represent a critical structure both for the onset and propagation of epileptiform activity and for infiltration by glioma cells. Although neuro-oncology and epilepsy surgery have traditionally been separate disciplines, we postulate that the analysis of the mechanisms underlying the tumor related epilepsy might equally yield significant insight into tumorigenesis. We expect that the contributions made from a multidisciplinary approach to unravel the complex pathways underlying the clinical features of gliomas related epilepsy will lead to meaningful progress for the patients affected by these devastating tumors.

Article 4. Electrophysiological signature of tumoral tissue from scale-free analysis of intraoperative ECoG during awake craniotomy for glioma-related epilepsy

4.2 Intraoperative electrocorticographic-guided surgery for patients with glioma

4.2.1 Introduction

Electrocorticography plays an important role in the localization of epileptogenic foci and evaluation of the effects of microsurgical procedures intraoperatively. Awake craniotomy in brain lesions has allowed an improvement of both oncologic and neurological outcomes however, the occurrence of intraoperative seizures were previously reported as a cause of failure of awake craniotomy. Furthermore, despite a high success rates of surgical outcomes in these series, approximately 20% of patients do not reach the complete postsurgical control of seizures. Therefore, the use of more precise preoperative evaluation and adequate surgical techniques is mandatory.

Resection of lesions near the eloquent cortex of brain necessitates awake craniotomy to reduce the risk of permanent neurologic deficits during surgery. Electrostimulation in awake brain mapping is widely used to guide tumor removal, but methodologies can differ substantially across institutions. Electrocortical stimulation (ECS) is the gold standard for functional brain mapping; however, precise functional mapping is still difficult in patients with language deficits. High gamma activity (HGA) between 80 and 140 Hz on electrocorticography is assumed to reflect localized cortical processing, whereas the cortico-cortical evoked potential (CCEP) can reflect bidirectional responses evoked by monophasic pulse stimuli to the language cortices when there is no patient cooperation. The use of "passive" mapping was thus proposed by combining HGA mapping and CCEP recording without active tasks during resections of brain tumors during the awake state (Tamura et al. 2016). Moreover, intrasubject and inter-subject variations of the minimum thresholds of positive naming areas and changes in the type of response and in the size of these areas according to the intensity were previously demonstrated which may limit the interpretation of data from electrostimulation in awake brain mapping (Roux et al. 2016). As such, ECoG was used to establish a low-threshold mapping and to monitor the occurrence of after-discharges. In this section, novel features data analyses methods were used to analyze task - evoked ECoG data.

4.2.2 Methods

Patients

Eight patients with a newly diagnosed intracranial glioma were enrolled; the patients underwent awake craniotomy for tumor resection with ECoG recordings as described in the previous section. In addition to the 5 min recording in the awake resting state, we equally acquired 5 min of recording for the task-state (patient listening to auditory stimuli as described in the Experimental Design section below).

Intra-operative ECoG electrode localization

In order to determine the accuracy of our method, for one subject, we equally performed an intra-operative MRI immediately following the ECoG recording. The cortical surface was segmented from the intra-operative MRI and both pre-op and intra-op MRIs were merged into a common coordinate space, using the patient's fiducials. The photograph of the ECoG grid was then merged with the cortical segmented surface using visible sulci and confirmed with intra-operative neuronavigation which allowed us to localize precisely the electrodes on the segmented cortical surface. We then transferred the electrode locations to the cortical surface segmented from the intra-operative MRI. The 3-dimensional brain shift was then estimated in the Euclidean space using the following formula:

$$d(p, q) = \sqrt{(p_1 - q_1)^2 + (p_2 - q_2)^2 + (p_3 - q_3)^2}.$$

where p (p_1, p_2, p_3) and q (q_1, q_2, q_3) are 2 points in the 3-D space. The intra-operative localization and confirmation of the technique with the intra-operative MRI revealed a median brain shift of 2.7 mm (Figure 4.11).

Experimental Design

Two auditory stimuli with distinct self-relatedness were used: the subject's own name and an unknown person's name. Each of the names was presented by a familiar voice (one of the patient's family members), and digitally recorded into an audio clip lasting for 0.5 s. The unknown names

were matched to the patient's own name by gender and number of syllables. A sparse event-related design was used for the task data acquisition. 60 own and 60 other names were delivered in a pseudo-random order with an inter-trial interval between 3 and 4 sec (Lipsman et al. 2014; Qin et al. 2012; Qin and Northoff 2011). All stimuli were programmed and delivered using E-Prime (Psychology Software Tools, Pittsburgh, PA) and the volume of the headphones was adjusted to the comfort level.

ECoG Signal Analysis

ECoG signals were manually inspected for bad channels and removed from analysis if the signal quality was too noisy, such as in loose contact. For the rest of the analysis, the signal was band-pass filtered between 0.5 and 70 Hz, and segmented according to the auditory stimuli with 0.5 second before and 2 second after the stimulus. The extracted epochs were averaged according to the start time of the auditory stimuli (time 0). The averaged ERP was then baseline corrected by subtracting the average value between -0.5 to 0 second.

Time-frequency decomposition of each trial along each channel was performed to construct the spectral-temporal structure according to the pre-defined stimulus. Morlet wavelet of the following form:

$$\varphi(\tau, t) = \exp\frac{i2\pi t}{\tau} \exp\frac{-t^2}{2\tau^2} \quad (1)$$

was convoluted with the ECoG channel time series to get a time-frequency estimate for every $f = 1/\tau$:

$$V(f, t) = \sum_{\tau'=-5\tau/2}^{5\tau/2} V(t + \tau') \varphi(\tau, \tau') \quad (2)$$

A total of 5 cycles (5τ) was used to estimate the amplitude and phase of the signal at each frequency for every point in time.

Event-related spectrum perturbation (ERSP) was used to visualize the event-related spectrum power dynamics in a range of frequencies. It generalizes the narrow-band event-related desynchronization/ synchronization (ERD/ERS) measures (Pfurtscheller and da Silva 1999). In brief,

$$F(f, t) = \frac{1}{n} \sum_{k=1}^n |V_k(f, t)|^2 \quad (3)$$

Let A be the baseline value for ERSP with $t_1 = -0.5s$ and $t_2 = 0s$

$$A = \frac{1}{t_2 - t_1} \sum_{t=t_1}^{t_2} F(f, t) \quad (4)$$

Then the relative ERSP across time and frequency was defined as:

$$ERSP(f, t) = \frac{F(f, t) - A}{A} \quad (5)$$

Significance of the deviation of the $ERSP(f, t)$ from the baseline power was assessed using a bootstrap method. For each trial, the spectrum estimate from randomly selected latency windows in baseline interval was constructed then averaged across trials. This procedure was applied several hundred times then a surrogate ‘baseline’ power distribution was constructed. The significance of the ERSP power changes was determined from the surrogate ‘baseline’ with $p=0.01$, and non-significant part was wiped out, i.e. ERSP was set to zero.

Trial-to-Trial Variability (TTV) was defined as the variability changes with respect to variability at the stimulus onset (time 0) (He and Zempel 2013). The standard deviation (SD) at each time point across above extracted trials was calculated, resulting a SD change across the segmented epoch. The reference was selected as the SD value at time 0, and each time point of SD was subtracted by the reference value and then divided by the reference value (He and Zempel 2013). [0.5 70] Hz was selected as the broad frequency band for TTV calculation. Moreover, frequency bands in Theta [3 8], Alpha [8 13], Beta [13 30], Gamma [30 70] were also used for TTV calculation. The procedure was performed similar to the above section, but with different frequency band for band-pass filtering.

4.2.3 Results

Our investigation of the cortical electrophysiological changes occurring in the setting of eight patients undergoing craniotomy for resection of glioma, revealed distinct spectral features across tumoral area compared to the surrounding tissues.

Reactivity to external stimulus is decreased in tumoral tissue

Due to the limited number of trials, all the stimuli including the self and non-self stimulus were pooled together, and the TTV on every selected electrode was presented in Figure 4.12.A for healthy tissue, and Figure 4.12.B for tumor tissue. The healthy tissue exhibited a clear TTV reduction, while the tumor tissue did not show a clear TTV on average. Moreover, the pre-stim

variance significantly and negatively correlated with TTV-index in healthy tissue, with $R = -0.70$ and bootstrap 95% confidence interval of $[-0.87 -0.45]$, while no significant correlation was shown in tumor tissue ($R = -0.27$, CI $[-0.59 0.02]$) (Figure 4.12. D, E).

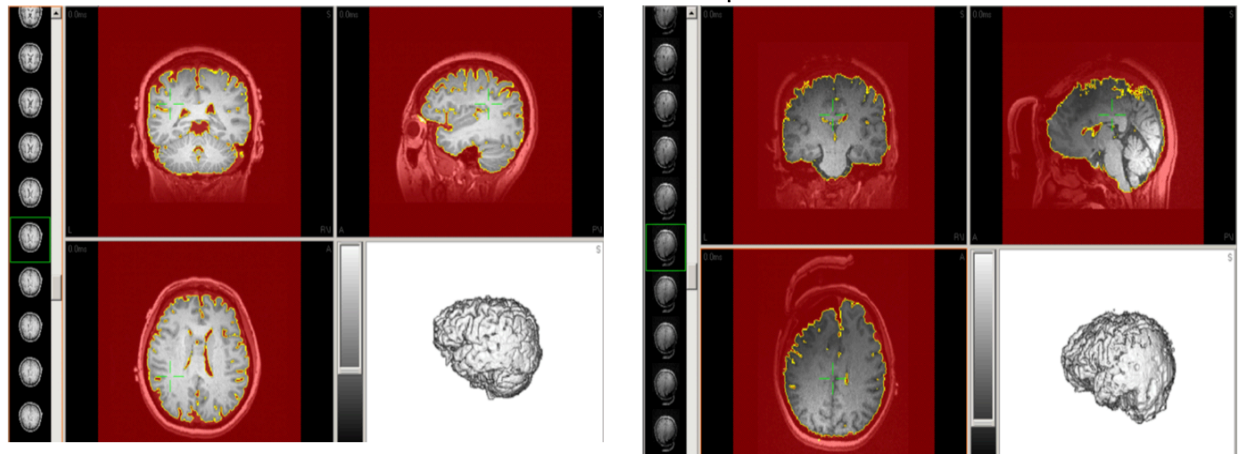
ECoG allowed us to investigate changes in resting state activity in tumor tissue electrodes showing lower power in faster frequencies within the pre-stim time period (Figure 4.12. F). Figure 3.7 G shows the bar plot which indicates the significant ($p < 0.0054$) alpha band power difference within pre-stim period between the healthy and tumoral tissue. Furthermore, given the previous results, we extended our analysis to the electrodes immediately surrounding the tumoral tissues (over the PT area). The pre-stimulus power comparison across different frequencies in the three tissues types showed that the peritumoral tissue has an intermediate feature when comparing to the HT and TT (Fig. 4.13).

Trial- to-trial variability for individualized features

The ECoG data on task-evoked activity demonstrated distinct levels of TTV reduction between these peritumoral electrodes which could be considered as index of the presence (no TTV reduction) or absence (TTV reduction) of infiltration of tumor cells into peritumoral tissue (Figure 4.14). Nonetheless, such hypothesis requires further confirmation by analysis of the specific tissue. Examples of patients with different TTV results are portrayed in Figure 4.14, 4.15 and 4.16.

Figure 4.11 ECoG localization method via intra-operative MRI.

A. Cortical surface segmented from the:



B. Pre-op and intra-op MRI's merged into a common coordinate space, using fiducials

C. 3-dimensional brain shift estimated in the Euclidean space

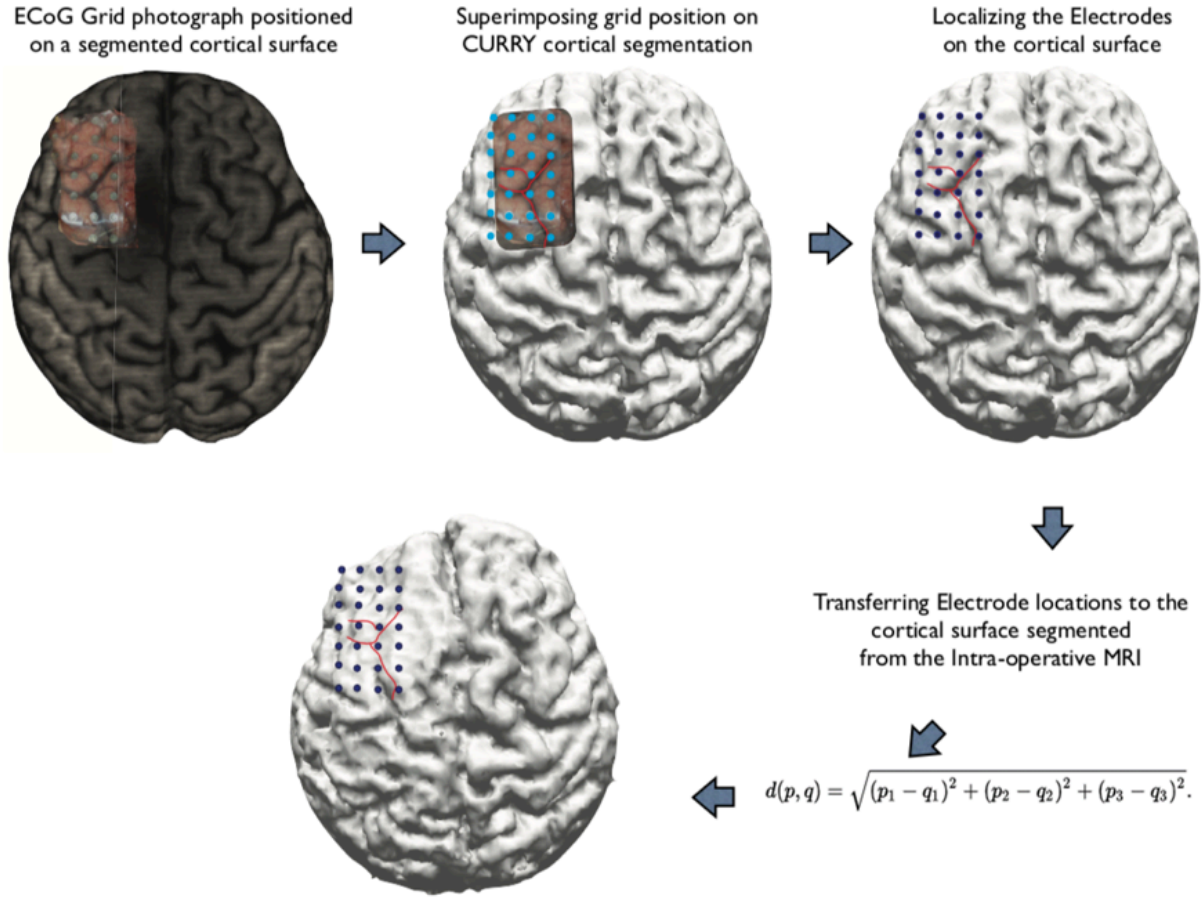
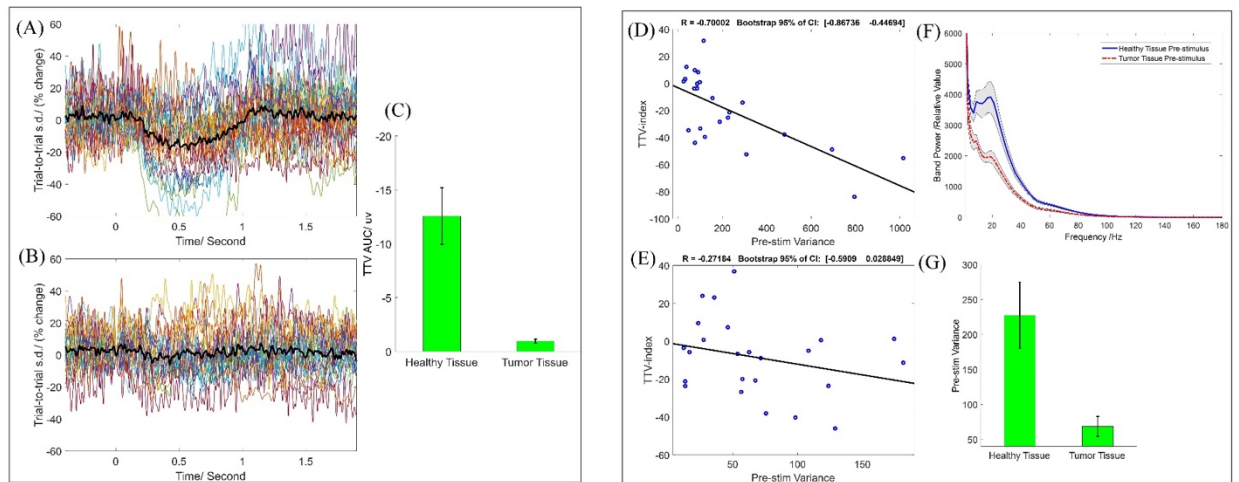


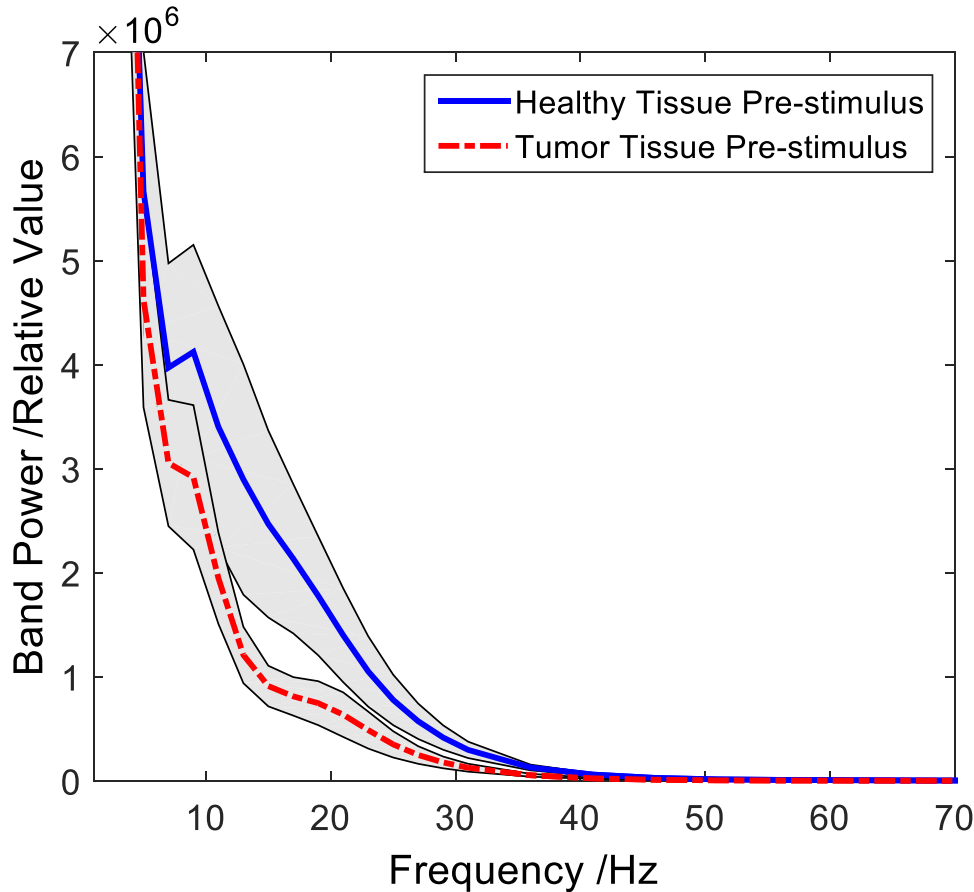
Figure 4.12 TTV and resting state activity in ECoG in healthy and tumor tissue.



A) TTV in healthy tissue, each curve represents one electrode, and bold-black line represents the averaged across all the electrodes. (B) TTV in tumor tissue. (C) the bar plot indicates significant

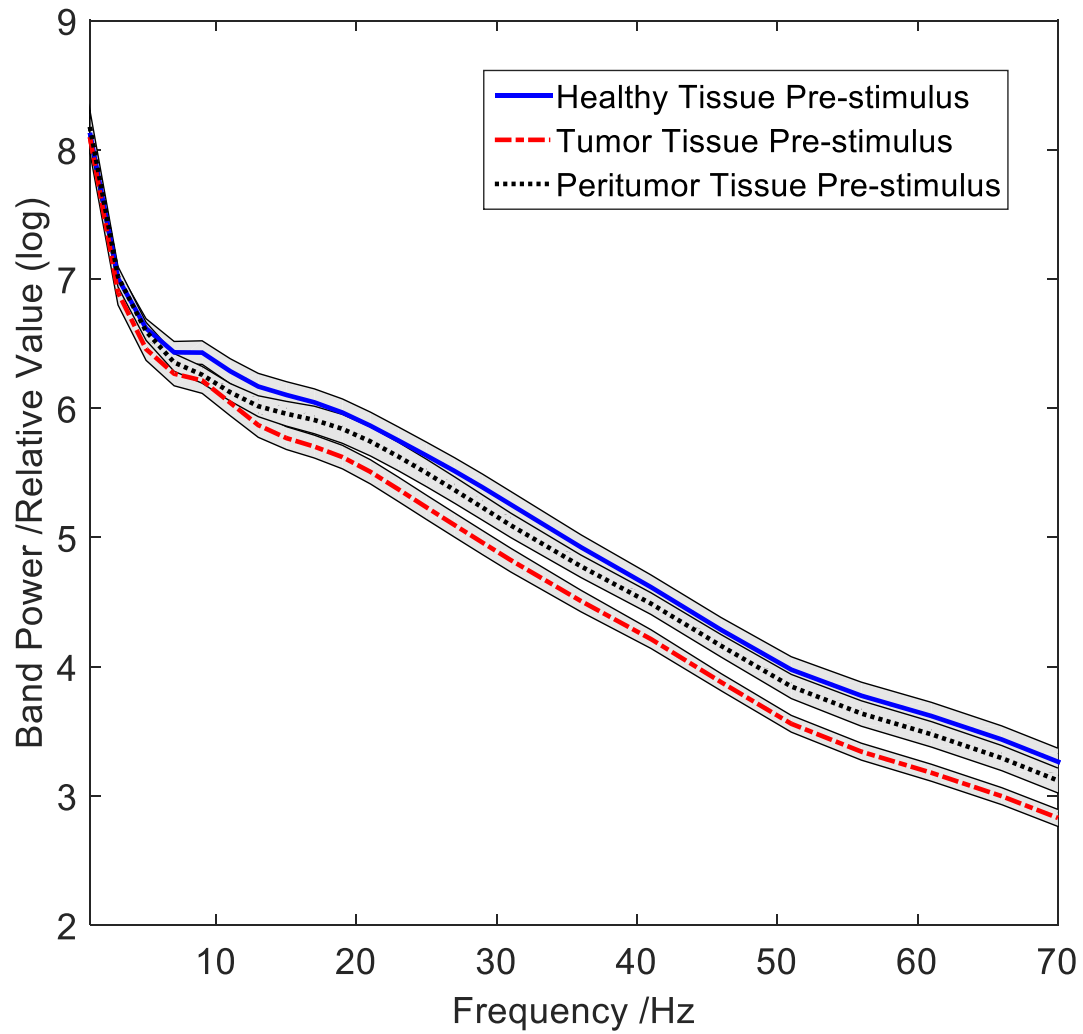
($p < 0.0138$) TTV difference between healthy and tumor tissue within time period of [200 800] ms. (D) correlation between pre-stim variance and TTV-index in healthy tissue within alpha band. (E) correlation between pre-stim variance and TTV-index in tumor tissue within alpha band. (F) Spectrum power difference between healthy and tumor tissue within pre-stim time period. (G) the bar plot indicates the significant ($p < 0.0054$) alpha band power difference within pre-stim period between healthy and tumor tissue.

