Diplomarbeit

A case report on the effect of priming a continuous theta burst stimulation (cTBS) used for functional language mapping with navigated transcranial magnetic stimulation (nTMS) in glioma patients

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Sascha Freigang

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Universitätsklinik für Neurochirurgie, Medizinische Universität-LKH Univ. Klinikum Graz

unter der Anleitung von

Univ.-Prof. Dr. med. univ. Michael Mokry

Univ.-Ass. Dr. med. Gord von Campe

Dr. med. univ. Kariem Mahdy Ali

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Zusammenfassung:

Gegenstand: Im Bereich der funktionellen Neurochirurgie werden Therapie-relevante Entscheidungen in Abhängigkeit diagnostischer Verfahren getroffen. Goldstandard für die Kartierung (Mapping) kortikaler Areale ist die direkte elektrische Stimulation (DCS). Im Fokus der Diplomarbeit zugrundeliegenden Studie liegt die transkranielle Magnetstimulation (TMS). Als Methode zum Mapping Sprach-eloquenter Areale bei Hirntumor-Patienten und Patientinnen wird diese im Vorfeld einer geplanten Wachoperation eingesetzt. Jede Hirnstimulation wird von der Erregbarkeit neuronaler Strukturen beeinflusst. Aus diesem Grund wird im Rahmen der Studie ein spezifisches TMS-Paradigma (cTBS) evaluiert und mit einer Vorabstimulation (priming) kombiniert.

Methode: 12 rechtshändige Patienten und Patientinnen mit Hirntumor in der linken Hemisphäre werden mittels navigierter transkranieller Magnetstimulation (nTMS) untersucht. Die TMS-Ziele werden anhand anatomischer und funktioneller Faktoren vor der Untersuchung definiert. Während TMS und Wach-Operation absolvieren die Patienten und Patientinnen einen Objekt-Benennungs-Test. Die TMS-Untersuchung wird in 2 Sitzungen (mit/ohne „priming“) durchgeführt. Abschließend werden die Ergebnisse beider Untersuchungsmodalitäten verglichen.

Ergebnisse: Der Fallbericht stellt im Wesentlichen das Studienprotokoll vor. Im beschriebenen Fall konnte während der TMS ein Sprachstopp bei Stimulation im Gyrus frontalis inferior und im Gyrus precentralis beobachtet werden. Geringe Defizite traten auch bei Stimulation anderer Regionen auf. In der Sitzung ohne „priming“ wurden 37,5% der Ziele als Sprach-eloquent bewertet, dieser Wert sank mit „priming auf 12,5%. Die Übereinstimmung von TMS und DCS hinsichtlich Detektion Sprach-eloquenter Areale ist vielversprechend.

Schlussfolgerung: Der Fallbericht besitzt keine statistische Signifikanz und die Ergebnisse müssen durch die Untersuchung weiter Patienten und Patientinnen verifiziert werden. Der Fallbericht zeigt jedoch, dass cTBS in der Lage ist, signifikante Sprachdefizite auszulösen. Auf der Suche nach einem geeigneten TMS-Protokoll zum Mapping Sprach-assoziierter Areale sind weitere Studien nötig um, neben cTBS als Paradigma sowie der Untersuchung der Effekte des „priming“, weitere Parameter wie Spulenposition und Intensität zu beurteilen.

Abstract:

Objective: Neurosurgical decision making relies on various diagnostic methods prior to surgery. Direct cortical stimulation (DCS) is considered the gold standard in brain mapping to gain information on functional cortical areas that might be affected by surgery. This thesis focuses on navigated transcranial magnetic stimulation (nTMS) as a method for preoperative language mapping in brain-tumour patients who will undergo awake surgery. Brain stimulation techniques critically depend on the prior state of neuronal excitability. The main goal of the study is to evaluate an inhibitory TMS paradigm called continuous theta burst stimulation (cTBS) for language mapping in combination with priming.