Figure 4.13 Pre-stimulus (-500 to 0ms) power comparison across different frequencies in A. healthy tissue and tumor tissue.



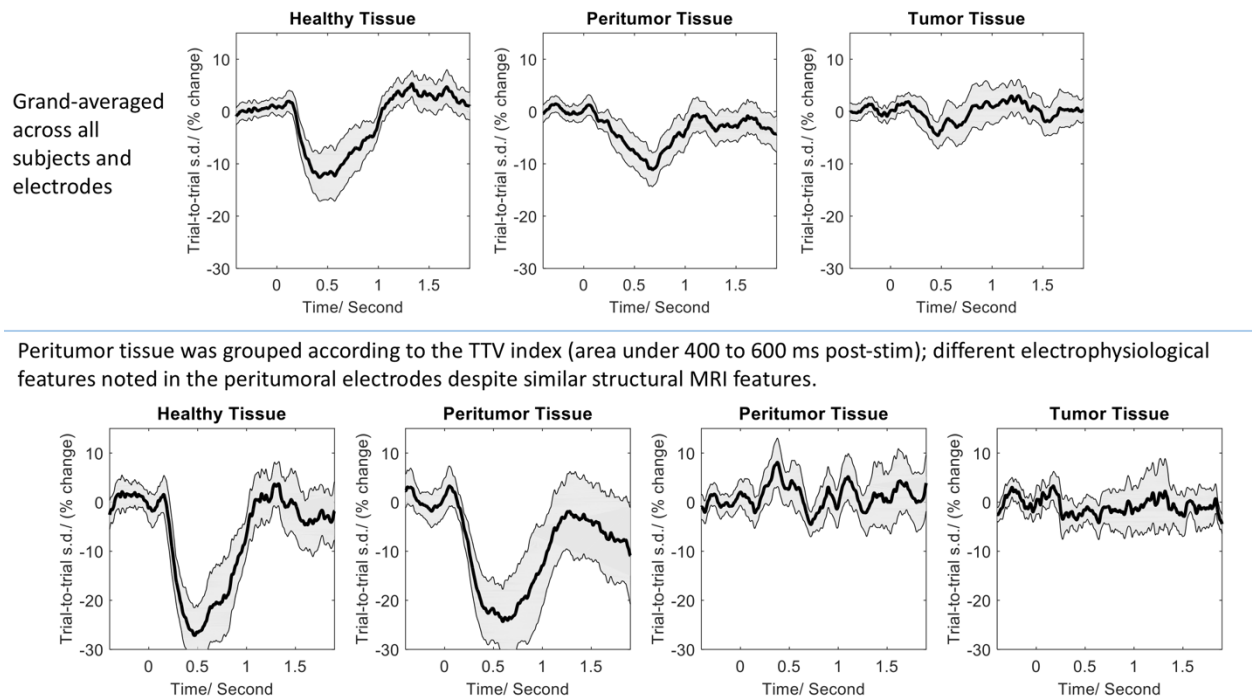
The figure shows the power spectrum in the pre-stimulus interval (-500 to 0ms) which exhibits highly significant reduction in specifically the power of alpha (8-13Hz) and beta (13-30Hz) in the tumor tissue (red line) when compared to healthy tissue (blue line).

B. Tumor, peritumoral and macroscopically healthy tissues



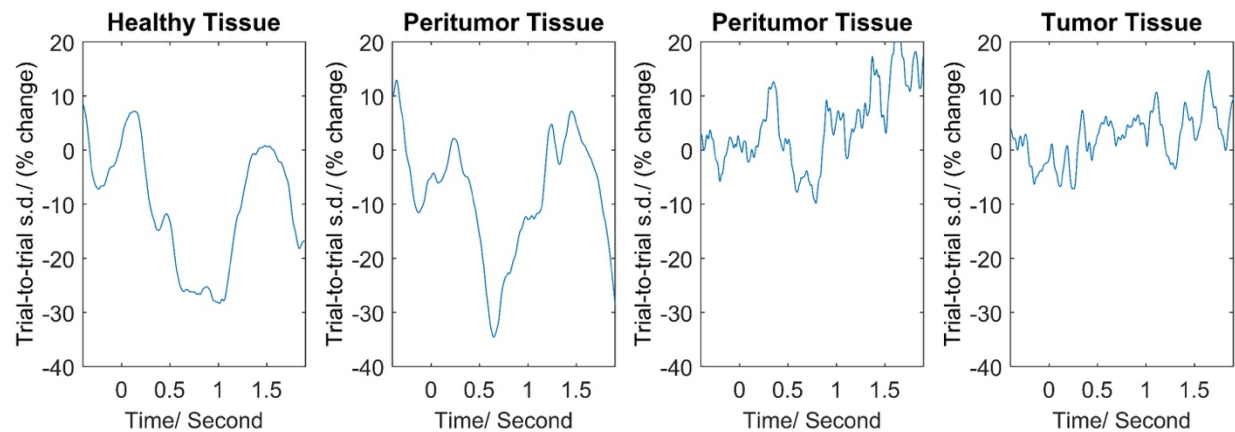
The figure shows the intermediate feature of the power spectrum in the peritumoral tissue when compared to the tumor tissue (red line) and the macroscopically healthy tissue (blue line).

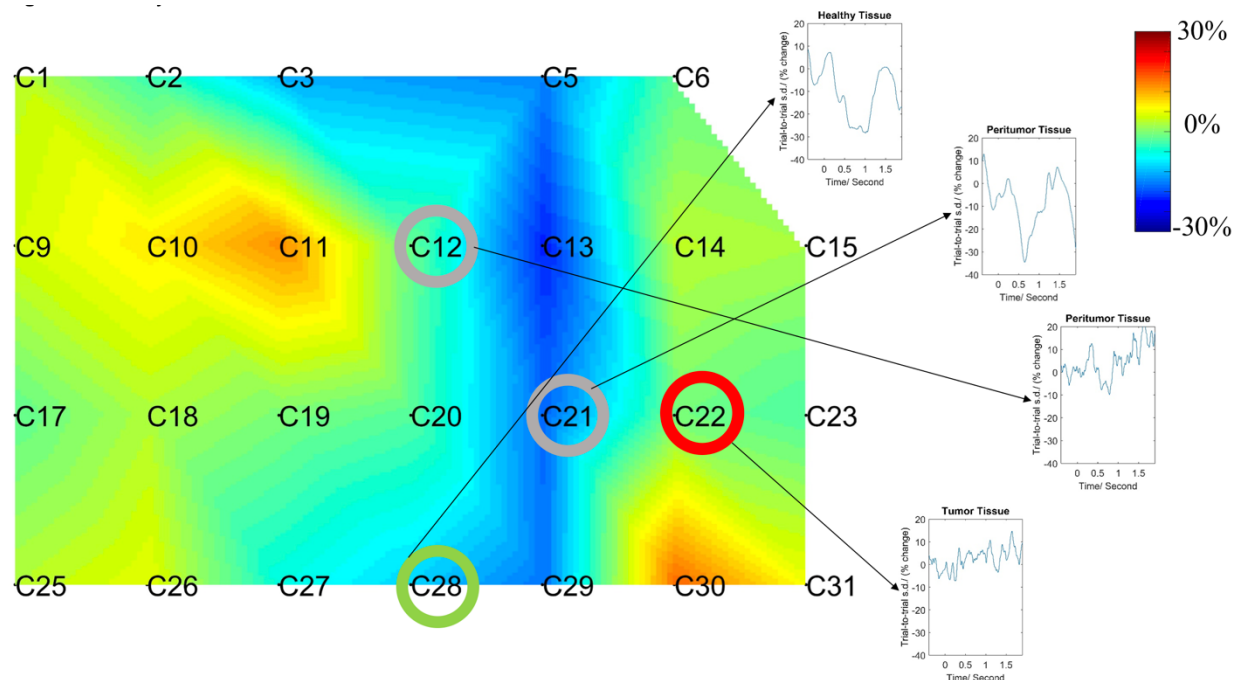
Figure 4.14 ECoG data on task-evoked activity measuring trial-to-trial variability (TTV) in the different tissues of glioma patients



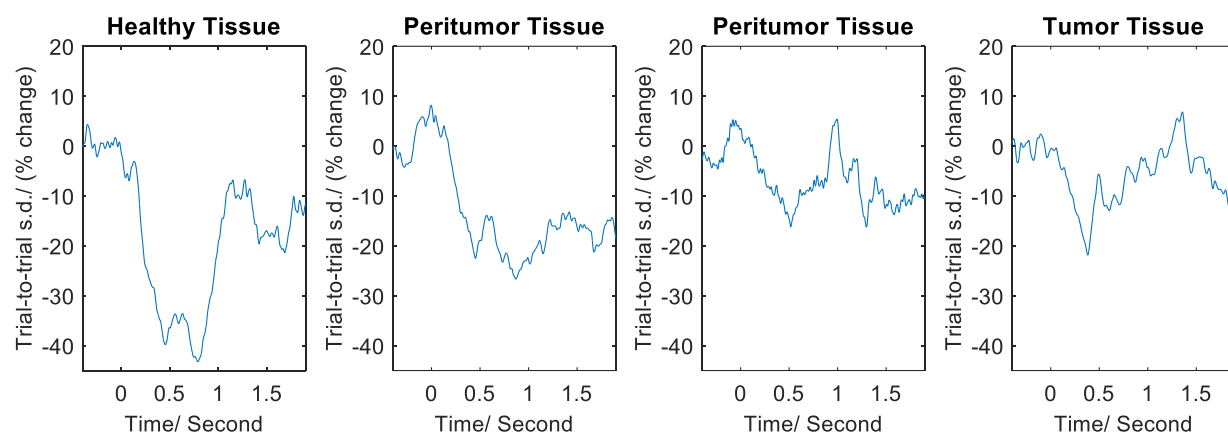
The figure shows the TTV ($n = 8$ patients with glioma) with the selection of 3 electrodes from each tissue. Most important, we grouped the peritumoral electrodes according to their TTV value (area under the curves between 400 and 600ms post-stimulus). Note that this shows two different kinds of peritumoral electrodes, those that show values similar to the healthy tissue (middle left and left) and those that show values similar to the tumor tissue (middle right and right). The difference between these peritumoral electrodes in TTV reduction can be considered as index of the presence (no TTV reduction) or absence (TTV reduction) of infiltration of tumor cells into peritumoral tissue.

Figure 4.15 Trial-to-trial variability in individual cases. **A.** Subject 1





B. Subject 2



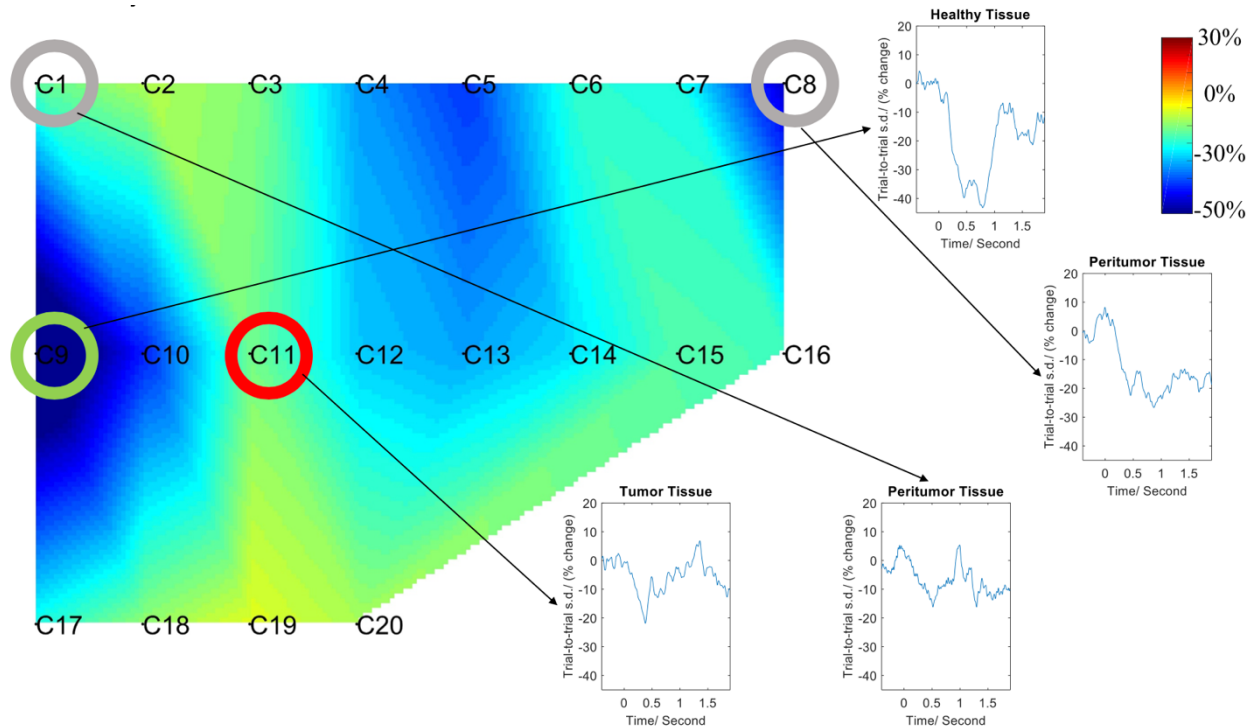
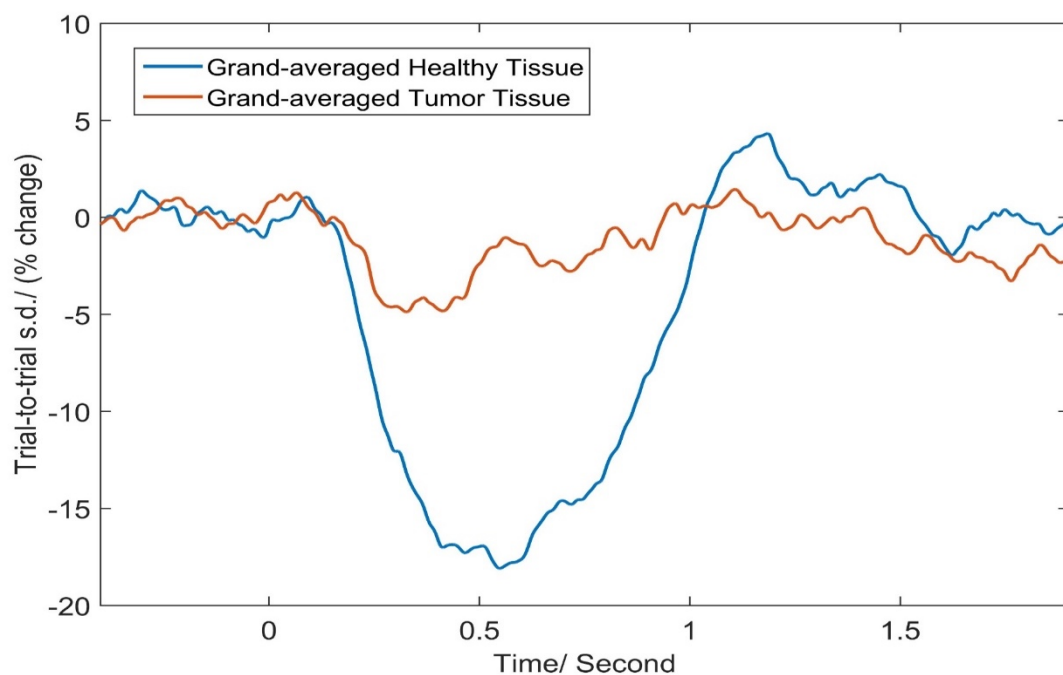
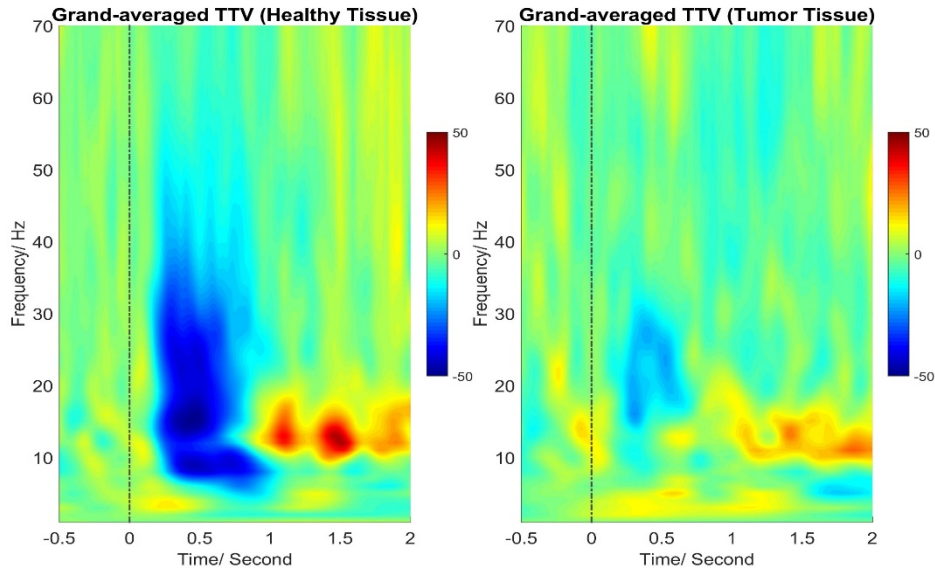


Figure 4.16. ECoG data from task-evoked activity in 8 subjects (3 electrodes each for healthy tissue and tumor tissue during stimulation (auditory stimuli, e.g., own and other name))





The upper figure shows the trial-to-trial variability (TTV) after stimulus onset - the data show that the tumor tissue does not exhibit any TTV reduction after stimulus onset. The lower figures show the corresponding event-related spectral perturbation (ERSP) for the TTV – the data show decreased power in alpha (8-13Hz) and beta (13-30Hz) (marked in blue) in the healthy tissue (left) whereas such decrease cannot be observed in tumor tissue (right). Overall, these data suggest that neural activity in the tumor tissue can no longer be properly modulated by the stimulus.

4.2.4 Discussion

In terms of the electrode localization, positioning the ECoG electrode grids is prone to errors given that the areas invaded by the tumor might not be clearly delineated and the sulcal patterns may not be easily segmented. Furthermore, the grid may be opaque or dense with electrodes and can obstruct the view of the underlying surface. The angle at which the 2-D photograph is taken can also be a source of error as it is being superimposed on a curved 3-D cortical surface. Lastly, the occurrence of brain shift could influence the precision of our technique however, the ECoG recording was performed as soon as the craniotomy was completed in order to minimize the effect of the brain shift. As the neuronavigation is used to decide the extent of resection, we confirmed our locations using the neuronavigation probe thus reducing the risk of error in electrode localisation. In order to quantify the brain shift influence, we calculated the extent of brain shift immediately after ECoG recording by using the intra-operative MRI. This approach was not done previously to our knowledge.

Stimulus-induced activity in glioma

An accurate estimation of the epileptogenic cortex and its removal requires the estimation of spatial spread of ECoG. Recent studies estimating the spatial spread of ECoG suggested that although brain's signals capture different features of the neural network, ECoG is a local signal (diameter of ~3 mm) (Dubey and Ray, 2019). Extrapolating our group's previous work in functional MRI, for the first time to our knowledge, the analysis of the signal from ECoG electrodes in this study concentrated on trial-to-trial variability (TTV). The spontaneous activity of the brain interaction with stimulus-induced activity is manifested in event-related amplitude and its TTV (Wolff, Di Giovanni, et al. 2019). TTV describes the variability in the amplitude of the stimulus-evoked response across trials, and while TTV reduction has been observed on both the cellular and regional levels, its exact behavioral relevance and neuronal basis remain unclear (Wolff, Di Giovanni, et al. 2019). Trial-to-trial response variability could also be considerably different across stimuli, suggesting that it could also provide an important contribution to the information conveyed by the neural responses in regards to the stimuli (Scaglione et al. 2011; Balaguer-Ballester, Tabas-Diaz, and Budka 2014).

4.2.5 Conclusion

In summary, we present for the first time distinct stimulus-induced changes in the tumoral and peritumoral neocortex of gliomas. We further observed that the TTV changes are not uniform among the various regions and we noticed a significant difference in the appearance of the TTV response in the peritumoral electrodes. As depicted in the result section, the TTV response noticed across PT electrodes can be closer to that of TT or HT-electrodes which we suspect is related to tumor cell infiltration. Although, no ictal or inter-ictal discharges were recorded, further studies with prolonged recordings and combined genetic analysis could test our hypothesis. The nature of such trial-to-trial dynamics remains to be defined and further inclusion of in-vivo neural recording to current data analysis approaches could help improve our insight into latent sources of trial-to-trial variability. As such, PT cortex might represent a pivotal structure both for the genesis of epileptic activity and for infiltration by glioma cells. This could play an important role for emerging techniques in brain mapping utilizing endogenous cortical physiology

Chapter 5 - PERSONALIZED PRECISE MULTIMODAL DEMARICATION OF PERITUMORAL TISSUE IN GLIOMA

As mentioned previously, gliomas are life-threatening brain tumors, and the extent of surgical resection represents one of the strongest influences on the patients' survival rate. However, proper distinction of infiltrated tissue remains elusive. The aim of this study is to combine multi-modal analyses to demarcate the PT from both TT and HT. We here investigated 40 patients with histologically confirmed glioma and compared resting state functional MRI (rs-fMRI) using voxel-based mean BOLD signal with the corresponding structural MRI (sMRI). RNA-sequencing, whole exome sequencing and histology analyses were performed on biopsy samples obtained from PT, HT, and TT. In this study we were able to demarcate a functionally-defined PT area where the mean BOLD signal decreased gradually near the edge of the tumor and extended beyond TT borders (as defined by sMRI) which was confirmed on an individual case-by-case basis. Correspondingly, genetic analyses showed a distinct gene expression pattern and mutational landscape in PT when compared to HT and TT. The genetic characterization of PT relative to HT and TT converged with the MRI-defined PT zones and was confirmed in three individual cases undergoing further histological analysis. The finding that a wider PT was associated with a higher PFS suggests that PT might act as an intermediate area between tumor and healthy tissue. In conclusion, combined multi-modal imaging and genetic analyses allowed an objective demarcation of the PT in glioma and a robust classification of the degree of infiltration of the PT which could improve both neurosurgical resection and radio-oncological therapy.