Method: nTMS language mapping will be performed on 12 right-handed patients scheduled for awake surgery to remove a left-hemispheric lesion (tumour). TMS targets are determined by fMRI hotspots and anatomical references. During TMS and awake surgery the patients perform an object naming task. cTBS will be applied in two sessions, with and without priming, separated by at least 1 week. Both mappings provided by TMS and DCS will be compared subsequently.

Result: The case report stands exemplary for the protocol used. TMS could induce speech arrest in the inferior frontal gyrus and precentral gyrus, with induced minor errors also in other sites. In the TMS session without priming, 37,5% of the stimulated targets had positive findings. With priming, the total error rate (positive response) decreased to 12,5%. The concordance of TMS and the DCS mapping was high.

Conclusion: Definite results of the underlying study still need to be confirmed using a larger subject cohort. Observations of the case report are: cTBS is capable of eliciting language errors; DCS and TMS concordance looks promising; priming decreases the effectiveness of eliciting language errors; TMS appears be a useful tool in the preoperative assessment in brain-tumour patients. Further studies are needed with regards to intensity, coil position and other TMS paradigms to create an optimal standardized protocol.

Contents:

Glossary and abbreviations

AAT - Aachner Aphasie Test

AF - arcuate fasciculus

AIS - axon initial segment

AMT - active motor threshold

APB - adductor pollicis brevis

AP- action potential

ATRX - adenosine triphosphate dependent helicase

BBB - blood-brain-barrier

BBTB - blood-brain-tumour-barrier

BCM - Bienenstock-Cooper-Munro

BDAE - Boston Diagnostic Aphasia Examination

BOLD - blood oxygen level dependent

Ca^{2+} - Calcium

CaDP - calcium level dependent plasticity

Cl^- - chloride

CNS - central nervous system

CSF - cerebrospinal fluid

cTBS - continuous theta burst stimulation

DCS – direct cortical stimulation

DES - direct electrical stimulation

DTI - diffusion tensor imaging

ECT- electroconvulsive therapy

EEG - electroencephalogram

EMG - electromyogram

EOR - extent of resection

FLAIR - fluid attenuation inversion recovery

fMRI - functional magnetic resonance imaging

GFAP - glial fibrillary acidic protein

GABA - gamma-aminobutyric acid

HADS-D - Hospital Anxiety and Depression Scale

Hz - Hertz

IDH - isocitrate dehydrogenase

IEM - intraoperative electrostimulation mapping

IFOF - inferior fronto-occipital fasciculus

iTBS - intermittent theta burst stimulation

K^+ - Potassium

KD - ketogenic diet

LGG - low grade glioma

Ki67 - antigen encoded by the MKI67 gene

LKH - Landeskrankenhaus

LTD - long term depression

LTP - long term potentiation

MEP - motor evoked potential

MEG - magnetencephalography

MRI - magnetic resonance imaging

MSO - maximum stimulator output

MTDDA - Minnesota Test for Differential Diagnosis of Aphasia

MUG - Medical University of Graz

Na^+ - Sodium

NCCEA - Neurosensory Center Comprehensive Examination for Aphasia

NMDAR - N-methyl-D-aspartate receptor

nTMS - navigated transcranial magnetic stimulation

ONT - object naming task

OS - Ojemann stimulation

PET - positron emission tomography

PFS - progression-free survival

PICA - Porch Index of Communicative Ability

PPT - picture presentation time

PS - Penfield stimulation

PTI - picture to trigger Interval

PT - phosphene threshold

RMT - resting motor threshold

R132h - specific mutation of IDH 1

RP - resting potential

rTMS - repetitive transcranial magnetic stimulation

RV - residual volume

SE- spin echo sequence
SMA - supplementary motor area
SRS - stereotactic radiosurgery
STG - superior temporal gyrus
STS - superior temporal sulcus
TE - echo time
TERT - telomerase reverse transcriptase
TES - transcranial electrical stimulation
TMS - transcranial magnetic stimulation
TMT - trail making test
TP53 - tumour protein p53
TR - repetition time
VAS - visual analog scale
VPC - virus producing cells
VOSP - Visual Object and Space Perception
WAB - Western Aphasia Battery
WHO - World Health Organization
5-ALA - 5-aminolevulinic acid