5.1 Introduction

Gliomas are one of the most common type of primary brain tumors, and are divided in several grades based on their molecular characterization (Louis et al. 2016). Glioblastoma (GBM) is an important subset that represents the most malignant form, with a median survival rate of 15 months (Thakkar et al. 2014). As tumors recur in 80% of cases within 2-3 cm from the original lesion, the main problem faced by both neurosurgical resection and radio-oncological therapy is

the demarcation of tumor cell infiltrated zones not yet macroscopically visible in structural imaging (Lemée, Clavreul, and Menei 2015).

These infiltrated zones are usually located adjacent to the main tumoral tissue and are therefore described as peritumoral tissue (Petrecca et al. 2013; Y. M. Li et al. 2016). Moreover, the infiltrating tumor cells located at the resection margins were shown to have a different phenotype with faster proliferation and higher invasion potential than those located in the tumor center (Lemée, Clavreul, and Menei 2015). Nonetheless, we already know that in GBM, tumor cell infiltration may be located more than 1 centimeter from the contrast-enhancing lesion and thus surgical options may be limited (Eidel et al. 2017; Lemée, Clavreul, and Menei 2015).

In order to better delineate and resect the tumoral areas, pre-operative structural Magnetic Resonance Imaging (s-MRI) has been used to demarcate TT given its relatively high-resolution (Despotović, Goossens, and Philips 2015), which allows a proper distinction of white and gray matter. However, a lack of contrast enhancement with gadolinium-based contrast agents for glioma does not necessarily imply the absence of tumor cells in this area as a minimum concentration of tumor cells is necessary to produce detectable alterations on structural MRI (Lemée, Clavreul, and Menei 2015). Furthermore, other techniques such as dynamic contrast enhanced MRI and diffusion weighted imaging have been used to differentiate TT from HT (Lemée, Clavreul, and Menei 2015; J. Zhao et al. 2015). These have been complemented by intra-operative measures such as intra-operative fluorescence (Belykh et al. 2016; Senders et al. 2017), 5-aminolevulinic acid (5-ALA) (S. Zhao et al. 2013), mass spectrometry (Eberlin et al. 2013), electrophysiology (Bandt et al. 2015; Boussen et al. 2016) and more recently, resting state functional MRI (rs-fMRI) (Rosazza, Zacà, and Bruzzone 2018; J. Zhang et al. 2018; D. C. Ghinda et al. 2018). Recent rs-fMRI studies usually employ functional connectivity (FC) which measures inter-regional relationships (Bullmore and Sporns 2009; Bassett and Bullmore 2009; Crossley et al. 2014). This approach may blur spatial resolution, as it potentially omits intra-regional features which would allow the characterization of PT on a voxel-based level (Zalesky et al. 2012; Hermundstad et al. 2013). Furthermore, studies integrating progressive genomics technologies, such as microarrays, identified differentially expressed genes (DEGs) between TT of diffuse low grade gliomas (DLGGs) and non-glioma tissue (S. Wang et al. 2017).

Nonetheless, the primary focus addressed by these measures was in identifying TT (for instance a strong 5-ALA fluorescence correlates with TT in HGG), without a clear demarcation of the PT.

As a more precise delineation of the PT could be valuable for subsequent adjuvant radiotherapy or individualized treatment, we here investigated a unique sample of glioma patients using a novel approach that combines multimodal imaging and high-throughput sequencing with histological analysis in order to demarcate the PT. Since rs-fMRI is a non-invasive study sensitive to the neovascularization associated with gliomas, our hypothesis is that intra-regional measures in rs-fMRI, i.e. mean blood oxygen-level dependent (BOLD) signal, could be helpful in demarcating PT relative to HT and TT (H. Huang et al. 2018). These regional differences could be confirmed by investigating the histology and genetic profile of samples from PT, which is necessary for optimizing the surgical and radio-oncological treatment and thus improve the patients' outcomes.

5.2 Methods

STUDY PATIENTS

The study was approved by the Huashan Hospital ethics board (#KY201209). Patients were eligible for inclusion in the study if they were 18 years and older, presented with a supratentorial lesion suggestive of a glioma, and had an indication for surgery as confirmed by an experienced neurosurgery team (JW, JL). From a total of 70 patients sequentially admitted for treatment, patients with other brain pathologies, patients who had undergone previous cranial irradiation or chemotherapy and patients for whom adequate biopsy tissue could not be obtained were excluded. The remaining 40 patients were included in the study; all patients provided written informed consent. All the forty of the included patients underwent biopsy with subsequent high-throughput sequencing; both RNA-Seq and whole-exome sequencing were performed in biopsies from 19 patients and RNA-Seq only were performed in biopsies from the remaining 20 patients. rs-fMRI data was acquired for 33 patients as for 7 patients the rs-fMRI data could not be analyzed because of the inability of the patients to complete the rs-fMRI study. Thus, 33 patients had both imaging (rs-fMRI and sMRI) and high-throughput sequencing; this is the core sample of our study (Figure 5.1 and Table S1 in Supplementary Appendix). All patients received a standard follow-up at 1, 3, 6, 9, 12 and 24 months after the surgical procedure. Patients received

postoperative adjuvant chemo- and/or radiotherapy according to histopathologic and molecular classification of the tumor.

MRI DATA

Conventional MR images, consisting of high resolution T1 MPRAGE and T2-weighted fluid-attenuated inversion recovery (FLAIR) MRI as well as rs-fMRI, were performed one or two days preoperatively to ensure an accurate correlation between the neuroimaging exams and the tissue samples. For the MRI data acquisition, all brain images were acquired one or two days preoperatively in the diagnostic room of an iMRI-integrated neurosurgical suite using a 3T Siemens MRI scanner (Siemens MAGNETOM Verio 3.0 T, Germany) with a standard 32-channel head coil. Gradient-echo EPI images of the whole brain were acquired (33 slices, repetition time/echo time [TR/TE] = 2,000/30 ms, slice thickness = 5 mm, field of view = 210 mm, flip angle = 90°, image matrix: 64 x 64, 3 x 3 x 4 mm resolution). Two hundred and forty scans (8 min) were acquired during the eyes-closed resting-state. High-resolution 3D T1-weighted MPRAGE and FLAIR MR images with neuronavigation sequences were acquired during the same scan session.

Preprocessing steps were implemented in Analysis of Functional NeuroImages (AFNI) software (<http://afni.nimh.nih.gov/afni>), including discarding the first 3 scans of each fMRI run; slice timing correction; spatial normalization into Talairach stereotactic space of the anatomical scan, alignment of the EPI and anatomical image using Fourier interpolation and resampling of the EPI to 3 x 3 x 3 mm resolution. The data extracted from the structural and functional MRI were plotted on the individual graphs after adjusting for the voxel resolution.

Two regions of interest (ROI) consisting of a 1 cm diameter sphere inside the tumor region (delineated on the standard anatomical images) and a contralateral mirror sphere were subsequently computed. We then extracted the mean BOLD signal value and standard deviation (SD) of the signal in the two ROIs, and differences between the two regions were calculated using a paired sample t-test. Subsequently, a linear ROI was drawn on the structural MRI from the tumor epicenter outwards while avoiding intervening sulci and areas with low BOLD signal, such as the inferior temporal lobe, for the identification of PT (Figure 5.2). The outer border of the TT was defined as the location with a large degree of change in the mean BOLD values after

which the mean BOLD value remained consistently lower. The results obtained via rs-fMRI were then directly compared with the corresponding structural MRI signal intensity after extracting the values from individual ROI's using the AFNI software and customized Matlab scripts. A BOLD-based similarity analysis (see below for details) was calculated for the rs-fMRI-based determination of the PT biopsy sites.

GENETIC AND MOLECULAR DATA

Image guided biopsies were performed as soon as possible after the craniotomy was completed, in order to minimize potential brain shift. Biopsy location was recorded using the intra-operative neuronavigation system prior to tumor resection, and the coordinates in a standard MRI space were used to extract the values for both structural and functional MRI. The accuracy of the neuronavigation approach used is well established and represents the standard method for image-guided resection of brain tumors (Barone, Lawrie, and Hart 2014; Jenkinson et al. 2018). A monitored anesthesia care approach or a general anesthesia approach was adopted for the surgical procedures as per previous publications (J. Lu et al. 2013; Jun-Feng Lu et al. 2012; D. Ghinda et al. 2016). The biopsy samples were collected for each patient according to the radiological defined boundaries as per the conventional structural MRI: FLAIR for non-enhancing diffuse low-grade gliomas and the T1 MPRAGE with contrast for contrast enhancing tumors. Three 4 mm³ biopsies were taken from the tumoral tissue, peritumoral tissue (within a 2 cm margin from tumor borders according to the RANO criteria) and as far as safely possible from the tumor (Ellingson, Wen, and Cloughesy 2017). Whole exome and RNA-sequencing and analysis were performed by Genetron Health Clinical Laboratories.

Whole exome sequencing

To measure the accurate expression diversity between the tumoral and healthy tissue, matched TT and macroscopically HT for each patient were collected, and their transcriptome was sequenced. Genomic DNA was isolated from fresh-frozen samples using QIAamp DNA Mini Kit (Qiagen, 51306) following the manufacturer's protocol. Genomic DNA libraries were constructed using KAPA Hyper Prep Kits (KAPA, KK8504) and captured using Agilent SureSelect Human All Exon V5 according to the manufacturer's protocol. High-throughput sequencing was performed on Illumina HiSeq X platform. Sequencing reads were mapped to a

human reference genome (hg19) using the Burrows–Wheeler Aligner (BWA) (H. Li and Durbin 2010). Duplicate removal, local realignment, and base quality recalibration were performed using PICARD (<http://broadinstitute.github.io/picard/>) and the Genome Analysis Toolkit (GATK) (DePristo et al. 2011). Somatic single nucleotide variations (SNVs) and small indels were called using Mutect (Cibulskis et al. 2013) and strelka (Saunders et al. 2012). Effects of variants were annotated using Variant Effect Predictor (McLaren et al. 2016). Only mutations of driver genes in glioma (Rubio-Perez et al. 2015), i.e., *IDH1*, *IDH2*, *TP53*, *ATRX*, *CIC*, *FUBP1*, *NOTCH1*, *PIK3CA*, *NF1*, *PIK3R1*, *ARID1A*, *ARID1B*, *PTEN*, *EGFR*, *BRAF*, *SETD2*, *TCF12*, *SMARCA4*, and *ZNF292*, were reserved for further analysis.

RNA-sequencing and analysis

Total RNA was isolated from fresh-frozen tissue samples using QIAGEN AllPrep DNA/RNA Mini Kit (Qiagen, 80204) following the manufacturer's protocol. The mRNA libraries were constructed using NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB, E7490) and NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB, E7420) according to the manufacturer's protocol. High-throughput sequencing was performed on Illumina HiSeq 4000 platform. Sequencing reads were mapped to a human reference genome (GRCh38) using the Hisat2-2.0.5 (Kim, Langmead, and Salzberg 2015). Expression levels of each protein-coding gene was calculated using Stringtie- 1.3.1c (Pertea et al. 2016) and differentially expressed genes (DEGs) were called using Cyber-T (Kayala and Baldi 2012). A gene was defined as a differentially expressed gene (DEGs) if (1) at least 2 fold change was observed between tumor tissue (TT) and healthy tissue (HT), and (2) q-value < 0.05 (Benjamini-Hochberg corrected test).

To confirm the transcriptome-based classification, we performed whole-exome sequencing on samples from 21 patients, and retrieved all mutations of driver genes in glioma, e.g., *IDH1*, *IDH2*, *TP53*, *ATRX*, etc., in TT and PT. After 2 patients were excluded for no mutations of driver genes were found, 19 patients were further analyzed. Three of the included patients had additional histological analysis of the individual samples. The molecular feature of TT and HT was extracted and compared with the PT biopsy in order to give a molecular-based classification of the PT. A tumour infiltration index (TII) was calculated to define the classification of PT based on mutations using the following formula:

$$TII = \frac{f_P - f_T/2}{f_T/2}$$

For each patient, a mutation of driver genes with the highest mutation frequency in PT was selected. f_T and f_P represent the frequency of this mutation in TT and PT. The TII value increased with the degree of tumor infiltration.

EXPRESSION SIMILARITY INDEX (ESI)

For each patient, an Euclidean distance was calculated to represent the similarity between two tissues for a group of selected genes. Based on these distances, an ESI was calculated to define the expression pattern of PT using the following formula:

$$ESI = \log_2 \frac{Dis_{HP}}{Dis_{TP}}$$

Here, Dis_{TP} is the distance between the TT and PT, and Dis_{HP} is the distance between the HT and PT. A positive ESI indicates that the PT is more similar to the TT than the HT, and vice versa. Similarly, in order to calculate the PT similarity to HT and TT using rs-fMRI, the mean BOLD values of a sphere with a 2 mm diameter was extracted from each of the three biopsy sites and the results were compared as per the following method:

$$PT \sim HT \text{ if } |\text{Mean BOLD}_{PT-HT}| < |\text{Mean BOLD}_{PT-TT}|$$

SURVIVAL ANALYSIS

Kaplan-Meier Overall Survival (OS) and Progression-free Survival (PFS) curves were plotted for the patients with combined rs-fMRI and genetic data according to the PT distance ≤ 10 or > 10 mm. The 10 mm cut-off point was obtained by the mean value of PT distance from all patients. To investigate whether the PT distance is related to the overall survival (OS) and progression free survival (PFS), a univariate Cox proportional hazard model was performed. In the univariate analysis, the independent variables included continuous and binary variables of distance PT. The generalized R^2 statistic was calculated based on the likelihood ratio statistic (LRT) for testing the global null hypothesis (Allison 2010) using the formula of:

$$R^2 = 1 - e^{-(LRT/n)},$$

where $LRT = -2\log L(0) - [-2\log L(p)]$, n is the sample size used, $\log L(0)$ is the log-likelihood for a null model with no covariates, and $\log L(p)$ is the log-likelihood for the fitted model with p

covariates. R^2 (between 0 and 1) is larger when the covariates are more strongly associated with the dependent variable. P-value of < 0.05 was considered as the threshold for statistical significance. Hazard ratio (HR) and 95% confidence intervals (CI) of HR were also calculated for each covariate. Analyses were conducted using Statistical Analysis Software (SAS version 9.4 for Windows) and R package (version 3.5.0) (Allison 2010).

5.3 Results

DIFFERENTIATING TUMORAL FROM HEALTHY TISSUE WITH RS-fMRI

The mean BOLD signal displayed a significant difference between the TT and the contralateral macroscopically non-infiltrated HT using the Wilcoxon rank-sum test ($P < 0.001$). In addition to the mean BOLD signal, we also investigated the SD which showed analogous significant difference between TT and HT ($P < 0.001$). Since, the mean BOLD signal was stronger and better marker than SD for distinguishing TT and HT (SD was 10 times less significant than mean BOLD signal), we focused our subsequent analyses on the former measure when comparing sMRI and rs-fMRI results on an individual basis. We also performed sub-group analysis according to the tumor grade and obtained similar results (Table S2 in Supplementary Appendix).

INDIVIDUALIZED DEMARCATION OF PERITUMORAL TISSUE USING RS-fMRI

The mean BOLD signal decreased gradually near the edge of the tumor and this intermediate region, demarcated by the transition from high to low mean BOLD values, was defined as the peritumoral zone. Based on the change in mean BOLD signal, the PT covers a spatially extended zone that is interposed between TT (high mean BOLD signal) and HT (low mean BOLD signal) (Figure 5.2). This process was performed on a case-by-case basis to individualize the definition of the peritumoral zone, which was measured on a voxel-by-voxel basis (mean of 6.8 mm). Based on these criteria, we were able to determine the spatial extent of PT relative to TT and HT on an individual basis and we obtained similar results with another ROI location (Fig. S1 in Supplementary Appendix).

CHARACTERIZATION OF PERITUMORAL TISSUE USING THE GENE EXPRESSION PATTERN

To extract the expression features of TT and HT, DEGs were retrieved and 465 significantly up-regulated and 795 down-regulated genes were found in the LGG group (Figure 5.3A). In contrast, expression levels of 195 up-regulated and 768 down-regulated genes were found in the GBM group (Figure 5.3B). In order to give a robust classification of the PT, the 100 most up-regulated and 100 most down-regulated genes were retrieved. To classify the PT as whether HT-like or TT-like, the expression levels of DEGs in PT were compared with those in HT and TT from the same patient using ESI_{100} . The proportion of HT-like PT (24 of 30 patients) was significantly higher than that of TT-like in LGG (Figure 5.3E, 6 of 30 patients, $P=0.01$, one tailed Fisher's exact test). Moreover, the proportion of TT-like PT is significantly higher in GBM patients than that in LGG patients (Figure 5.3E, $P=0.05$, one tailed Fisher's exact test), which suggests a more invasive infiltration pattern in GBM. To further test whether the classification was sensitive to DEGs selection, we examined various DEGs, ranging from 20, 30, 40 and 50 most significantly up- and down-regulated genes to all DEGs. We found the same classification in 37 patients for all sets of markers (Fig. S2 in the Supplementary Appendix, $P<0.001$, one tailed binomial test). These results indicate that gene expression pattern could provide a robust classification of PT and that the number of selected DEGs is not a confounding factor to demarcate PT.

CHARACTERIZATION OF PERITUMORAL TISSUE USING GENETIC ALTERATIONS

The TII was calculated for each patient to normalize the difference of mutation frequency, and it was found that the transcriptome-defined TT-like PTs were significantly increased in the high-infiltration group ($P=0.03$, one tailed Fisher's exact test, Figure 5.4C). Furthermore, transcriptome-defined TT-like PTs showed a significantly higher tumor infiltration (median = 0.64) than HT-like PTs (median = -0.88, $P=0.03$, Mann-Whitney U test, Figure 5.4D). These results support a transcriptome-based classification of PT and suggest that the transcriptome could accurately illustrate the molecular characterisation of PT.

CONVERGENCE BETWEEN RS-fMRI AND GENETICS IN PERITUMORAL TISSUE

Since both rs-fMRI and genetic data allowed to distinguish PT on an individual basis, we converged the findings after calculating a similarity index for PT (Figure 5.5A). This allowed us

to directly compare whether the similarities or differences in molecular features of PT (relative to HT or TT) corresponded to the rs-fMRI-based determination of PT relative to HT or TT. The mean BOLD signal (rs-fMRI) and the molecular data (ESI) showed a significant association ($P < 0.001$) in their determination of PT relative to HT and TT (Figure 5.5B). To further confirm our results, we also performed histopathological analysis on available biopsies for the PT, TT and HT of three patients and obtained similar results (Figure 5.2C and Fig. S3 in Supplementary Appendix).

ANALYSIS OF CLINICAL OUTCOMES ACCORDING TO THE PT DISTANCE IN THE MAIN GROUP

The Kaplan-Meier PFS curves showed a significant difference in PFS ($P = 0.02$) and no significant difference in OS ($P = 0.11$) between patients with PT distance of ≤ 10 mm and > 10 mm (Figure 5.5C and Table S3). The actuarial median PFS (obtained from Kaplan-Meier curve) was 26 months with 95% CI of 18-30 months (Fig. S4 and Fig. S5 for OS in Supplementary Appendix). Using the univariate Cox proportional hazard model, we found significant associations between PT distance (continuous or binary variable) and PFS. Patients with lower PT distance ($P = 0.009$, HR=0.81) or with PT distance ≤ 10 mm ($P = 0.04$, HR=3.74) are more likely to develop recurrence, comparing to others (Table S4). We did not find significant associations between the PT distance (continuous or binary variable) and OS ($P > 0.05$) (Table S5). Accordingly, the average PT distance in LGG (grade II) is 10.9 mm with an 18% recurrence whereas in HGG is 8.75mm with an 81% recurrence rate at last follow-up. Although we did not find a significant association between LGG, HGG and OS ($P = 0.59$) in the Cox proportional hazard model, patients with HGG ($P = 0.002$, HR=6.94) demonstrate higher probabilities of developing recurrence, compared to those with LGG.

5.4 Discussion

We here propose, for the first time to our knowledge, an objective measure of the PT. Our findings of distinguishing TT and HT at the group level were significant for mean BOLD signal. The results remained consistent within sub-group analysis, according to the tumor grade. Based on our successful group results in distinguishing TT and HT, we then shifted to an individual, case-by-case approach to demarcate PT from TT using the mean BOLD signal. We observed that in most of the cases, the high mean BOLD signal of TT decreases at the structurally-defined

boundary of TT. Nonetheless, the change in mean BOLD signal is not abrupt but occurs gradually, extending over several mm before it reaches the level of the mean BOLD signal in HT. The zone featured by a gradual decrease in mean BOLD signal was defined as PT as it is interlaid between TT (high mean BOLD signal) and HT (low mean BOLD signal).

The BOLD signal is based on neuro-vascular coupling (NVC) (Logothetis et al. 2001; Goense and Logothetis 2008). As such, the mean BOLD signal, as applied here, is ideal to demarcate PT in glioma since NVC is known to be impaired in the setting of glial tumors. As shown in different immunohistochemical studies of the PT, the peritumoral zone undergoes significant modifications including neovascularization (Mangiola et al. 2013; Sica et al. 2011; R. Tamura et al. 2018). For instance, markers of neovascularization (CD105, nestin, phosphorylated extracellular signal-regulated kinases, and c-Jun NH₂ terminal kinases) are expressed in the PT irrespective of the presence of tumor cells, and their expression is associated with poor prognosis (Mangiola et al. 2013; Sica et al. 2011; R. Tamura et al. 2018). Future studies may thus wish to combine the here applied methods with vascular-angiographic methods to support such a claim of the vascular-physiological basis of PT.

Since the mean BOLD signal gradually decreases in this PT zone, we assume that such functionally-defined PT represents a changing region with different levels of tumor cell infiltration, although the infiltrated cells are not yet reaching macroscopic visibility and are thus not yet detectable in sMRI. This assumption is further supported by characterization of PT's molecular basis through the use of genomic and functional genomic information. Although it has been reported that significant differences were found between glioma and healthy brain tissues, few studies were designed using matched tumoral and macroscopically healthy tissue from the same patient. Thus, the reported differences may be affected by the individual characteristics of each case (tumor location, genomic alterations, growth kinetics etc.). Also, previous studies have found that mutations of driver genes could be examined to monitor the dynamics of tumors (Garcia-Murillas et al. 2015; Dawson et al. 2013). Thus, detection of these mutations in PT could serve as a marker of tumor infiltration.