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1. Introduction

“The limit of my language means the limits of my world” (1)

This quote from the Austrian philosopher Ludwig Wittgenstein shows the importance of the ability to produce and understand language and their fundamental role as element for quality of life (2). These abilities can be impaired by neoplastic diseases of the Central Nervous System (CNS) when language areas are affected; especially entities such as Gliomas can affect language function due to their infiltrative growth (3).

The main objective for an optimal treatment of patients suffering from intracranial tumours, must be the preservation of quality of life. As the survival time might be limited by the disease, the direct and intermediate term postoperative condition is important. Therefore, the avoidance of significant postoperative deficits is a major goal in any oncological neurosurgical procedure (4). Language represents a special challenge when it comes to surgical treatment like tumour resection because testing language production and comprehension requires an awake patient and thus creates a need for awake craniotomies (5).

In fact, the maintenance of the language function is a major priority of neurosurgical decision making. Mapping language eloquent cortical and subcortical areas before brain tumour resection is a crucial part of every surgical procedure within these regions (5). The mapping of those areas is complex, including different kinds of neurophysiological and neuropsychological applications; it requires a profound knowledge of the anatomical organization of language associated systems within the brain and their underlying physiological processes (4).

Due to the complex nature of language networks and individual anatomic variability, a multimodal approach is needed prior to brain tumour surgery (6,7). A precise assessment of the interindividual functional anatomy and clear expectations of their distribution within the surgical area will decrease the rate of complications and postoperative deficits; it allows a more valid evaluation of chances for success, which is a critical part of therapeutic decision making (8).

Preoperative imaging is the basis of any neurosurgical planning (4,5). Beside the representation of anatomical structures, functional regions of the brain are of particular interest for glioma surgery. Functional MRI (fMRI) is the most commonly used mapping method in both clinical use and neuroscience (5). However, fMRI is an indirect method for mapping of functional areas and does not show the relevance of areas to specific functions (9,10). Therefore, accuracy and reliability of functional maps generated on fMRI basis alone has been widely questioned in the diagnostics of brain tumour patients (7,11,12). In fact, the correlation of fMRI and intraoperative language mapping is thought to be approximately 33% (9). In order to improve preoperative language mapping, navigated Transcranial Magnetic Stimulation (nTMS) is a promising approach which has already shown useful results (8). In nTMS the combination of imaging and non-invasive brain stimulation is a key component to a more detailed and accurate presurgical assessment.

The principle of TMS is the modulation of neuronal activity by applying a magnetic induced electric current to the cortical surface (8,13). Depending on the paradigm, a transient lesion caused by TMS will mimic loss of function and allows an evaluation of areas and their role for specific functions (12,14,15).

The objective of this thesis is a comprehensive evaluation of TMS as a technique and its neurophysiological effects, the basic physiological mechanisms within the cerebral cortex, the recent theories of anatomical and functional language models and clinical language assessments, as well as the integration of nTMS diagnostics into the treatment of glioma patients. This thesis, as part of a pilot study, will also present results in form of an exemplary case report.

Even though nTMS is already in use at specialized centres, a consensus for an optimal language mapping protocol is still lacking. Therefore, a focus of the underlying study is the evaluation of a specific TMS paradigm called continuous theta burst stimulation (cTBS), which is used in two different settings. Both will be compared in regard to practicability and reliability. Beside practical evaluation, conclusions will be made to evaluate the connection between neuronal plasticity and the priming of TMS. Priming is an additional stimulation applied before mapping of functional areas *per se* is performed (16). While a formal comparison of primed TMS and intraoperative mapping procedures is not yet available, the study also enables an insight into stimulation effects and neuronal function on a theoretical basis.

2. Theoretical assessment

2.1 Neurophysiological fundamentals of the cerebral cortex

The CNS is a system of tremendous complexity. It consists of several types of cells that differentiate according to their development, functional units and anatomical topography (17). This chapter aims to give a review of cortical anatomy and function.

The shape and surface of the brain are made up by the telencephalon (17). It is the biggest part of the human brain; a large portion of the other parts is hidden by telencephalic structures (17). The telencephalon is divided into two hemispheres (17). Each hemisphere is divided into 5 parts: frontal lobe, temporal lobe, parietal lobe, occipital lobe and insula (17). The lobes include cerebral cortex/grey matter and the white matter (17,18). The grey matter is the superficial part on the surface of the brain, composed mainly by neuronal cell bodies, and is structured into gyri and sulci as seen in figure 1 (19). Because of the gyral surface, 2/3 of the cerebral cortex is hidden in the sulci and just 1/3 is exposed on the surface (17). The white matter consists of myelinated axons which build a complex system of pathways within the CNS (17,18). Both, grey and white matter, are further classified based on characteristics which will be outlined in the following.

Apart from neurons, neuroglia account for a major percentage of cellular volume in the CNS (17). In general, glial cells have different functions like forming the cerebral structure (astroglia), chemical homeostasis, nutrition, axonal isolation (oligodendroglia), immuno-competency (microglia) and contributing to the blood-brain-barrier (BBB) (4,17,20–22). The ratio of neurons to neuroglia varies in each part of the human brain; certain types of glia contribute differently to this ratio (23,24). Within the cerebral cortex, the amount of glial cells is roughly four times higher compared to the number of neuronal cells (24). Four main types of glia cells exist: Astrocytes, oligodendrocytes, microglia and ependymal cells (17,18,20). In most regions, astrocytes are the majority of glial cells (17,20).

Furthermore, the cerebral cortex is classified by phylogenetic findings. It can be divided into allocortex, which is the evolutionary oldest part, consisting of paleo- and archicortex; as well as the neocortex (17). The neocortex is the major part of the grey matter (17,19). It consists of a typical histological pattern of six layers (17,19,21), is 2-5 mm wide and covered by pia mater (21). Figure 2 shows the organization of the six layers. The thickness of each layer varies within the different functional areas; this is due to the fact, that different types of neuronal cells are represented (17,19,21).

Different layers of cells are grouped in columns of a diameter of 0.5 mm (17). Pyramidal cells and non-pyramidal cells like cortical interneurons are the main types of neuronal cells (17). The classification was based on the shape of the Golgi apparatus and the length of the axons and their function (21). Pyramidal cells are efferent neurons which are found in the layers III and IV and innervate cortical regions or subcortical structures (19). Pyramidal cells represent 85% of the cortical neurons (18); through recurrent collaterals, they often influence cortical interneurons as well (17,21). Cortical interneurons remain in circumscribed cortical areas and can be found in all cortical layers (17). While pyramidal cells are excitatory neurons, using the neurotransmitter glutamate, cortical interneurons serve most likely inhibitory effects via the neurotransmitter gamma-aminobutyric acid (GABA) (17). Cortical interneurons inhibit pyramidal cells in their vicinity and are responsible for the demarcation of the columns (17). The inhibitory effect of cortical interneurons has an influence on the mechanism of TMS affecting the cortex which will be outlined later (25).

According to the previously described differences of cell types and layer thickness, Korbinian Brodmann created a cytoarchitectural map of the human cerebral cortex in 1909, which is still in use as a reference for functional areas (19). An overview of the so called Brodmann areas can be seen in figure 3. Each area correlates with a functional area of the cortex (19). The functional areas are grouped into primary, secondary and association areas. For example, the primary motor cortex corresponds to Brodmann area 4 and covers the precentral gyrus (19,22). An example for a secondary area is Brodmann area 18, which is located in the medial and occipital gyri as the secondary visual cortex.