To identify the genetic alterations in the PT, building on previous genetic studies (Network 2015; Yan et al. 2009; Kondo et al. 2014; S. Wang et al. 2017), we first demonstrated that the expression levels of DEGs and genomic alterations can provide a reliable group-based difference between TT and HT. Second, we applied the same genetic measures to PT and compared them with those in TT and HT on an individual basis. From the RNA-sequencing and whole exome sequencing data, we found significant differences in the genome and transcriptome between TT and HT, which allowed for a robust classification of the degree of infiltration of the PT. As such, our genetic findings strongly support the demarcation of a zone where tumor cells are already cellularly-genetically abnormal albeit not yet in a macroscopically visible way. Most importantly, for clinical purposes, demarcation of PT relative to TT and HT, in both rs-fMRI and genetics, could be shown on an individual basis. This is clinically central for obtaining a more precise localization of a biopsy target to improve the boundary detection for subsequent neurosurgical resection and radio-oncological therapy.

Clinical markers – Implication for neurosurgical resection and radio-oncological therapy

To further support such a claim, we converged our rs-fMRI and genetic findings of the PT zone using the similarity or difference of PT relative to either HT or TT in both rs-fMRI and genetic data (Figure 5.5B). This revealed a good convergence between rs-fMRI and genetic findings in almost all individual cases: the PT was either similar or different to HT or TT in the same way in both methods, rs-fMRI and genetics. This strongly suggests that the functionally-defined PT zones are related to distinct cellular features. Albeit conducted in only three subjects, the assumption of abnormal cell types in PT could be confirmed by our histological data, as they converged with the rs-fMRI and molecular results. Nonetheless, the genetic analyses are based on a restricted sample area that might not be representing the entire PT/TT/HT area. Our study revealed the heterogeneity of the tissue surrounding the core of the tumor tissue, and denotes the need for further studies involving serial biopsies to better define and delineate the PT area. Furthermore, the study is limited to a small glioma patient population, with a short follow-up period and although the MRI-defined PT region is consistent with its genetic features, and its range is related to the patient's progression-free survival, the lack of correlation with OS needs further investigation.

The fact that a wider PT was associated with a higher PFS could imply that PT acts as a buffer or protective zone, and thus a shorter distance implies less protection against tumor infiltration. This finding is clinically highly important, as it entails that we should focus our therapeutic endeavors not only on TT, as structurally demarcated from HT, but also on a zone interlaid between HT and TT, namely PT. As our outcome data show a relation between PT extent and progression free survival, further PT analysis could be highly important for improving clinical outcomes in both neurosurgical and radio-oncological therapy to avoid tumor recurrence, which usually occurs at the margin of the surgical resection (Petrecca et al. 2013; Lemée, Clavreul, and Menei 2015). The unique features of PT suggest it could represent a distinct region where the normal brain protection mechanisms are attempting to contain the tumor infiltration. Further studies are needed to explore this hypothesis.

5.5 Conclusion

In conclusion, through the present study we demonstrate an individualized objective demarcation of peritumoral tissue in glioma which has the potential of carrying high clinical importance, paving the way for direct application in clinical contexts. In the future, we plan to also investigate non-invasive vascular markers of infiltrated tissue and distinguish it from non-infiltrated tissue across the radiologically-defined macroscopic boundaries of TT and HT in glioma using arterial spin labelling (ASL). Based on previous results that show increased blood flow, i.e., hyperperfusion, in TT in GBM (Chawla et al. 2007; Qiao et al. 2015) which did not investigate PT though), we hypothesize increased regional cerebral blood flow (rCBF) in infiltrated tissue within PT and TT as distinguished from non-infiltrated PT and HT (Figure 5.6 for pilot data of arterial spin labeling - ASL). It shall be noted that ASL is a functional measure of blood flow which must be distinguished from structural or histological signs of vascularity.

5.6 Figures And Legends:

Figure 5.1 Study Description A. Overall study schema, B. Study cohort description, C. Study flowchart.

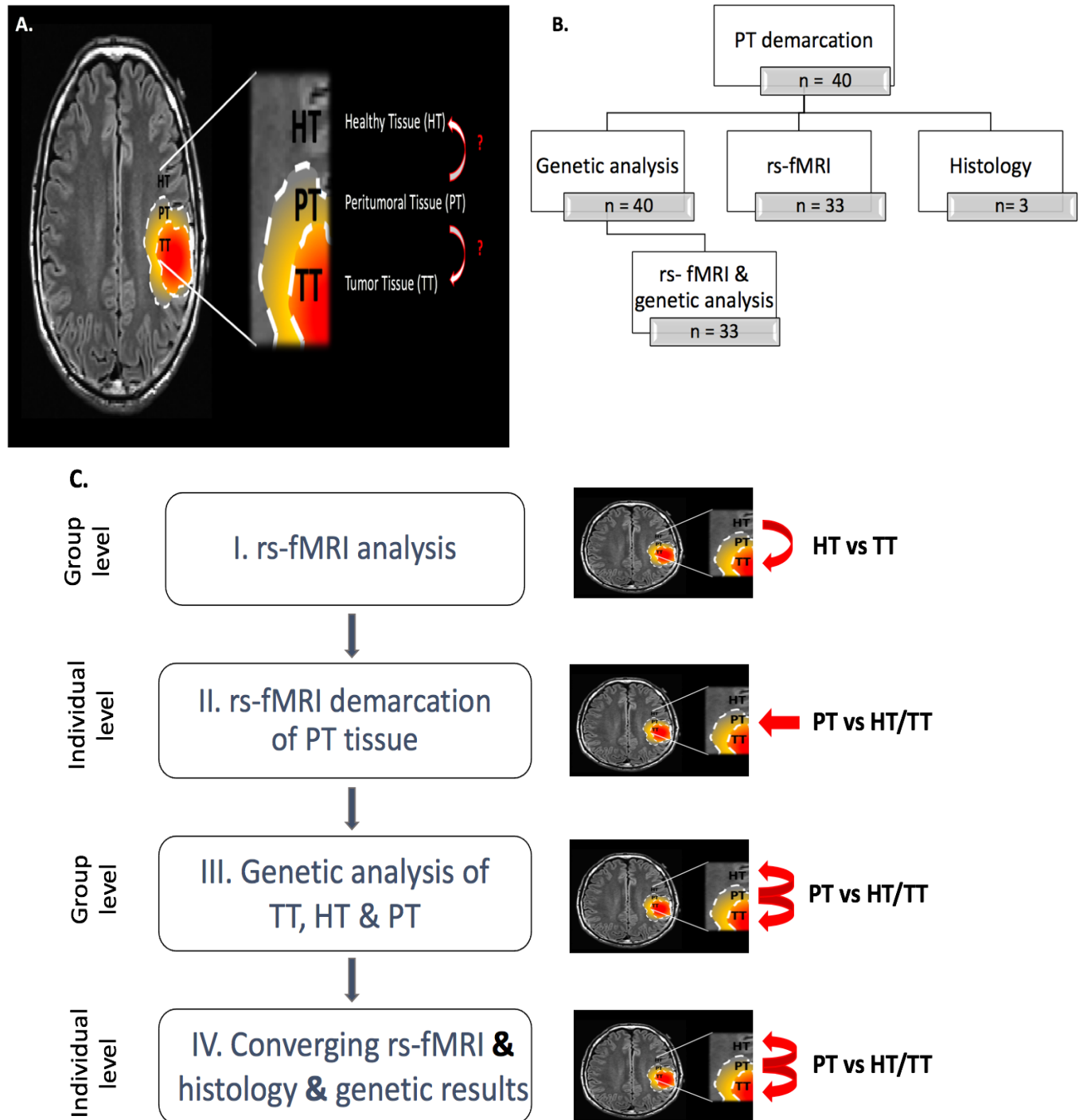
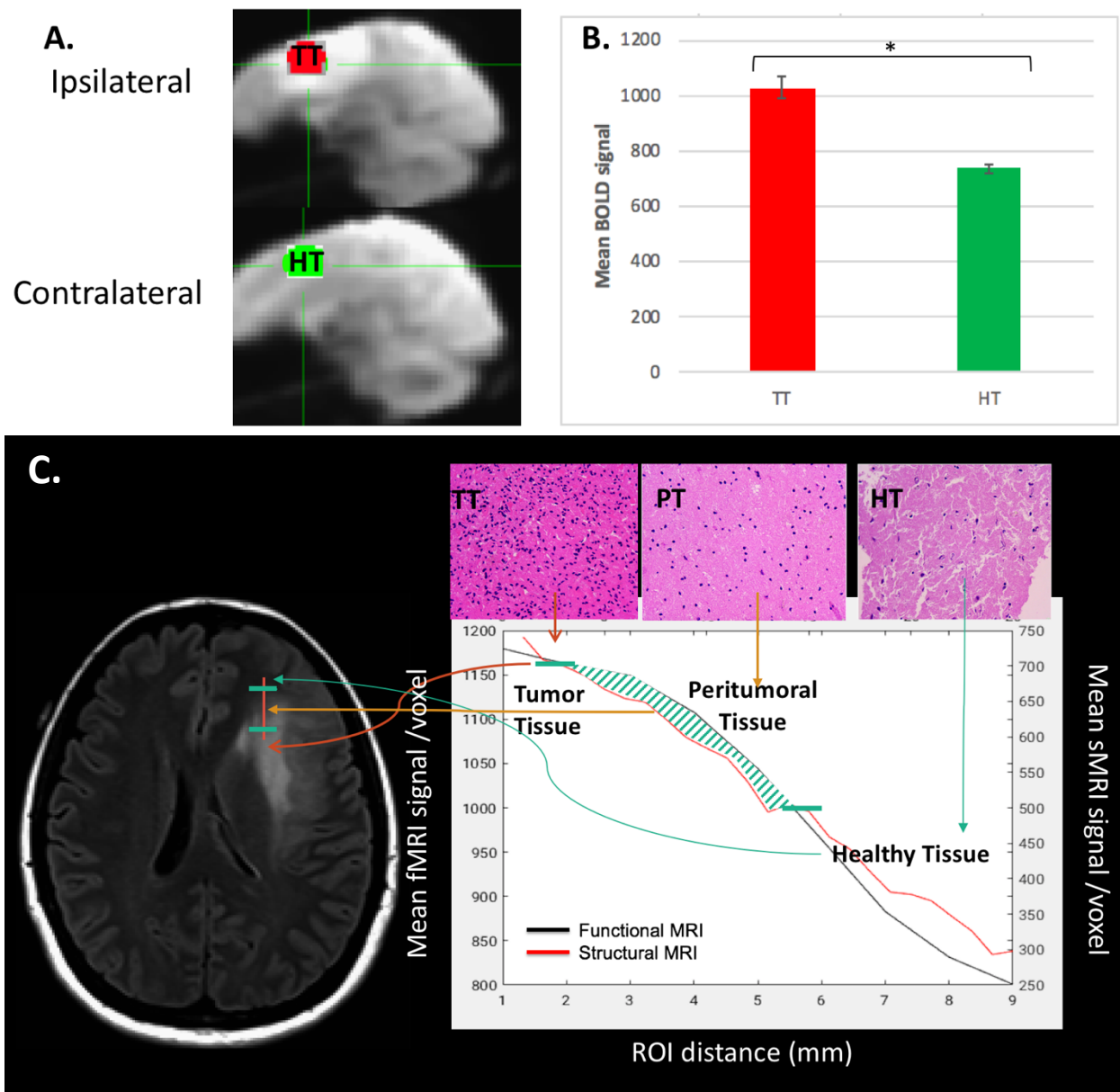
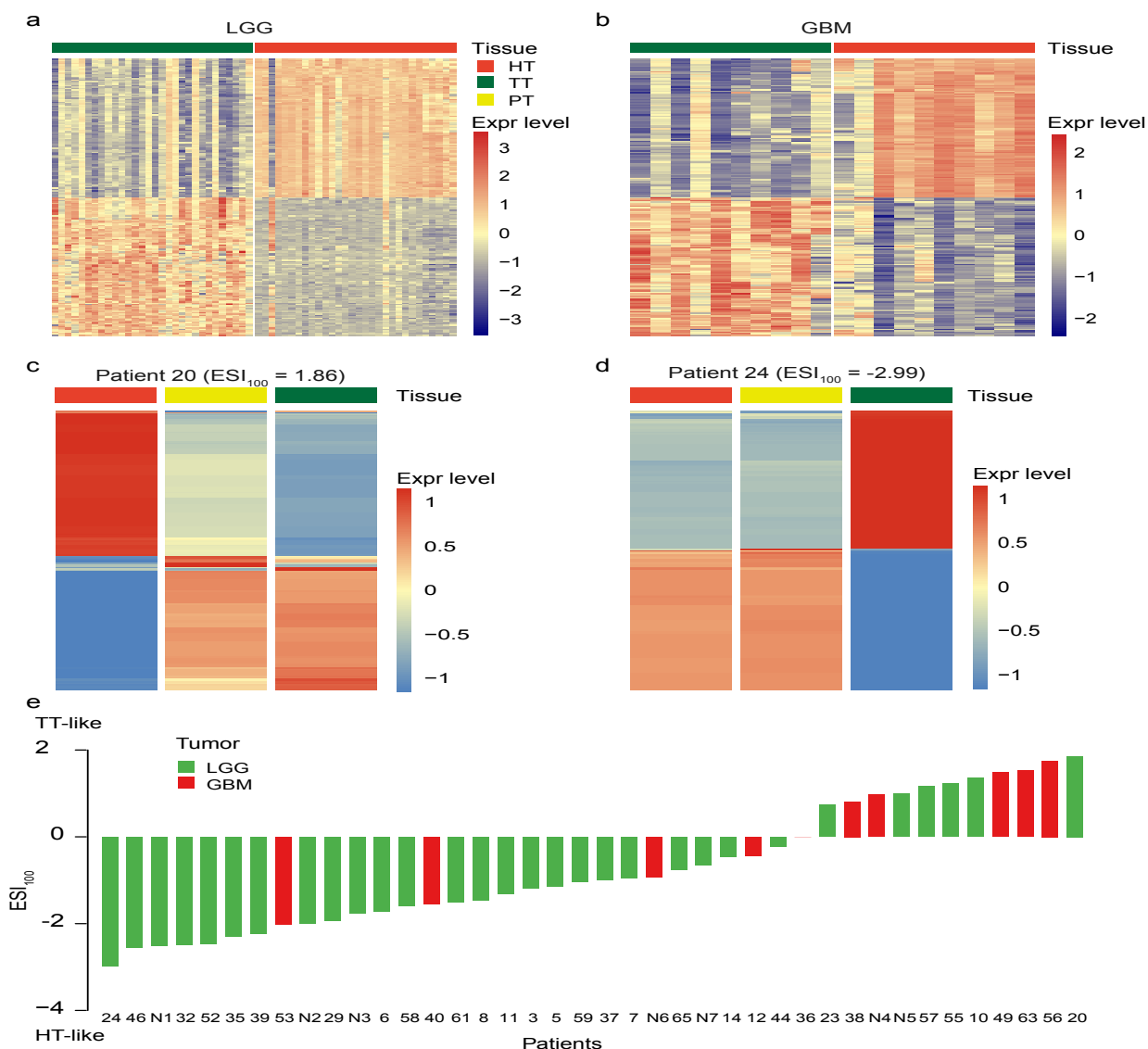


Figure 5.2 Demarcation of Macroscopically Healthy (HT), Peritumoral (PT) and Tumoral Tissue (TT) with rs-fMRI.



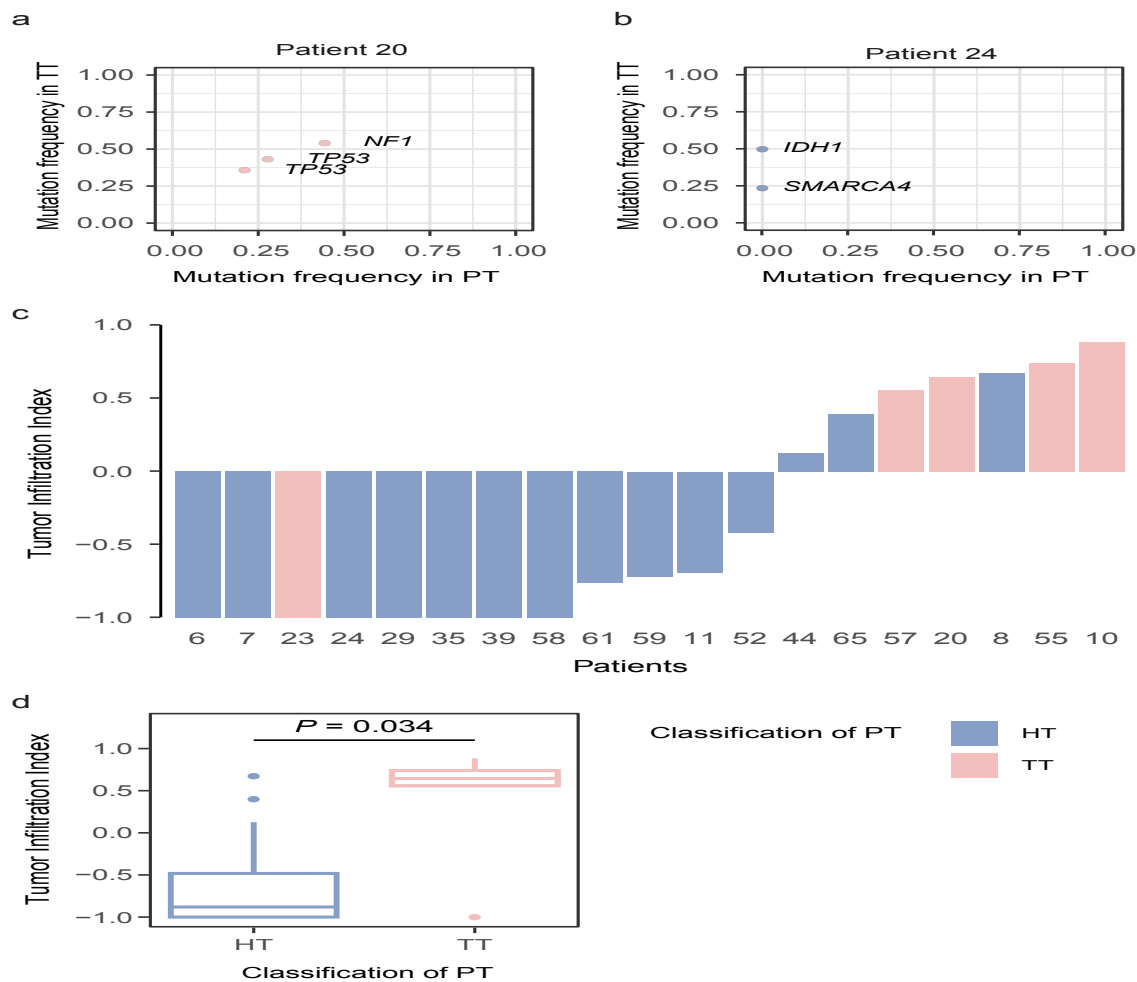
A. Tumoral and contralateral region of interest (ROI); B. Mean BOLD signal difference between tumoral and contralateral ROI ($P < 0.001$); C. Single subject demarcation of peritumoral tissue (PT) (all single subject data available in supplement).*

Figure 5.3 Genetic Results - Evidence from Differentially Expressed Genes



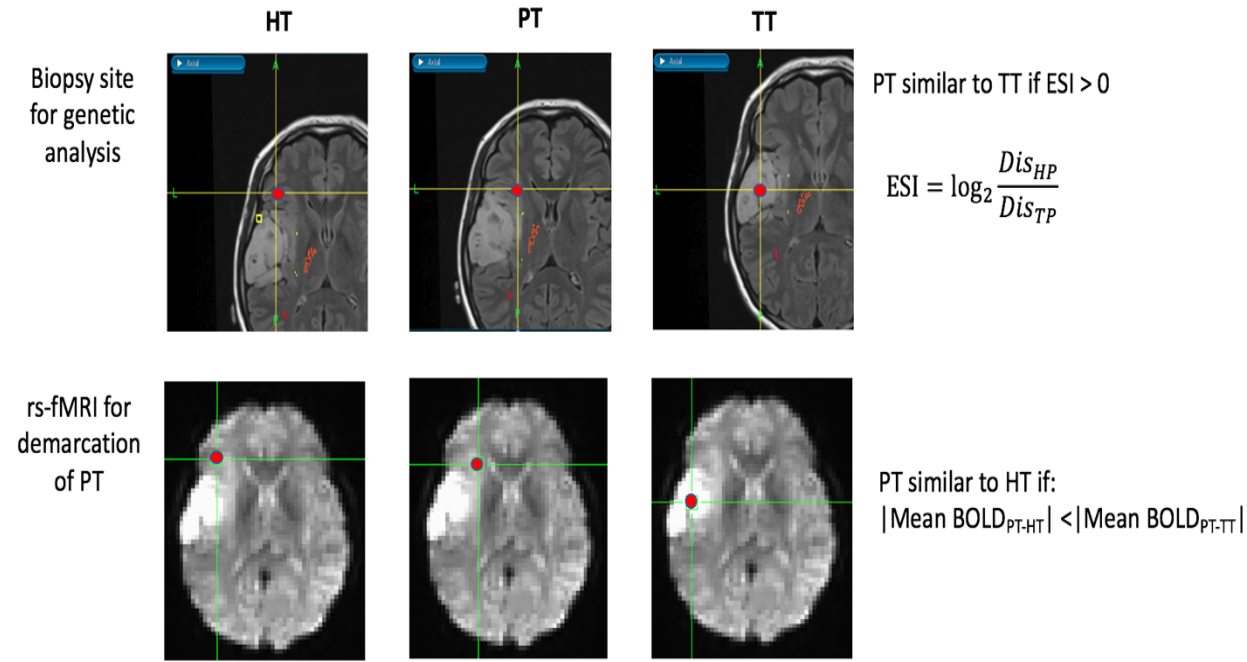
A. 465 significantly up-regulated and 795 down-regulated genes were found in the LGG patients. B. 195 up-regulated and 768 down-regulated genes were found in GBM patients. C. A positive ESI indicates that the gene expression pattern of PT is similar to TT. For example, a similar expression pattern was found between TT and PT in patient no. 20, and thus the PT was classified as TT-like ($ESI_{100} = 1.86$). D. In contrast, the PT was classified as HT-like in patient no. 24 since the expression pattern was similar to HT rather than TT ($ESI_{100} = -2.99$). E. The 100 most up-regulated and 100 most down-regulated genes were retrieved to classify the PT as whether HT-like or TT-like using ESI_{100} .

Figure 5.4 Genetic Results - Evidence from Genetic Alterations

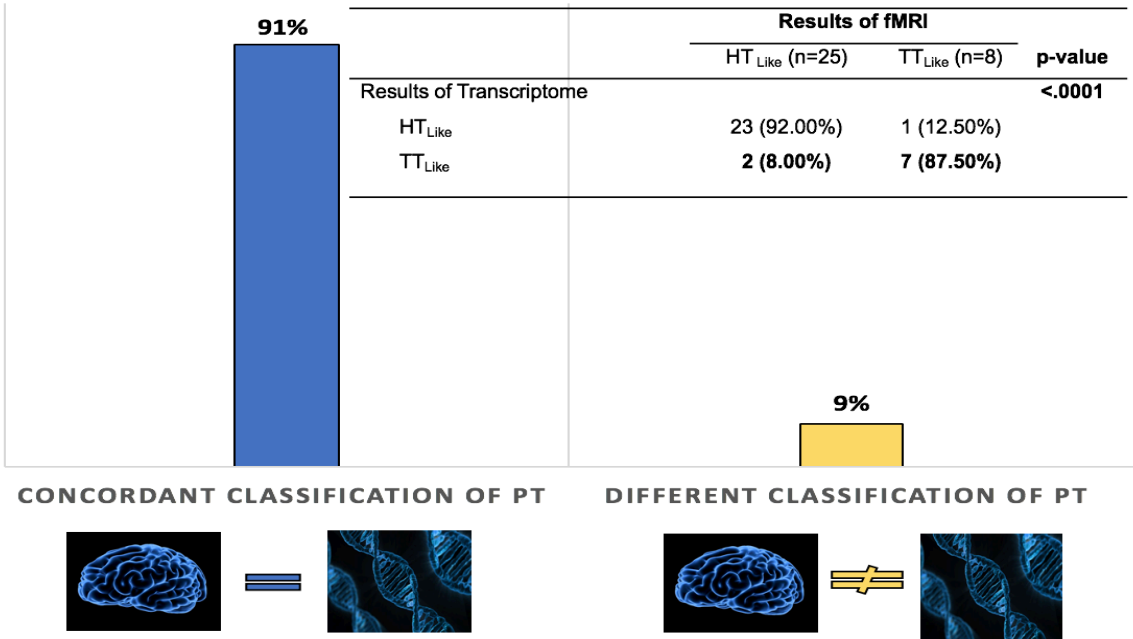


Tumoral infiltration in PT was found in some patients, characterized by identification of the same mutation in both TT and PT. A. For instance, in patient 20, mutations of *NF1* and *TP53* were identified in both TT and PT with similar mutation frequencies. B. In contrast, absence of mutations in PT indicates less tumoral infiltration as seen in patient 24. C. To quantify the degree of tumor infiltration, we retrieved the mutation with highest frequency in PT, and compared its frequency between PT and TT. A positive/negative TII indicates high/low degree of tumor infiltration. D. Transcriptome-defined TT-like PTs showed a significantly higher tumor infiltration than HT-like PTs.

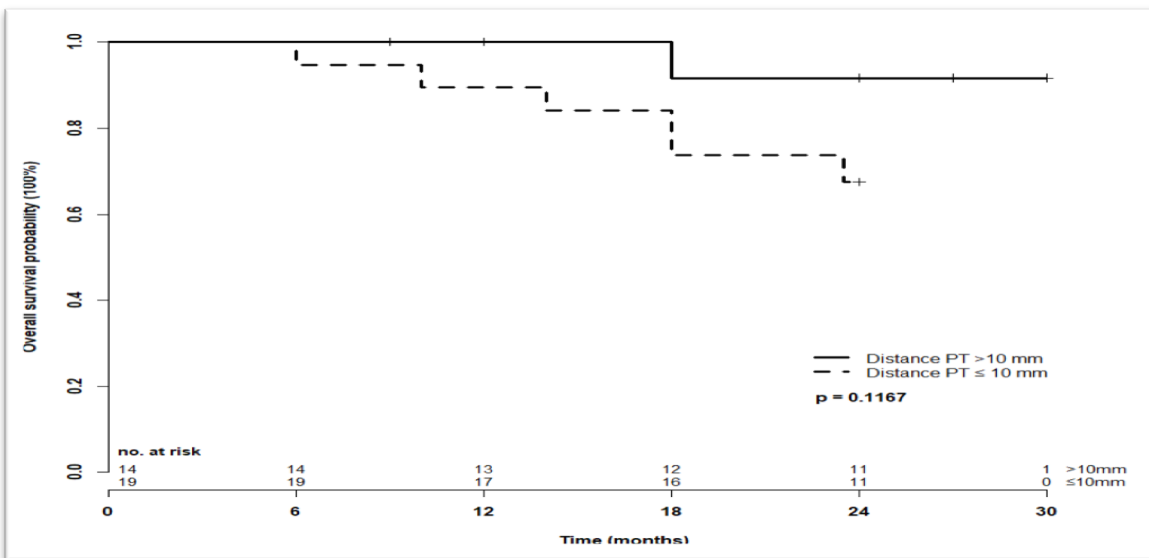
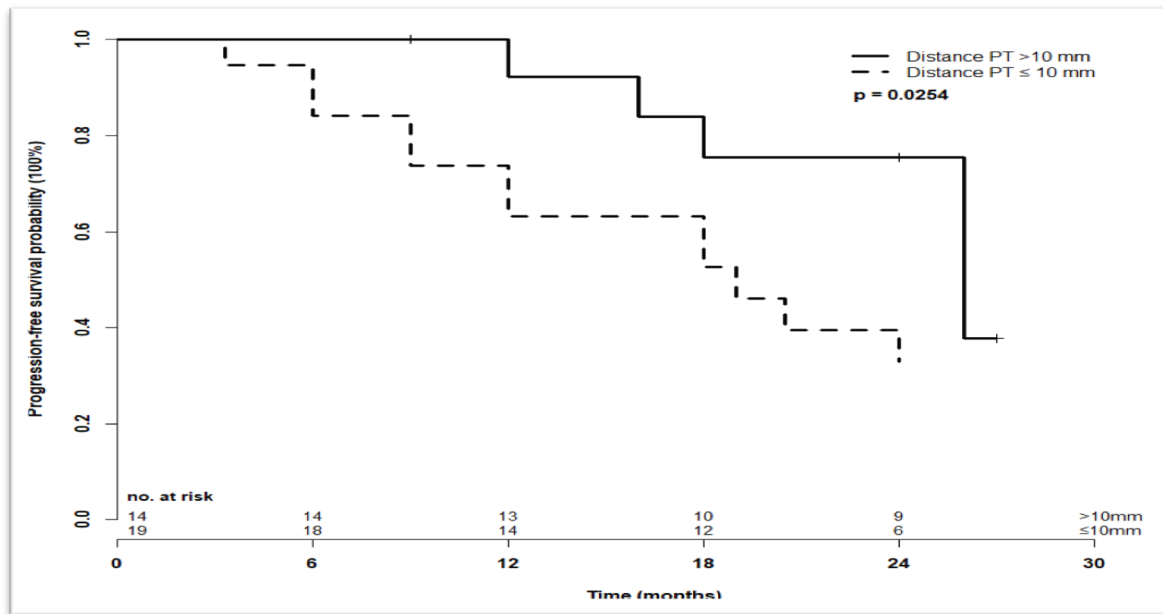
Figure 5.5 Peritumoral Tissue Demarcation
A.



B.

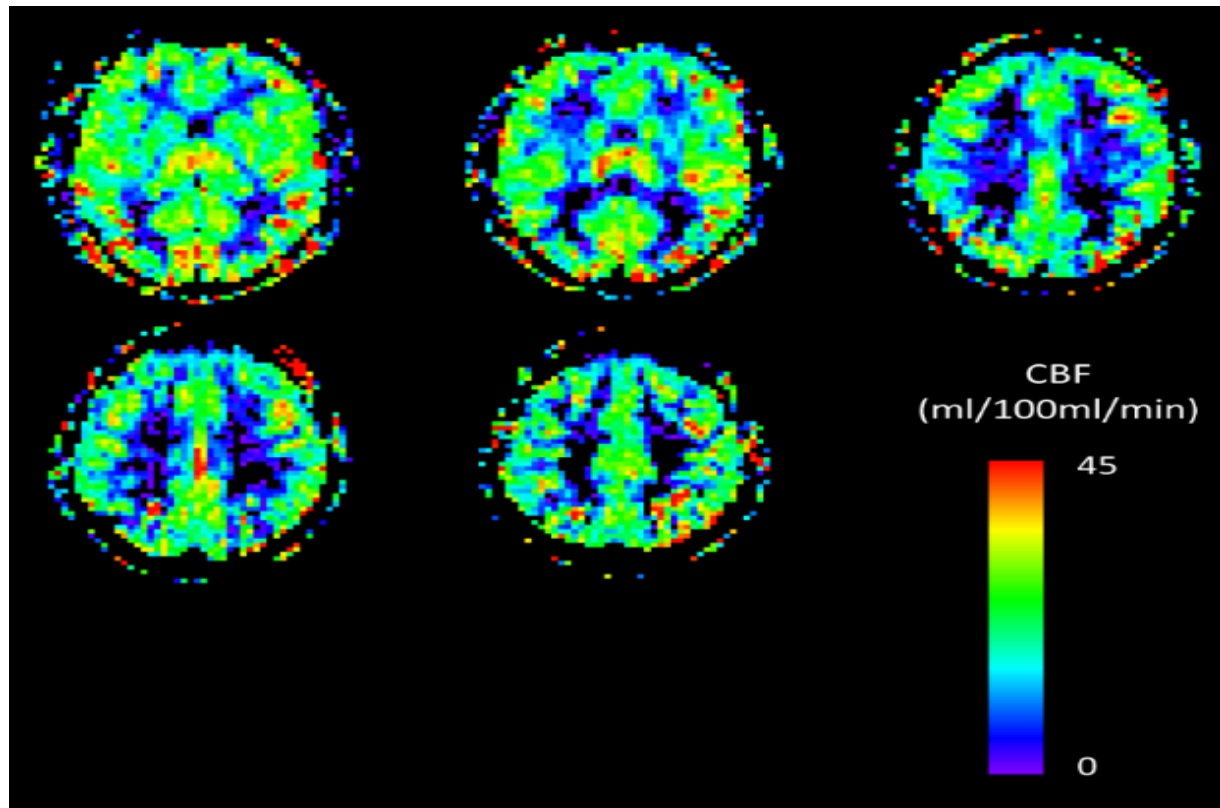


C.



A. Method for grouping the biopsy site according to rs-fMRI and genetic analysis. B. rs-fMRI and genetic analysis correlation showing significant association between fMRI and the results of transcriptome ($p < 0.001$, using Fisher exact test). C. Clinical outcomes: Kaplan-Meier PFS and OS curves according to PT distance ≤ 10 or > 10 mm.

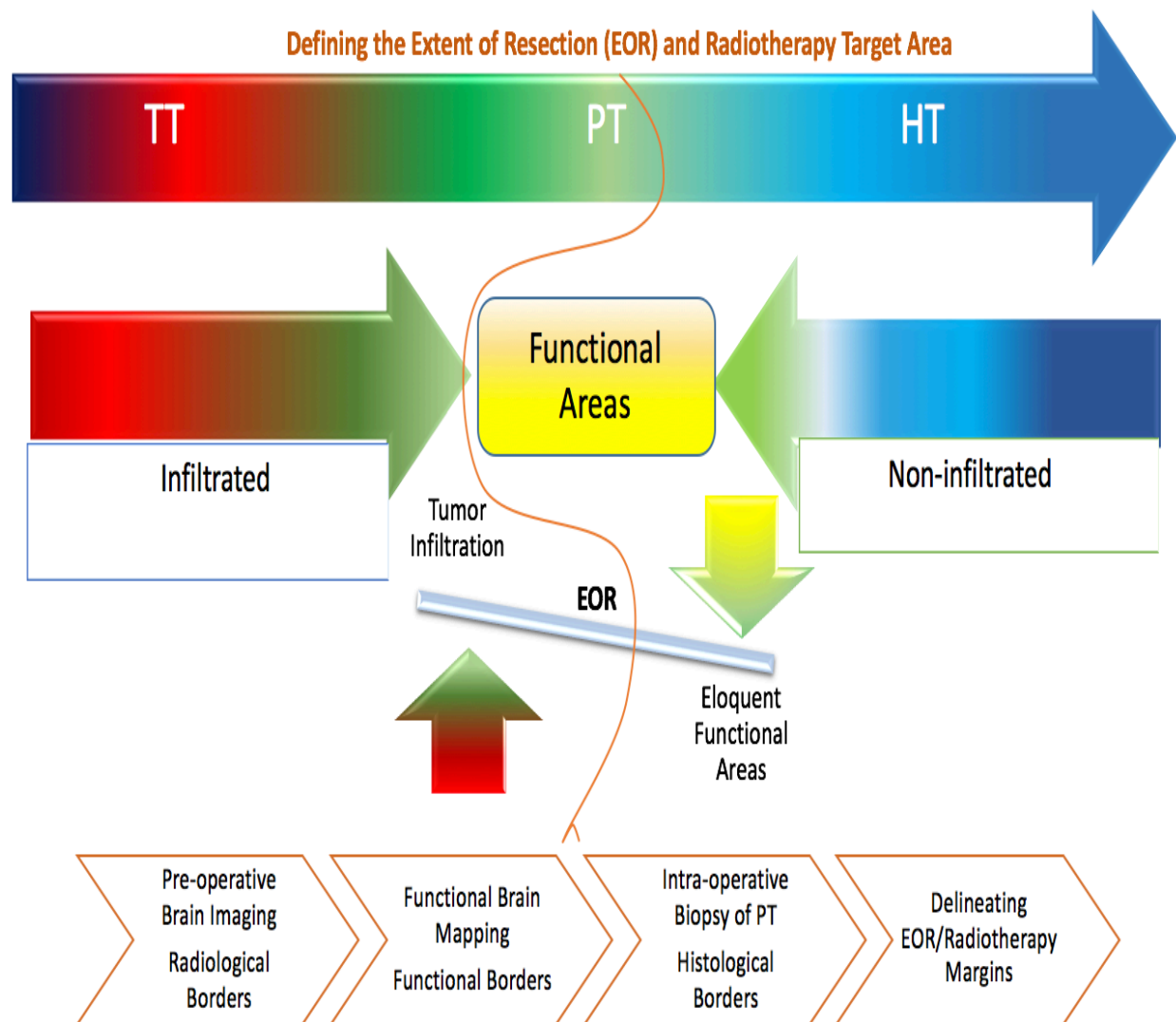
Figure 5.6 Arterial spin labelling (ASL) in a healthy pilot subject (acquired in Ottawa).



Arterial spin labeling (ASL) MRI was performed on a Siemens 3T Biograph mMR system at the Royal Ottawa Hospital. The ASL pulse sequence was PICORE Q2TIPS, with the following parameters: spatial resolution=3.4 x 3.4 x 6.0 mm³, repetition time=4 s, echo time=12 ms, inversion time one=600 ms, inversion time two=1600 ms, saturation stop time=1500 ms, and scan time=3.3 min. The pulse sequence produces inline cerebral blood flow maps in units of ml/100ml/min

CONCLUSION AND FUTURE DIRECTIONS

This research investigated various neuronal, molecular and electrophysiological measures in patients with low- and high-grade gliomas in order to better demarcate the interplay between the macroscopically-detectable tumor tissue and the surrounding non-macroscopically-affected tissue. This multimodal approach provides the much needed opportunity to broaden our knowledge on the electrophysiological features and cerebral network reorganization occurring in the presence of glioma. Taken together, the various findings indicate a potential clinical utility of combined functional-structural and molecular assessment for investigating the local and diffuse alterations occurring in the setting of glioma. For the first time to our knowledge, we proposed an objective definition of the peritumoral tissue through the use of rs-fMRI. Future research including sequential assessments to identify glioma induced and surgical related changes could provide new ways of delineating functional resection boundaries in light of anticipated neurological consequences. Moreover, more detailed insights and correlations with the pathophysiological and molecular mechanisms of glioma are needed for developing specific diagnostic and therapeutic markers that could be applied intra-operatively in order to guide the surgical management. In future, we plan to also investigate non-invasive vascular markers of infiltrated tissue across the radiologically-defined macroscopic boundaries of TT and HT in glioma using arterial spin labelling. As described in the proposed management model in the figure below, future research may allow to model the dynamic interplay between the tumour's molecular and genetic features, the brain network plasticity and the individualized surgical intervention in order to tailor the extent of resection according to the potential of network reorganization and anticipated functional consequences. This could lead to the clinical implementation of our proposed novel diagnostic and therapeutic markers in order to bring further therapeutic options for patients affected by gliomas.



Article 1. Network Plasticity and Intraoperative Mapping for Personalized Multimodal Management of Diffuse Low-Grade Gliomas



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Network Plasticity and Intraoperative Mapping for Personalized Multimodal Management of Diffuse Low-Grade Gliomas

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Gliomas are the most frequent primary brain tumors and include a variety of different histological tumor types and malignancy grades. Recent achievements in terms of molecular and imaging fields have created an unprecedented opportunity to perform a comprehensive interdisciplinary assessment of the glioma pathophysiology, with direct implications in terms of the medical and surgical treatment strategies available for patients. The current paradigm shift considers glioma management in a comprehensive perspective that takes into account the intricate connectivity of the cerebral networks. This allowed significant improvement in the outcome of patients with lesions previously considered inoperable. The current review summarizes the current theoretical framework integrating the adult human brain plasticity and functional reorganization within a dynamic individualized treatment strategy for patients affected by diffuse low-grade gliomas. The concept of neuro-oncology as a brain network surgery has major implications in terms of the clinical management and ensuing outcomes, as indexed by the increased survival and quality of life of patients managed using such an approach.

Keywords: awake surgery, functional brain mapping, intraoperative mapping, anatomofunctional connectivity, low-grade gliomas, neuroplasticity, direct electrical stimulation

INTRODUCTION

Neurosurgical resection remains the standard of care for gliomas, and the extent of resection (EOR) is one of the most important factors affecting the patients' survival and quality of life for both high- and low-grade gliomas (1–9). The diffuse low-grade gliomas (DLGGs) portray a distinct clinical and radiological behavior and display particular gene expression signatures. DLGG is thought to represent a chronic invasive lesion that migrates along the white matter pathways, and eventually undergoes malignant transformation leading ultimately to death (10).

The concept of individualized surgery in neuro-oncological treatment of glial tumors is based on the goal of achieving a maximal tumor resection without inducing new neurological deficits. Analogously, for tumors located in proximity to critical functional areas, the use of intraoperative cortical and subcortical electro stimulation mapping (IEM) during awake craniotomy evolved over time and allows a substantial increase in the survival and quality of life of patients (1, 6, 9, 11–14).

The joint efforts of neuroscientists, researchers, and clinicians have provided an unprecedented ability to localize lesions and to assess the human brain function at the microscopic, mesoscopic,

Article 2. Contribution of combined intraoperative electrophysiological investigation with 3-T intraoperative MRI for awake cerebral glioma surgery: comprehensive review of the clinical implications and radiological outcomes

Contribution of combined intraoperative electrophysiological investigation with 3-T intraoperative MRI for awake cerebral glioma surgery: comprehensive review of the clinical implications and radiological outcomes

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OBJECTIVE This study aimed to assess the clinical efficiency of combined awake craniotomy with 3-T intraoperative MRI (iMRI)-guided resection of gliomas adjacent to eloquent cortex performed at a single center. It also sought to explore the contribution of iMRI to surgeons' learning process of maximal safe resection of gliomas.

METHODS All patients who underwent an awake craniotomy and iMRI for resection of eloquent area glioma during the 53 months between January 2011 and June 2015 were included. The cases were analyzed for short- and long-term neurological outcome, progression-free survival (PFS), overall survival (OS), and extent of resection (EOR). The learning curve was assessed after dividing the cohort into Group A (first 27 months) and Group B (last 26 months). Statistical analyses included univariate logistic regression analysis on clinical and radiological variables. Kaplan-Meier and Cox regression models were used for further analysis of OS and PFS. A p value < 0.05 was considered statistically significant.

RESULTS One hundred six patients were included in the study. Over an average follow-up period of 24.8 months, short- and long-term worsening of the neurological function was noted in 48 (46.2%) and 9 (8.7%) cases, respectively. The median and mean EOR were 100% and 92%, respectively, and complete radiographic resection was achieved in 64 (60.4%) patients. The rate of gross-total resection (GTR) in the patients with low-grade glioma (89.06% ± 19.6%) was significantly lower than that in patients with high-grade glioma (96.4% ± 9.1%) (p = 0.026). Thirty (28.3%) patients underwent further resection after initial iMRI scanning, with a 10.1% increase of the mean EOR. Multivariate Cox proportional hazards modeling demonstrated that the final EOR was a significant predictor of PFS (HR 0.225, 95% CI 0.070–0.723, p = 0.012). For patients with high-grade glioma, the GTR (p = 0.033), the presence of short-term motor deficit (p = 0.027), and the WHO grade (p = 0.005) were independent prognostic factors of OS. Performing further resection after the iMRI (p = 0.083) and achieving GTR (p = 0.05) demonstrated a PFS benefit trend for the patients affected by a low-grade glioma. Over time, the rate of performing further resection after an iMRI decreased by 26.1% (p = 0.005). A nonsignificant decrease in the rate of short-term (p = 0.101) and long-term (p = 0.132) neurological deficits was equally noted.

CONCLUSIONS Combined awake craniotomy and iMRI is a safe and efficient technique allowing maximal safe resection of eloquent area gliomas with possible subsequent OS and PFS benefits. Although there is a learning curve for applying this technique, it can also improve the surgeon's ability in eloquent glioma surgery.

<http://thejns.org/doi/abs/10.3171/2015.12.FOCUS15572>

KEY WORDS glioma; awake craniotomy; intraoperative MRI; extent of resection; survival; clinical outcome; quality of life; eloquent areas; intraoperative neurophysiological monitoring

ABBREVIATIONS BOLD = blood oxygen level-dependent; DTI = diffusion tensor imaging; EOR = extent of resection; GTR = gross-total resection; HGG = high-grade glioma; iMRI = intraoperative MRI; LGG = low-grade glioma; MEP = motor evoked potential; OS = overall survival; PFS = progression-free survival; QOL = quality of life.

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* Dr. Ghinda and Mr. Zhang contributed equally to this work.

Article 3. How much is enough—Can resting state fMRI provide a demarcation for neurosurgical resection in glioma?

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Review article

How much is enough—Can resting state fMRI provide a demarcation for neurosurgical resection in glioma?



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Neuroplasticity

ABSTRACT

This study represents a systematic review of the insights provided by resting state functional MRI (rs-fMRI) use in the glioma population. Following PRISMA guidelines, 45 studies were included in the review and were classified in glioma-related neuronal changes ($n = 28$) and eloquent area localization ($n = 17$). Despite the heterogeneous nature of the studies, there is considerable evidence of diffuse functional reorganization occurring in the setting of gliomas with local and interhemispheric functional connectivity alterations involving different functional networks. The studies showed evidence of decreased long distance functional connectivity and increased global local efficiency occurring in the setting of gliomas. The tumour grade seems to correlate with distinct functional connectivity changes. Overall, there is a potential clinical utility of rs-fMRI for identifying the functional brain network disruptions occurring in the setting of gliomas. Further studies utilizing standardized analytical methods are required to elucidate the mechanism through which gliomas induce global changes in brain connectivity.

1. Introduction

Gliomas represent the most common primary intracranial tumour and its most aggressive form, glioblastoma, represents a lethal primary brain tumour with fewer than 5% of patients surviving at 5 years despite optimal treatment (Ostrom et al., 2014). Conversely, diffuse low-grade gliomas (DLGG) are a slow growing tumour with insidious onset that migrates along the white matter pathways, eventually undergoing malignant transformation leading ultimately to death (Duffau and Taillandier, 2015; Ghinda and Duffau, 2017). The prognosis of patients with low-grade glioma can vary from years to decades as it is determined by multiple factors, including molecular and genetic features (Oberheim Bush and Chang, 2016). Furthermore, the quality of life and the neurocognitive status are important factors in the management decision process with multiple reports providing evidence of cortical

reorganization and brain plasticity occurring in the setting of gliomas raising the need of more targeted and personalized treatments (Duffau, 2013a; Fiscaro et al., 2016; Southwell et al., 2016).

Neurosurgical resection remains the standard of care for gliomas and mounting evidence suggests that more extensive surgical resection is associated with longer life expectancy for both low- and high-grade gliomas, thus representing an important prognostic marker for patient outcome (Ius et al., 2012; Sanai et al., 2011; Tarapore et al., 2011; Wen and Kesari, 2008). Although there is a lack of class I evidence, in a recent systemic review on the role of surgery in the management of DLGG, accumulating evidence supports a benefit of gross total resection when safe and feasible since it can result in increased overall survival (OS) (Aghi et al., 2015). Given this, a central question for such a surgical approach is to confirm that total resection has been achieved. Although neurosurgeons have access to a number of methods to do this,

Abbreviations: BOLD, blood oxygen level-dependent; ICA, independent component analysis; ROI, region of interest; rs-fMRI, resting-state functional magnetic resonance imaging; tb-fMRI, task-based functional magnetic resonance imaging; RSN, resting-state network; RSC, resting state connectivity; LN, language network; NVU, neurovascular uncoupling; ICA, independent component analysis; ReHo, regional homogeneity; ALFF, amplitude of low-frequency fluctuation; fALFF, fractional ALFF; CSM, cortical stimulation mapping; LGG, low-grade glioma; DLGG, diffuse LGG; HGG, high-grade glioma; HC, healthy control; FC, functional connectivity; DMN, default mode network; PMC, premotor cortex; PCC, posterior cingulate cortex

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Electrophysiological Signature of tumoral tissue from scale-free analysis of intraoperative ECoG during awake craniotomy for glioma-related epilepsy

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12 Health Research, University of Ottawa, Ottawa, Ontario, Canada

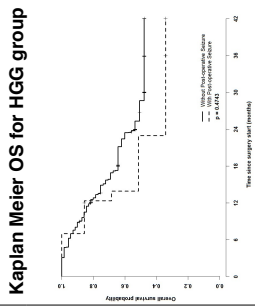
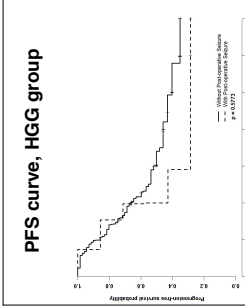
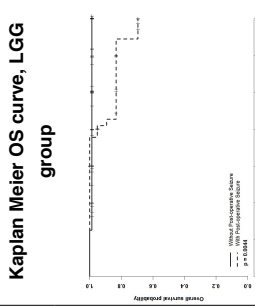
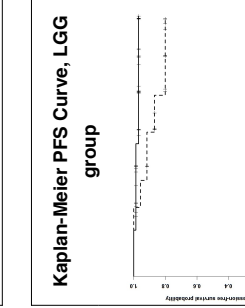
13 **Keywords:** glioma related epilepsy¹, electrocorticography², glioma³, tumor⁴, electrophysiology⁵,
14 awake craniotomy⁶, scale free measures⁷.

15
16 **Word Count:** 2 611

17 Abstract

18
19 **Background:** Electrocorticography (ECoG) has been utilized in many epilepsy cases however, the
20 use of this technique for evaluating electrophysiological changes within tumoral zones is sparse.
21 Nonetheless, epileptic activities seem to arise from the neocortex surrounding the gliomas suggesting
22 a link between epileptogenesis and glioma cell infiltration in the peritumoral area. The purpose of
23 this study was to implement novel scale free measures to assess how cortical physiology is altered by
24 the presence of an invasive brain tumor. **Methods:** Twelve patients undergoing an awake craniotomy
25 for resection of a supratentorial glioma were included. ECoG data over the main tumor and the
26 exposed surroundings was acquired intra-operatively just prior to tumour resection. Six patients
27 presenting with seizures had data acquired both in the awake and anesthetic state. The corresponding
28 anatomical location of each electrode in relation to the macroscopically-detectable tumor was
29 recorded using the neuronavigation system based on structural anatomical images obtained pre-
30 operatively. The electrodes were classified into tumoral, healthy or peritumoral based on the
31 macroscopically - detectable tumoral tissue from the pre-operative structural MRI. **Results:** The

Poster 1. Persistent or New Post-operative Seizures as Prognostic Marker for Survival in Glioma Patients

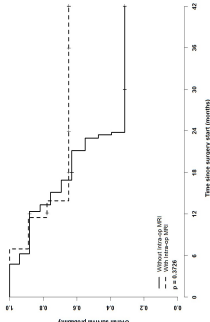
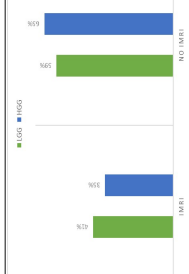
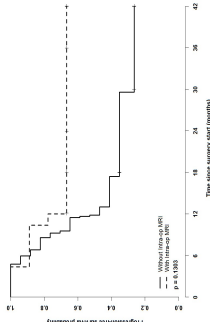
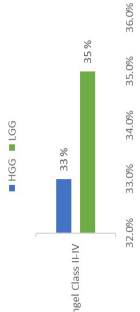
 Persistent or New Post-operative Seizures as Prognostic Marker for Survival in Glioma Patients Diana Cristina Ghinda MD; Zhengda Yu; Jin-song Wu MD, PhD Huashan Hospital, Neurosurgical Department, Fudan University, Shanghai, China; Ottawa Hospital, Neurosurgery Division, Ottawa, Canada 	Introduction Infiltrated peritumoral neocortex appears to be a key structure for glioma-related epileptogenesis that may explain both the anti-epileptic effects of oncological treatments and the increase in seizure frequency as tumors progress. Methods 182 consecutive patients aged 18–70 years were randomly assigned to either the trial group (MRI group) or conventional neuronavigation. 177 patients included for survival analysis [78(44%) HGG and 99(56%) LGG] after the patients with less than 6 months of postoperative follow-up and neuropathologically confirmed nonglioma lesions were excluded. Benign histologies were excluded from the secondary end-point follow-up.	Kaplan Meier OS for HGG group  PFS curve, HGG group  In the HGG group, an OS (p=0.4743) and PFS (p=0.5773) benefit trend was noted in the group of patients with HGG without post-operative seizure. Results 59(59.6%) and 26 (33.3%) patients presented with seizures in the LGG and respectively HGG group (p=0.001). No patient had intractable epilepsy and a pre-operative seizure duration of more than 6 months was present in 27% and 13% for LGG and respectively HGG groups (p=0.108).	Kaplan Meier OS curve, LGG group  Kaplan-Meier PFS Curve, LGG group  In the LGG group, log-rank test demonstrated a statistically significant OS (p=0.0044) and PFS (p=0.0172) benefit in the patients without seizure after surgery.	Learning Objectives By the conclusion of this session, participants should be able to: 1) Discuss the benefit of tumor related seizure assessment 2) Outline the importance of good seizure control 3) Recognize the need of a thorough follow-up of the seizure-related symptoms
	Conclusions Persistent or new post-operative seizures might indicate tumor recurrence/progression and thus serve as a prognostic marker for survival.	References 1. Kerkhof M, Vecht CJ. Seizure characteristics and prognostic factors of gliomas. <i>Epilepsia</i>. 2013;54 Suppl 9:12–7. 2. Englot DJ, Berger MS, Barbaro NM, Chang EF. Factors associated with seizure freedom in the surgical resection of glioneuronal tumors. <i>Epilepsia</i>. 2012;53(1):51–7. 3. Englot DJ, Han SJ, Berger MS, Barbaro NM, Chang EF. Extent of surgical resection predicts seizure freedom in low-grade temporal lobe brain tumors. <i>Neurosurgery</i>. 2012;70(4):921–8		

Poster 2. Assessment of the Contribution of 3-Tesla intra-operative MRI Guided Surgery for Patients with Seizure- related Glioma in a Prospective Triple-blinded Study



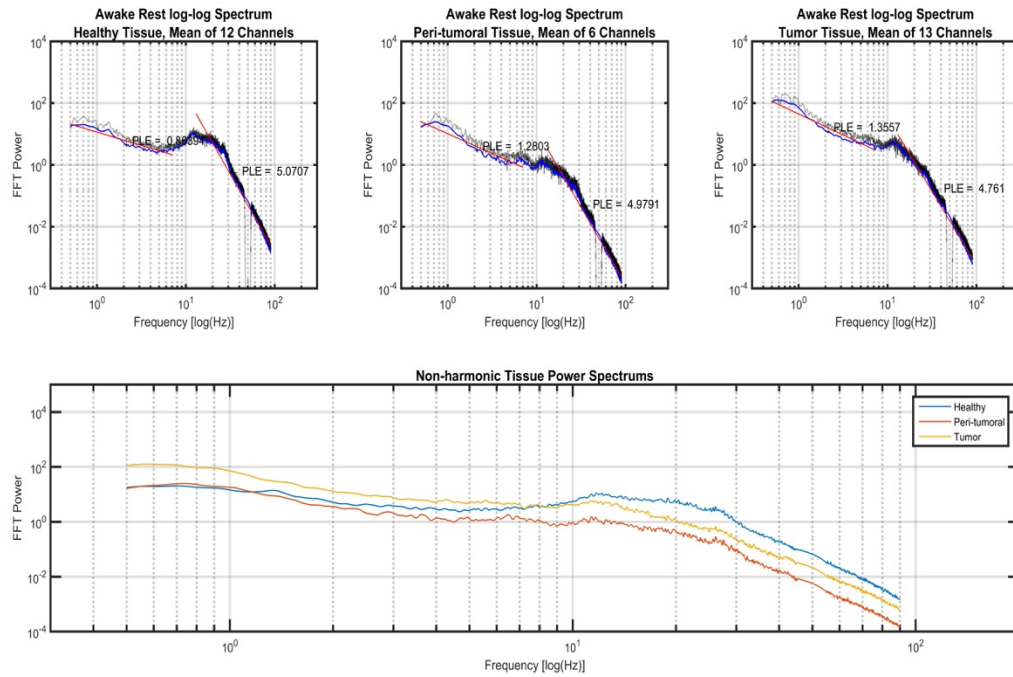

Assessment of the Contribution of 3-Tesla intra-operative MRI Guided Surgery for Patients with Seizure-related Glioma in a Prospective Triple-blinded Study

Diana Cristina Ghinda MD; Zhengda Yu; Jin-song Wu MD, PhD
Huashan Hospital, Neurosurgical Department, Fudan University, Shanghai, China;
Ottawa Hospital, Neurosurgery Division, Ottawa, Canada

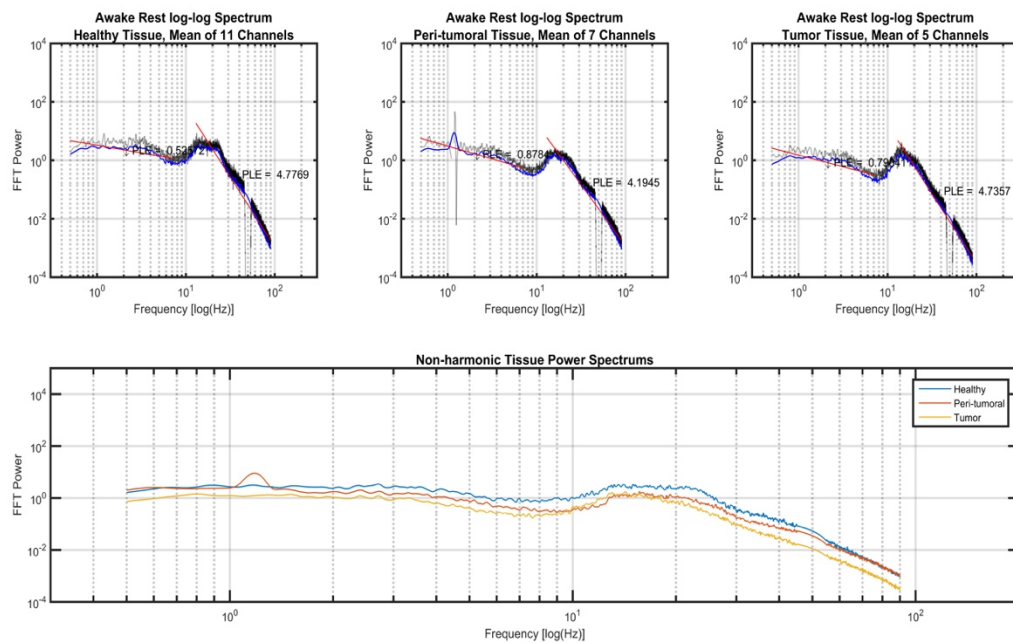
Introduction Glioma-related seizures are a daily concern in the management of patients. As incomplete resection can result in local tumor recurrence and persisting seizures, application of iMRI could be beneficial.	Results No patient had intractable epilepsy and a pre-operative seizure duration of more than 6 months was present in 19(22%) patients. 56 (65.9%) patients had an Engel Class 1 outcome. Overall, there was no significant association between iMRI use and tumor grade ($p=0.390$) in patients with persistent seizures. 18 (40.9%) patients from the iMRI group underwent further resection after initial iMRI scanning, for which the use of iMRI allowed a 11.4 % and 13% increase of the mean EOR for the LLG and HGG patients.	OS in HGG group stratified by iMRI use 	Conclusion iMRI can provide a survival benefit for HGG patients presenting with seizures but this technique doesn't necessarily result in seizure freedom. The use of other electrophysiological measures allowing a better understanding and assessment of glioma-related epileptogenesis would be beneficial.
Methods 85 patients with at least one de novo epileptic seizure at presentation and a neuropathological confirmed glioma enrolled in a prospective randomized iMRI trial were included in this study. The cases were analyzed for short and long-term neurological outcome, progression-free survival (PFS), overall survival (OS), seizure freedom (Engel Class I) and extent of resection (EOR). Wilcoxon non-parametric test or Fisher exact test was applied for continuous or categorical variables.		PFS in HGG group 	Learning Objectives By the conclusion of this session, participants should be able to: 1) discuss the role of intraoperative MR imaging in patients with glioma related seizure 2) outline possible future preoperative and intraoperative considerations for improving seizure control after tumor resection.
Engel Class II-IV for patients in iMRI group according to grade 	35% patients with LGG and 33% patients with HGG had persistent seizures at follow-up.	PFS and OS analysis of the patients with HGG, demonstrated a survival benefit trend for patients undergoing iMRI intervention.	References 1. Campbell SL, Buckingham SC, Sontheimer H. Human glioma cells induce hyperexcitability in cortical networks. <i>Epilepsia</i>. 2012;53(8):1360-70. 2. De Groot M, Reijneveld J, Aronica E, Heimans J. Epilepsy in patients with a brain tumour: focal epilepsy requires focused treatment. <i>Brain</i>. 2012;135:1002-1016. 3. Buckingham SC, Robel S. Glutamate and tumor-associated epilepsy: glial cell dysfunction in the peritumoral environment. <i>Neurochem Int</i>. 2013; 63(7):696-701. 4. Englot DJ, Berger MS, Barbaro NM, Chang EF. Factors associated with seizure freedom in the surgical resection of glioneuronal tumors. <i>Epilepsia</i>. 2012;53(1):51-7. 5. Englot DJ, Han SJ, Berger MS, Barbaro NM, Chang EF. Extent of surgical resection predicts seizure freedom in low-grade temporal lobe brain tumors. <i>Neurosurgery</i>. 2012;70(4):921-8

Appendix 1 Power Law Exponent results in individual cases for remaining patients.

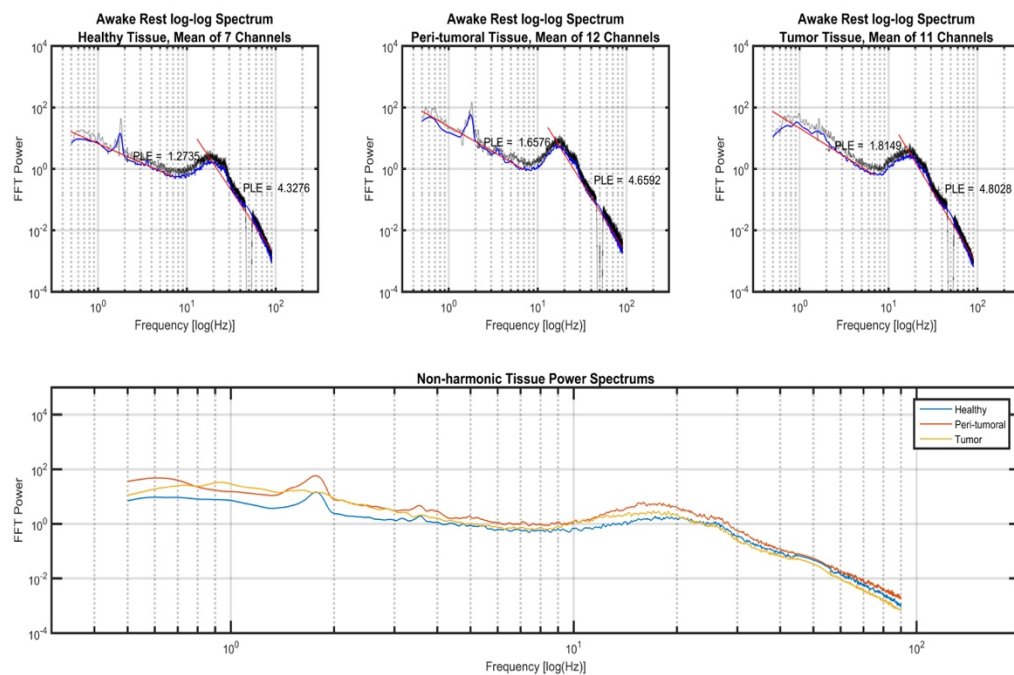
A. Patient 7



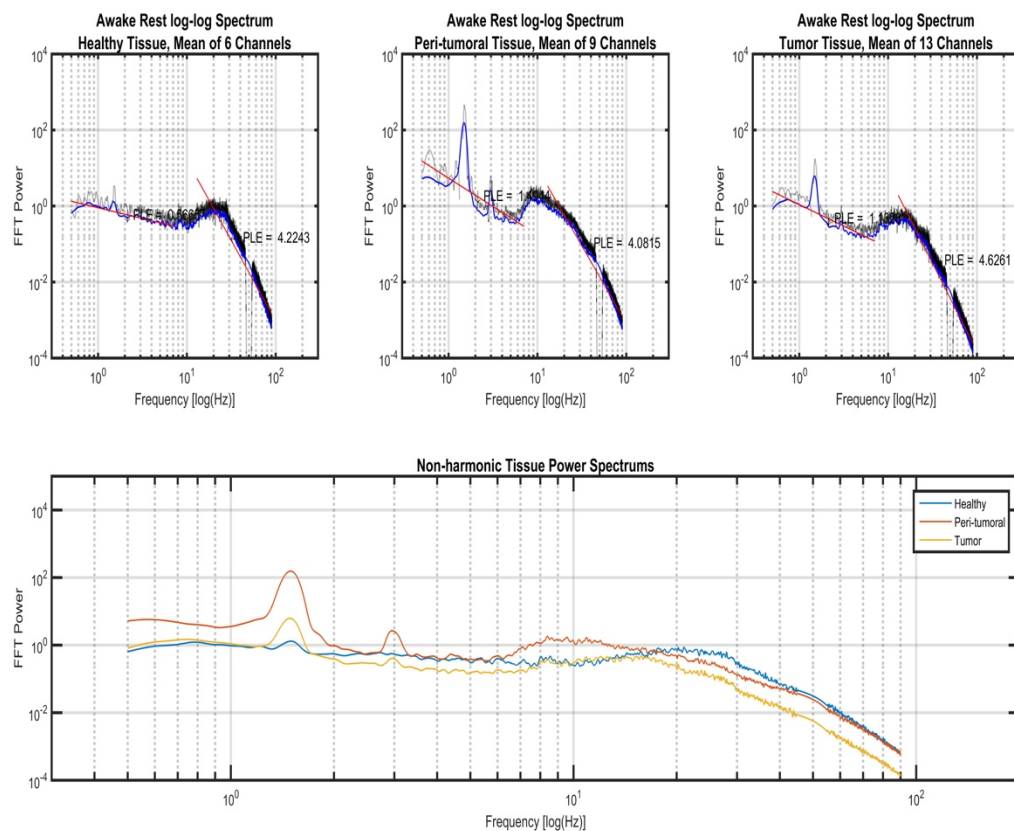
B. Patient 8



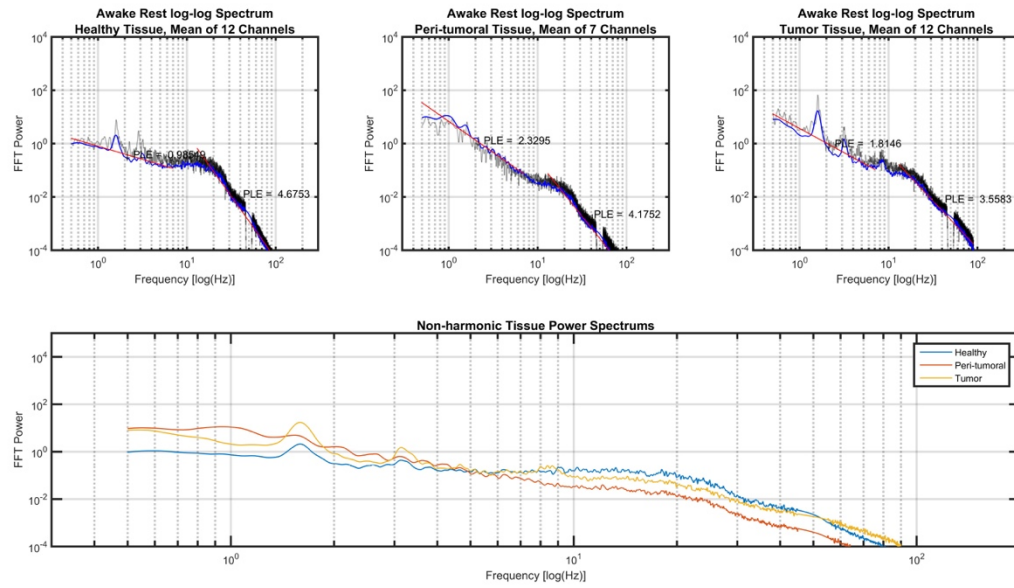
C. Patient 9



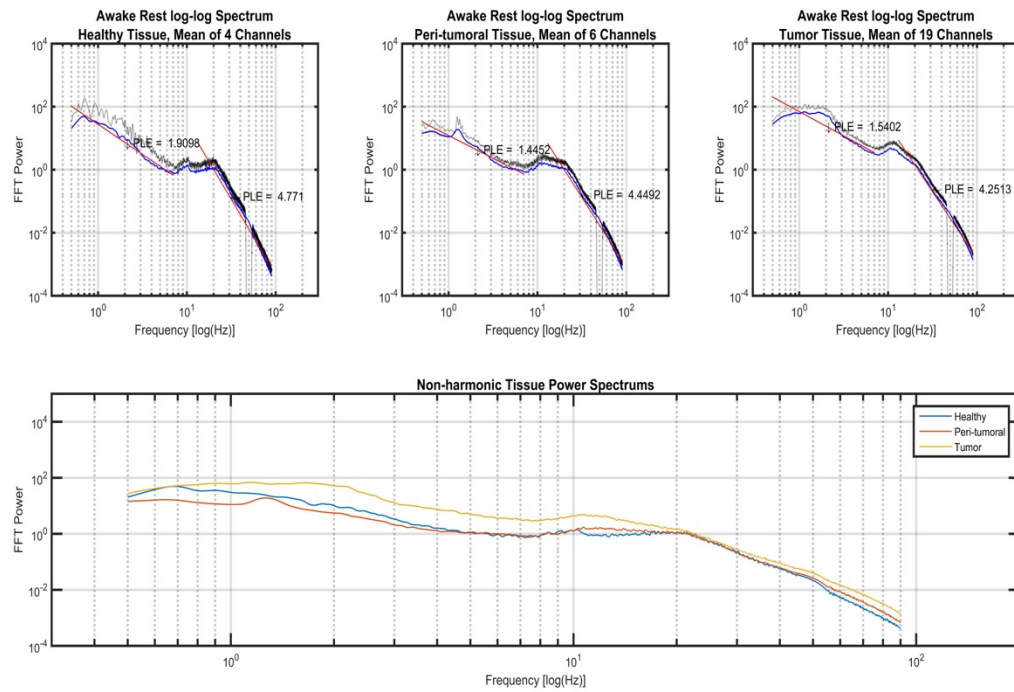
D. Patient 10



E. Patient 11



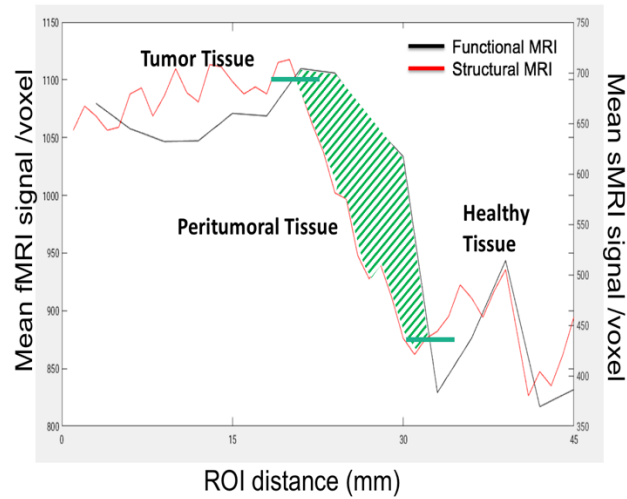
F. Patient 12



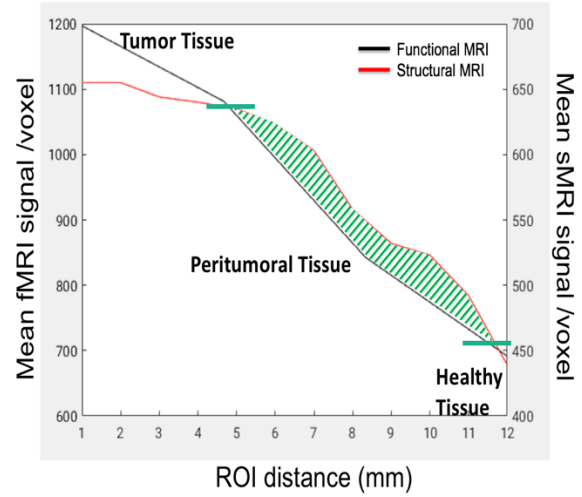
II. SUPPLEMENTARY FIGURES:

Fig S1. Individualized Peritumoral Tissue demarcation with rs-fMRI (two ROI locations) for 5 patients. The rest of the individual figures are available upon demand.

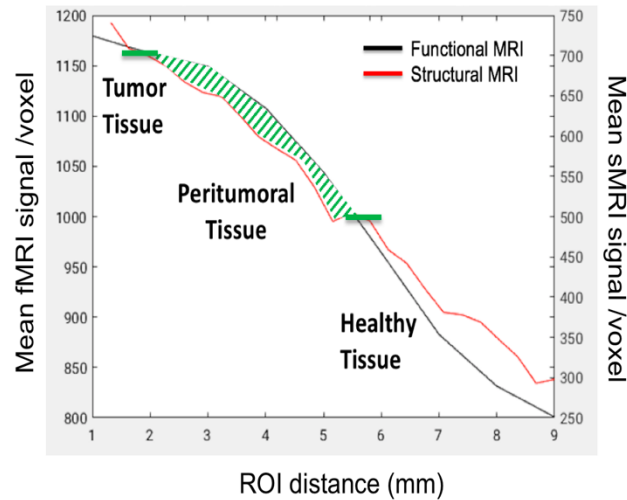
1. A. PT demarcation



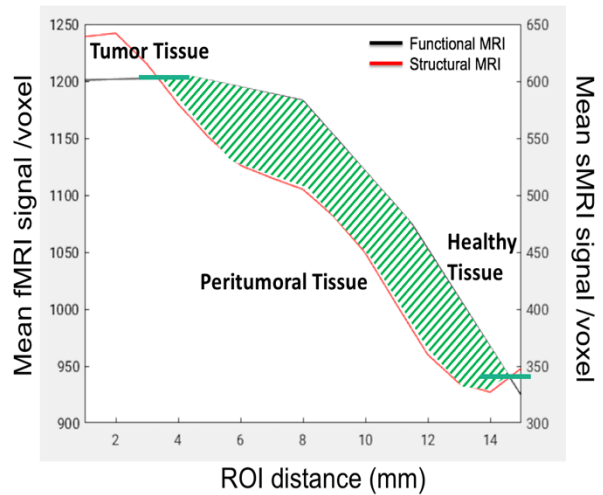
B. Control



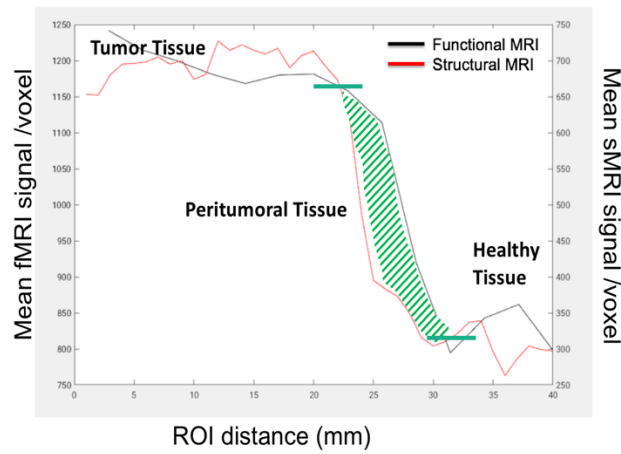
2. A. PT demarcation



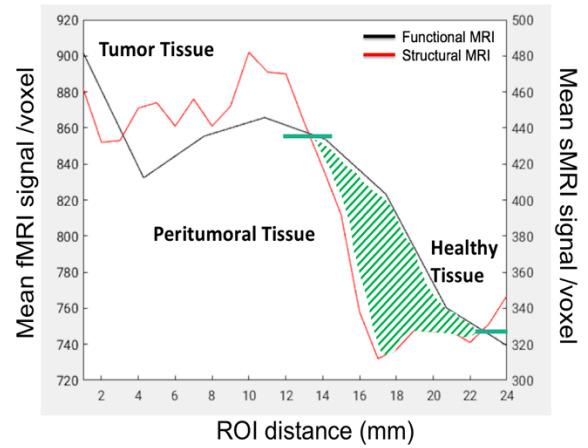
B. Control



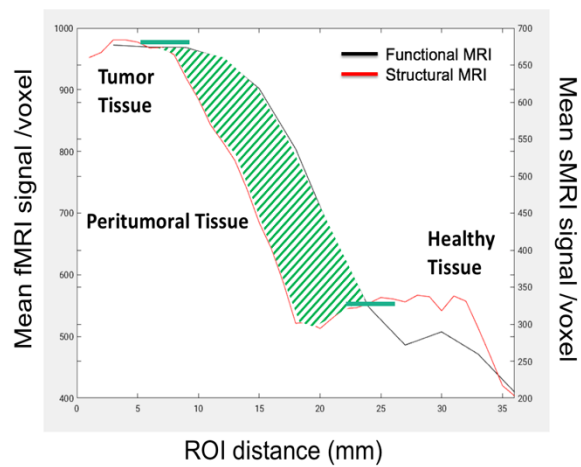
3. A. PT demarcation



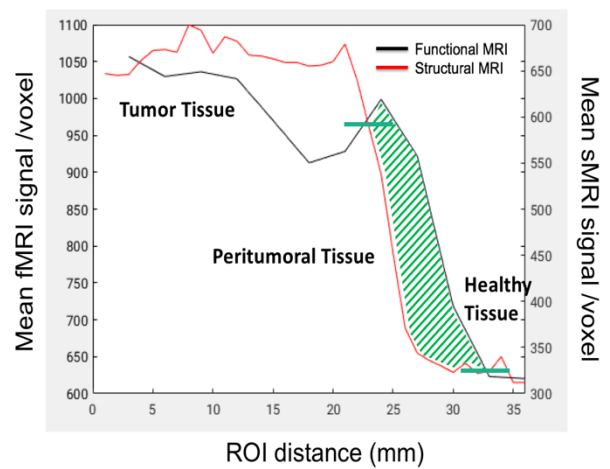
B. Control



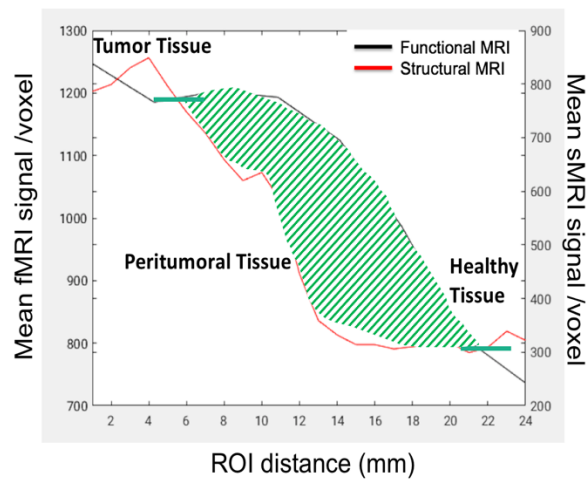
4. A. PT demarcation



B. Control



5. A. PT demarcation



B. Control

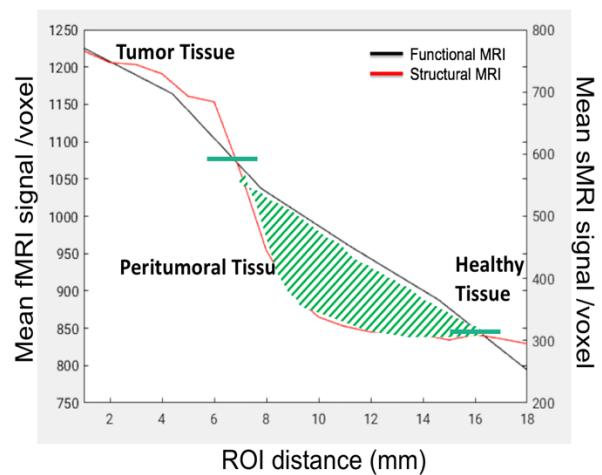


Fig S2. Peritumoral Tissue classification according to DEGs, ranging from 20, 30, 40 and 50 most significantly up- and down-regulated genes.

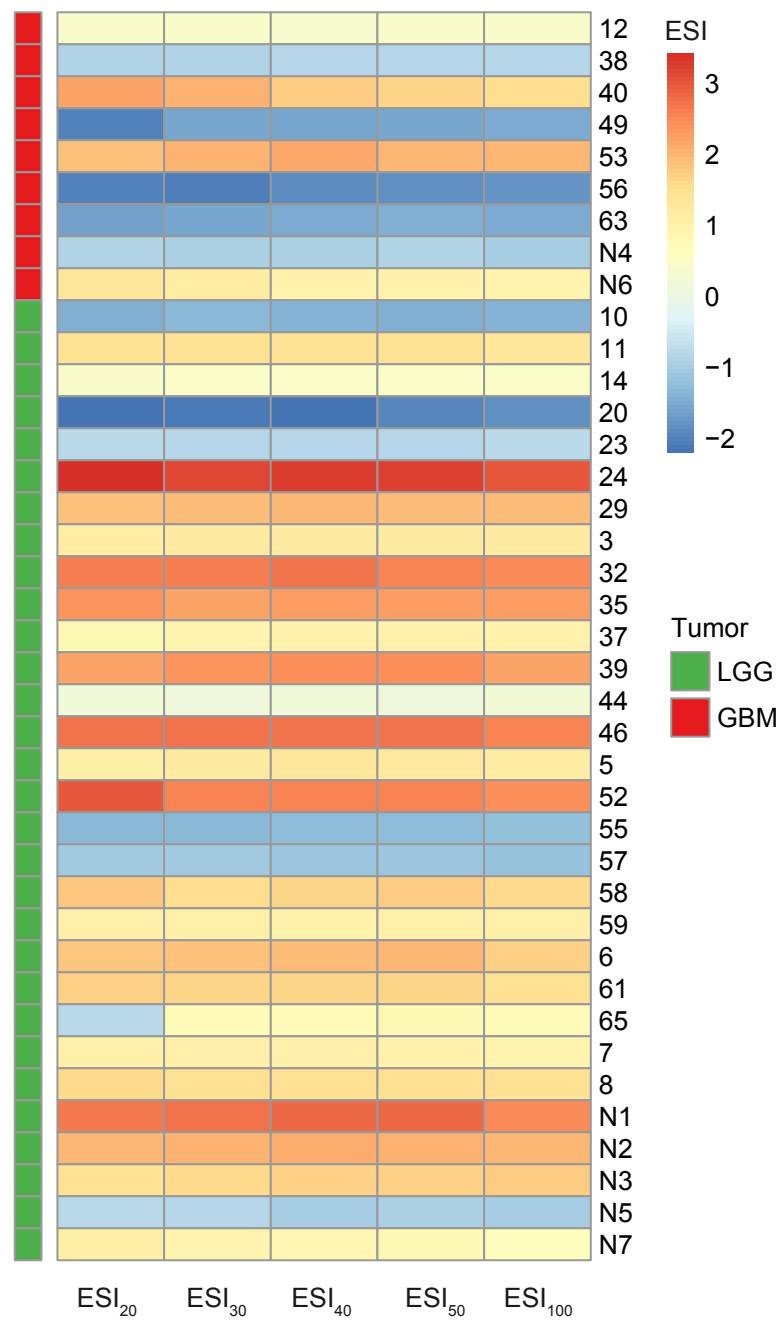
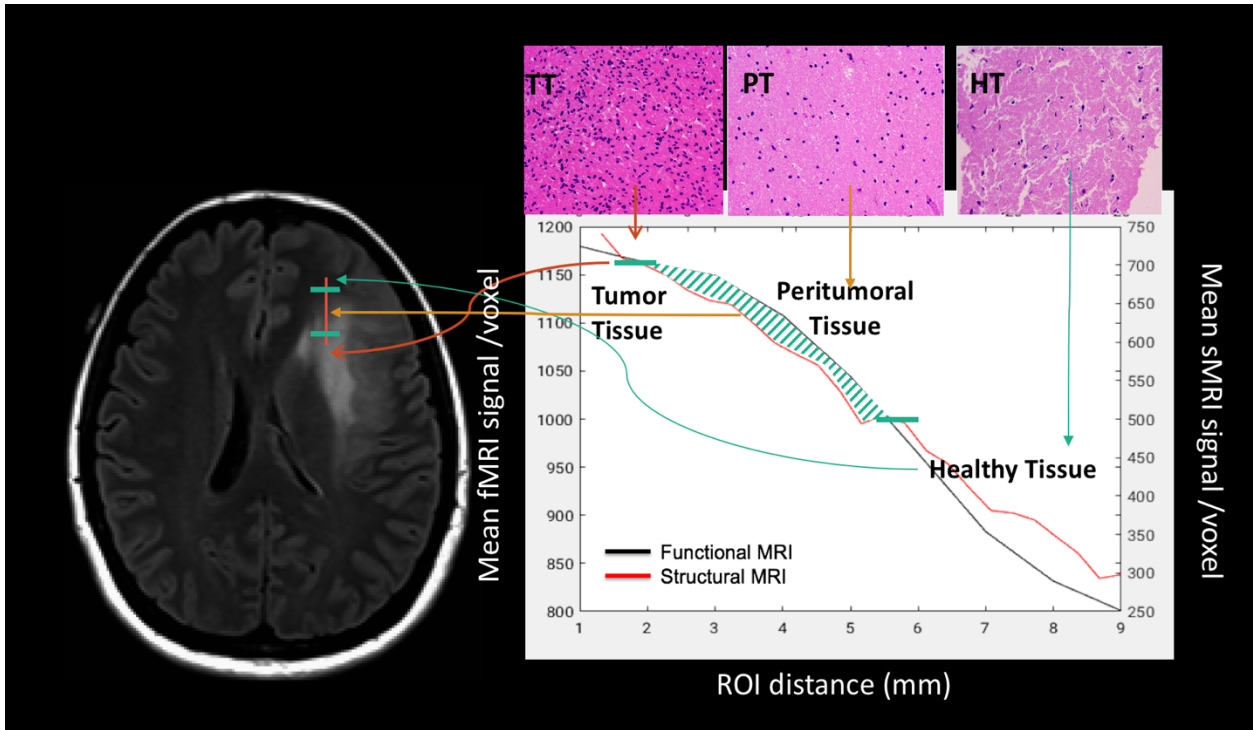
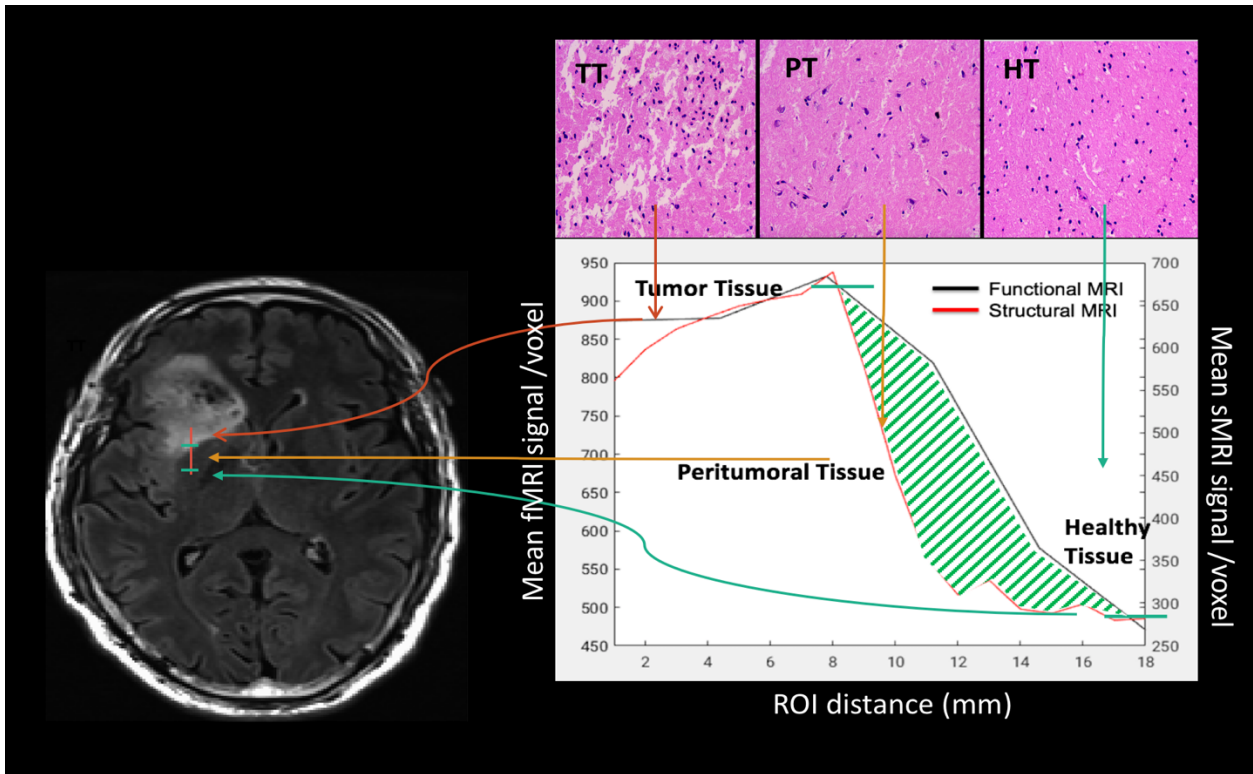


Fig S3. Individualized histology results for the three patients.

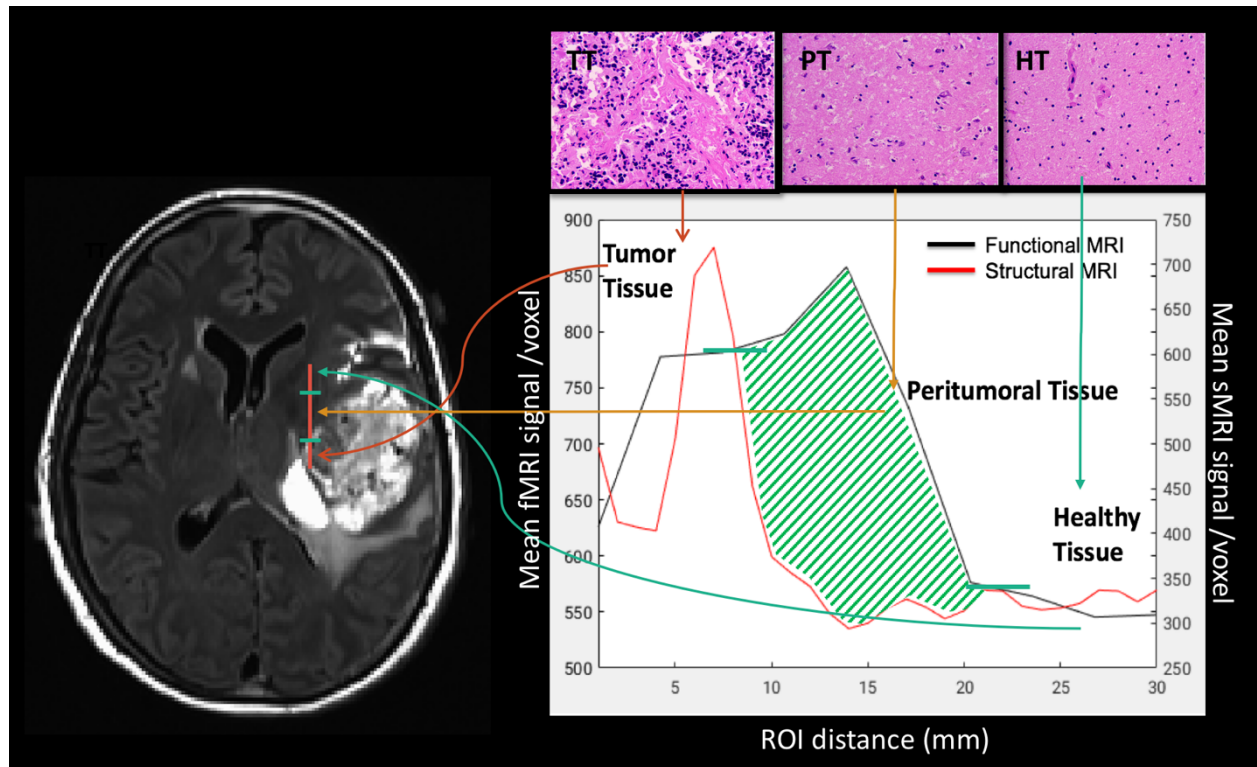
A. Patient 1.



B. Patient 2



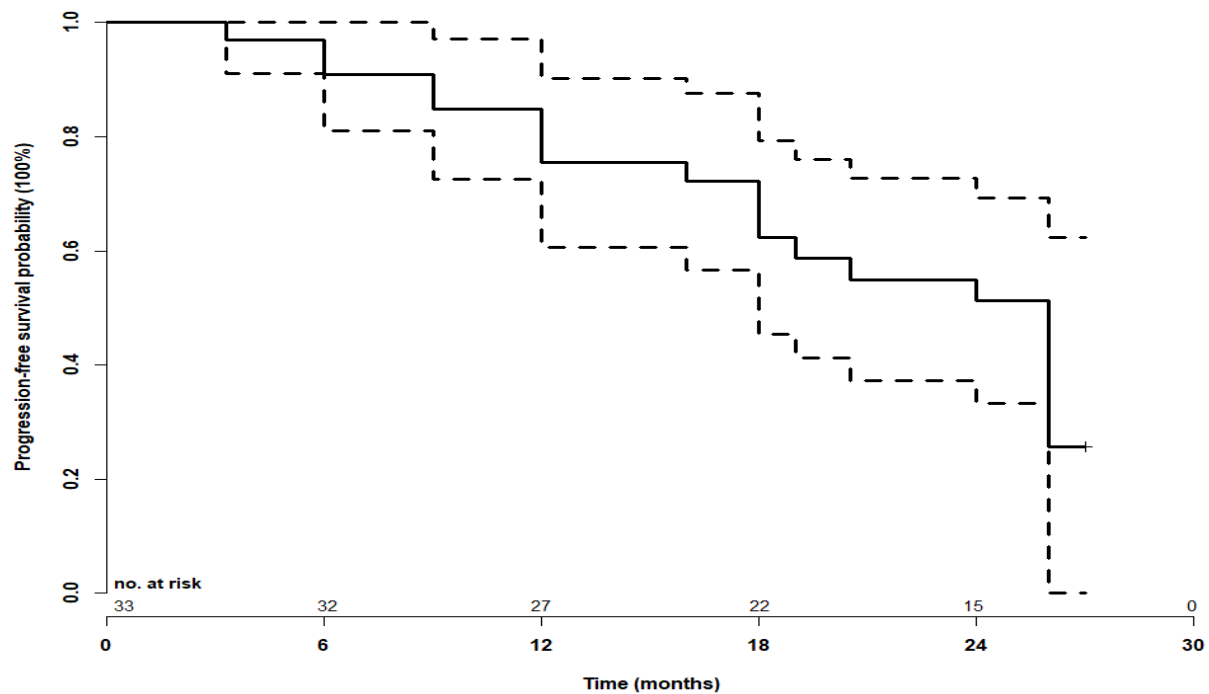
C. Patient 3



The presence of macroscopically not yet visible abnormal cell types could be confirmed in three single cases where we obtained additional histological analyses of the three zones converging with the rs-fMRI and molecular results. This supports our assumption that our rs-fMRI-based identification of PT already contains abnormal cell types as based on alterations in transcriptome and genome.

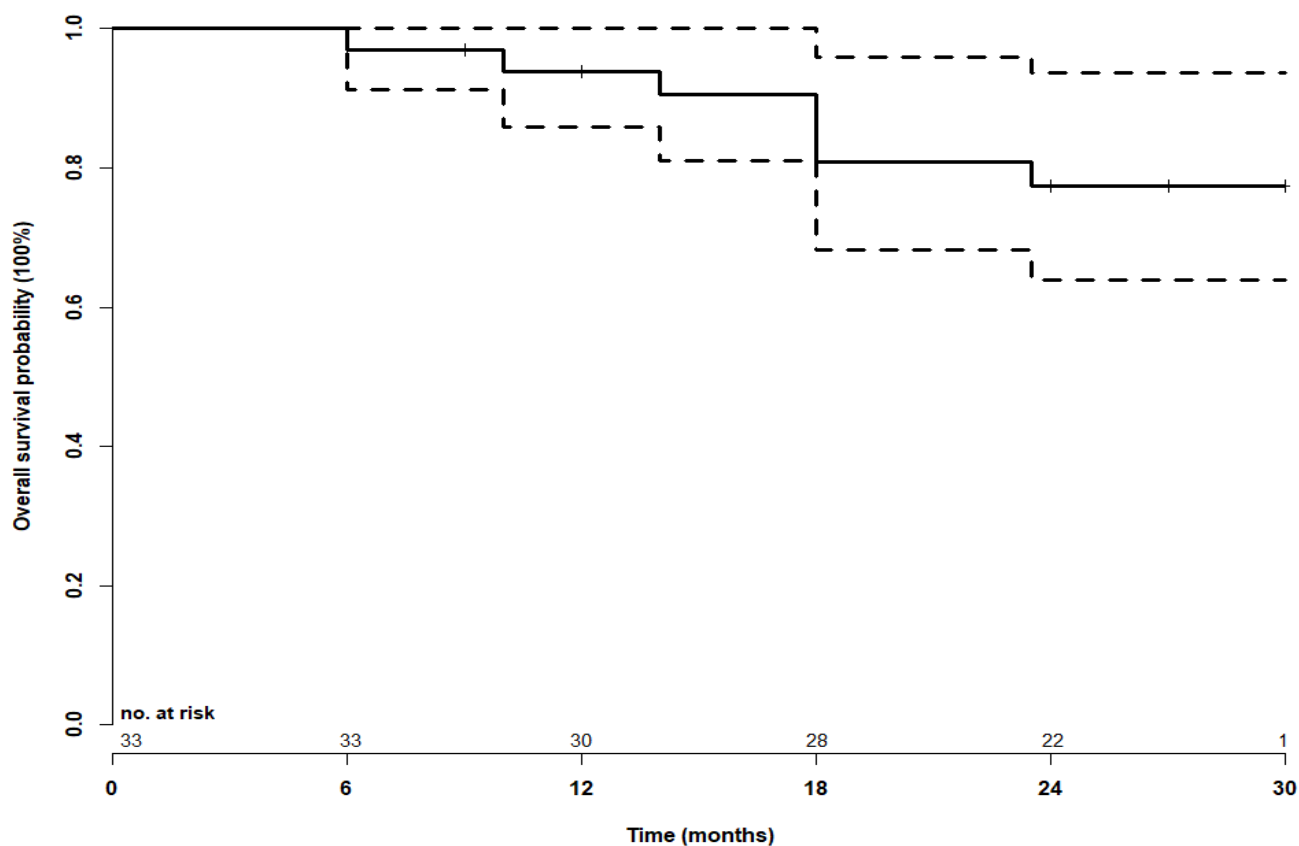
Fig S4. Kaplan-Meier Progression-free Survival (PFS) Curve

The following shows Kaplan-Meier PFS curve for the main group (n=33). The actuarial median PFS (obtained from Kaplan-Meier curve) was 26 months with 95% CI of 18-30 months. There were 16 patients with recurrence among total 33 patients.



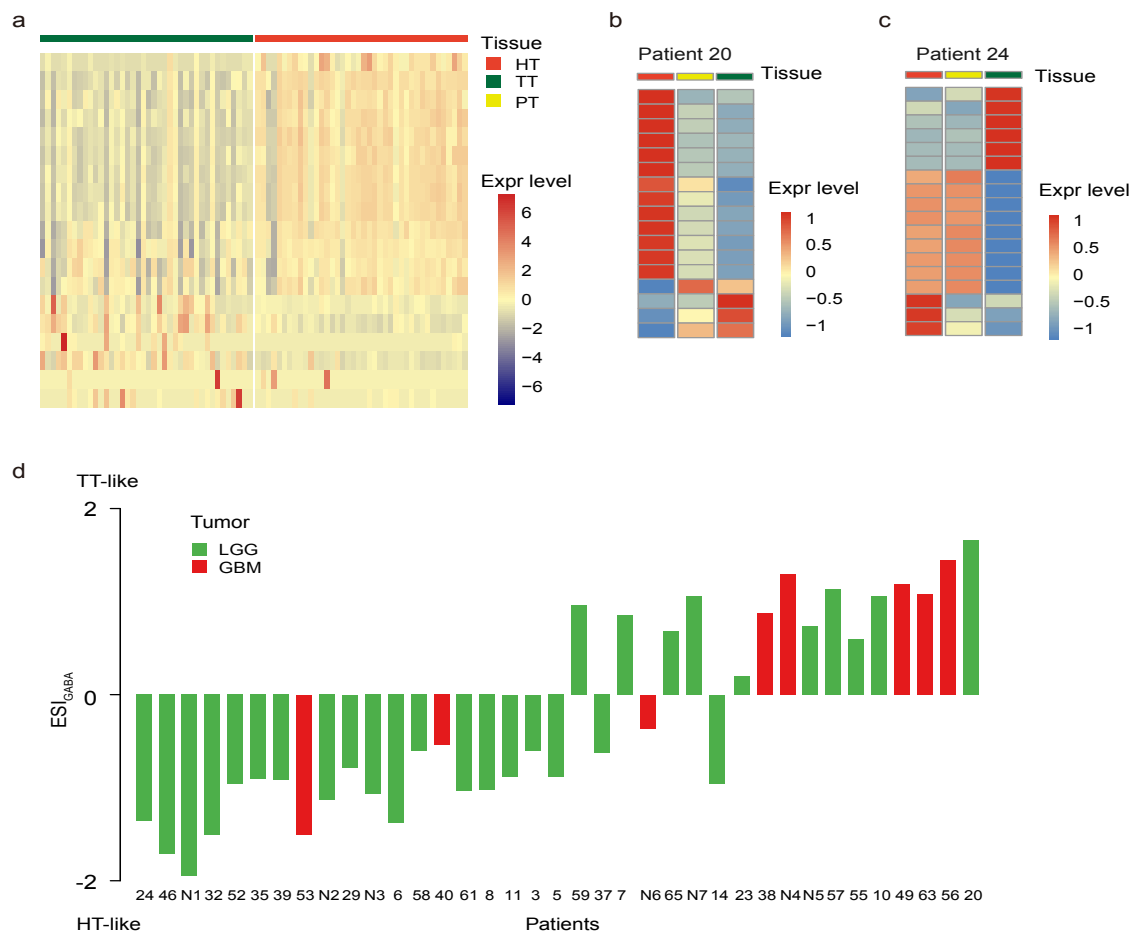
Time in Months	PFS (95% CI)
	Total patients (n=33)
Recurrence/Total	16/33 (51.5%)
(censored rate)	
Actual Median PFS	19.0 (15.8-20.6)
(months)	
Actuarial Median PFS	26.0 (18.0-30.0)
(months)	
6 months	90.9% (81.1-100%)
12 months	75.4% (60.6-90.2%)
18 months	62.3% (45.4-79.2%)
24 months	51.3% (33.4-69.2%)

Fig S5. Kaplan-Meier Overall Survival (OS) Curve



Time in Months	Overall survival (95% CI)
	Total patients (n=33)
Dead/Total (censored rate)	7/33 (78.8%)
Actual Median OS (months)	24 (19.3-23.2)
Actuarial Median OS (months)	NA (all probabilities >50%)
6 months	97.0% (91.3-100%)
12 months	93.8% (85.9-100%)
18 months	80.9% (68.2-95.9%)
24 months	77.4% (63.9-93.7%)
30 months	77.4% (63.9-93.7%)

Fig S6. Genetic results - evidence from genetic alterations in GABA. PT was classified as TT-like/HT-like if the expression pattern of γ -aminobutyric acid (GABA)_A receptors genes (a) in PT is similar to HT (b) / TT (c). In three patients, *DIS_{TP}* and *DIS_{HP}* are too alike to infer the classification. Among the remaining 37 patients, an ESI (ESI_{GABA}) was calculated using expression level of (GABA)_A receptor genes, and significant correlation was observed between ESI_{GABA} and ESI_{DEG100} ($r = 0.87$, P -value = 2×10^{-13} , Pearson's correlation test). The same classifications of PT from 33 patients were in accordance with those inferred from DEGs (d, P -value = 5×10^{-7} , one tailed binomial test). In summary, irrespective if DEGs or genes related to tumor growth were selected as biomarkers, the gene expression pattern could thus give a robust classification of PT.



III. SUPPLEMENTARY TABLES:

Table S1: Demographic and Clinical Factors of Patients in main group (n=33)

N = 33	
<u>Demographics</u>	
Age (years)	
N	33
Mean $\pm$ SD	46.0 $\pm$ 13.0
Median (Inter-quartiles)	43 (36, 54)
Min, Max	27, 72
Gender	
Female	16 (48.48%)
Male	17 (51.52%)
WHO Grade	
II	17 (51.52%)
III	8 (24.24%)
IV	8 (24.24%)
Location	
Left frontal and temporal lobe	2 (6.06%)
Left frontal lobe	5 (15.15%)
Left temporal and insular lobe	6 (18.18%)
Left parietal and occipital lobe	2 (6.06%)
Left parietal lobe	1 (3.03%)
Left temporal lobe	1 (3.03%)
Right frontal and insular lobe	1 (3.03%)
Right frontal lobe	9 (27.27%)
Right insular lobe	1 (3.03%)

N = 33	
Right parietal lobe	2 (6.06%)
Right temporal lobe	3 (9.09%)
Pre-operative seizure	
Absence	12 (36.36%)
Presence	21 (63.64%)
IDH1	
Wide type	18 (54.55%)
Mutation	15 (45.45%)
1p19q	
Retain	17 (51.52%)
Co-del	5 (15.15%)
Unknown	11 (33.33%)
MGMT	
Un-methylation	10 (30.30%)
Methylation	19 (57.58%)
Unknown	4 (12.12%)
<u>Vital status</u>	
Vital status	
Alive	26 (78.79%)
Dead	7 (21.21%)
Recurrence	
No	17 (51.52%)
Yes	16 (48.48%)
Duration of follow-up (months)	
N	33
Mean $\pm$ SD	21.3 $\pm$ 5.5
Median (Inter-quartiles)	24 (18, 24)

<i>N = 33</i>	
Min, Max	6, 30
<u>Clinical factors at post-operation</u>	
KPS at 6 months post-operation	
N	33
Mean $\pm$ SD	92.1 $\pm$ 16.9
Median (Inter-quartiles)	100 (100, 100)
Min, Max	40, 100
Seizure at 6 months post-operation	
Absence	31 (93.94%)
Presence	2 (6.06%)
Neurological function at 6 months post-operation	
Absence	23 (69.70%)
Presence	10 (30.30%)
Post-operative treatment	
No adjuvant	9 (27.27%)
Chemotherapy	4 (12.12%)
Radiotherapy	3 (9.09%)
Radiotherapy and Chemotherapy	17 (51.52%)
<u>Other clinical factors</u>	
Distance PT (mm)	
N	33
Mean $\pm$ SD	9.9 $\pm$ 3.0
Median (Inter-quartiles)	10 (8, 12)
Min, Max	4, 16
Distance PT categories	
>10 mm	14 (42.42%)

<i>N = 33</i>	
≤10 mm	19 (57.58%)
Index AIIDEG	
N	33
Mean ± SD	0.450 ± 1.284
Median (Inter-quartiles)	0.78 (-0.54, 1.30)
Min, Max	-2.17, 2.48
Index IDH1 Mutation	
N	33
Mean ± SD	1.085 ± 1.751
Median (Inter-quartiles)	0.00 (0.00, 1.68)
Min, Max	0.00, 5.67

Table S2: Summary analysis on SD and mean BOLD signal (fMRI) between ipsilateral tumoral tissue (TT) and contralateral macroscopically non-infiltrated healthy tissue (HT) in main group (n=33) as well as subgroup analysis in patients with low-grade glioma (LGG), and in patients with high-grade glioma (HGG), respectively. To compare SD or mean BOLD signals between TT and HT, Wilcoxon rank-sum test was used. P-value <0.05 was considered statistically significant.

	TT	HT	p-value
<u>In Total (N=33)</u>			
SD			0.0007
N	33	33	
Mean $\pm$ SD	0.198 $\pm$ 0.176	0.262 $\pm$ 0.207	
Median (Q1, Q3)	0.14 (0.12, 0.22)	0.22 (0.18, 0.26)	
Range	0.09, 1.10	0.15, 1.36	
Mean BOLD signal			<.0001
N	33	33	
Mean $\pm$ SD	1009.326 $\pm$ 235.999	732.239 $\pm$ 98.544	
Median (Q1, Q3)	1047.86 (913.00, 1191.45)	735.97 (686.89, 799.09)	
Range	463.90, 1310.28	452.98, 970.77	
<u>In LGG Only (n=17)</u>			
SD			<.0001
N	17	17	
Mean $\pm$ SD	0.142 $\pm$ 0.040	0.230 $\pm$ 0.070	
Median (Q1, Q3)	0.13 (0.12, 0.16)	0.20 (0.18, 0.26)	
Range	0.10, 0.27	0.16, 0.39	
Mean BOLD signal			<.0001
N	17	17	
Mean $\pm$ SD	1092.687 $\pm$ 155.301	761.305 $\pm$ 94.026	

	TT	HT	p-value
Median (Q1, Q3)	1116.40 (978.56, 1226.43)	735.97 (706.15, 820.82)	
Range	769.23, 1310.28	609.80, 970.77	
<i>In HGG Only (n=16)</i>			
SD			0.5591
N	16	16	
Mean ± SD	0.258 ± 0.239	0.297 ± 0.290	
Median (Q1, Q3)	0.22 (0.12, 0.27)	0.22 (0.18, 0.26)	
Range	0.09, 1.10	0.15, 1.36	
Mean BOLD signal			0.0400
N	16	16	
Mean ± SD	920.755 ± 277.212	701.356 ± 96.497	
Median (Q1, Q3)	1006.34 (664.58, 1160.02)	717.73 (642.97, 773.08)	
Range	463.90, 1248.45	452.98, 832.54	

Table S3 A. Kaplan-Meier PFS results according to PT distance ≤10 or >10 mm

Time	PFS (95% CI)	
	PT ≤10mm (n=19)	PT >10mm (n=14)
<i>Recurrence/Total</i>	<i>12/19 (36.8%)</i>	<i>4/14 (71.4%)</i>
<i>(censored rate)</i>		
<i>Actual Median PFS</i>	<i>18 (13.0-19.9)</i>	<i>24 (17.1-24.0)</i>
<i>(months)</i>		
<i>Actuarial Median PFS</i>	<i>19 (9-24)</i>	<i>26 (16-27)</i>
<i>(months)</i>		
6 months	84.2% (69.3-100%)	100%
12 months	63.2% (44.8-89.0%)	92.3% (78.9-100%)
18 months	52.6% (34.4-80.6%)	75.5% (54.9-100%)
24 months	32.9% (16.6-65.3%)	75.5% (54.9-100%)

Table S3 B. Kaplan-Meier OS results according to PT distance ≤ 10 or >10 mm

Time	Overall survival (95% CI)	
	PT ≤ 10 mm (n=19)	PT >10 mm (n=14)
Dead/Total (censored rate)	6/19 (68.4%)	1/14 (92.9%)
Actual Median OS (months)	24 (17.9-23.1)	24 (19.1-25.5)
Actuarial Median OS (months)	NA (all probabilities $>50\%$)	NA (all probabilities $>50\%$)
6 months	94.7% (85.2-100%)	100%
12 months	89.5% (76.7-100%)	100%
18 months	73.7% (56.3-96.4%)	91.7% (77.3-100%)
24 months	67.5% (49.1-92.9%)	91.7% (77.3-100%)
30 months	67.5% (49.1-92.9%)	91.7% (77.3-100%)

Table S4. Univariate Cox Proportional hazard model of PFS on PT distance variable

Predictive factors	P-value	HR	95% CI of HR		R ² (%)
Distance PT (mm, continuous)	0.0091	0.805	0.684	0.947	18.77%
Distance PT ≤ 10 vs. 10mm	0.0416	3.742	1.052	13.31	14.46%
6					

Table S5. Univariate Cox Proportional hazard model of OS on PT distance variable

Predictive factors	P-value	HR	95% CI of HR		R ² (%)
Distance PT (mm, continuous)	0.1057	0.816	0.638	1.044	7.70%
Distance PT ≤ 10 vs. 10mm	0.1600	4.563	0.549	37.92	7.92%
6					

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