The association areas are located in the frontal lobe, supramarginal and angular gyrus, serving higher cognitive functions such as language, writing, mathematical and visual reasoning as well as multisensory perception (20,22).

Consisting of myelinated axon tracts, the white matter is located below the cerebral cortex (17). Three different types of fiber tracts may be distinguished: commissural tracts, projection tracts, association tracts (17,18). Commissural tracts connect both hemispheres (18). The major commissural fiber tract is the corpus callosum which connects neocortical areas of the left and right hemisphere (17,18). Further commissural tracts are: the anterior commissure, the hippocampal commissure, the habenular commissure and the posterior commissure (17). Projection tracts connect cortical areas to subcortical structures and other areas of the CNS like basal ganglia or brain stem (18). Most of the projection tracts, e.g. the corticospinal tract and subthalamic fasciculus, are located in the internal capsule (18,20).

Association tracts connect areas of the ipsilateral hemisphere, e.g. the visual cortex to the angular gyrus (18). These tracts allow a directed stream of information processed in primary and secondary areas, ending in association areas and thereby enabling higher cognitive functions (17). Three different systems of association tracts may be distinguished: short association fibers, long association fibers and the cingulum (17). The most important tracts are: inferior longitudinal fasciculus, connecting the occipital and temporal lobes; superior longitudinal fasciculus, connecting the frontal and occipital lobes and comprising the arcuate fasciculus which is crucial for language processing and production; the uncinate fasciculus, connecting the frontal and temporal lobes; the inferior fronto-occipital fasciculus, connecting the frontal and occipital lobes (17,18). Figure 4 is a schematic representation of these tracts.

Beside the structural organization of cortical and subcortical areas, the biological mechanisms of excitability are crucial for understanding methods such as TMS and DCS. Neuronal functions, as well as processing and transmitting of information, are based on electrical and chemical signals (22). The ability of neurons to create and transmit signals is linked to ionic intra- and extracellular potentials (22,26). Potentials are built up by the difference in the ion distributions between intracellular- and extracellular space (22). The difference in distribution is based on the phospholipid membrane properties, hence the potentials are called membrane potentials; resting potential (RP) and action potential (AP) are the fundamental factors of neuronal function and cortical excitability is basically determined by the RP (22,26).

The RP is the membrane potential of non-excited cells (27) and amounts roughly -65 mV in neurons (22). It is built up by an ion concentration gradient of potassium (K^+), sodium (Na^+), chloride (Cl^-) and calcium (Ca^{2+}); the gradients are established by the sodium-potassium- and calcium-pumps (22,26). The exchange of K^+ ions causes the negative RP (22). The intracellular concentration of K^+ is 20 times higher than in the extracellular space (22). Due to K^+ channels in the phospholipid membrane, K^+ can diffuse from intracellular to extracellular space, following the ion concentration gradient built up by the ion pumps (26). This exchange creates a negative intracellular charge and an electrical potential across the membrane (26). The negative intracellular potential is located on the inner side of the phospholipid membrane (26). This potential causes K^+ ions to go back from extracellular- to intracellular space and results in a potential equilibrium (22).

The complex combination of the potential equilibrium of each previously mentioned ion creates the RP of neurons (22). The phospholipid membrane is relatively permeable, which means that the RP of around -65 mV is a combination of the potential equilibrium of basically K^+ and Na^+ (26). However, the RP is close to the potential equilibrium of -80 mV for K^+ because the cellular membrane is 40 times more permeable for K^+ ions than for Na^+ (22). In summary, the ion pumps create a negative intracellular charge which causes the RP by moving ions between the neuronal intra- and extracellular space (22,26).

The reversal of the negative RP is the AP (22). The AP is defined as a transient change of the membrane potential of an excitable cell (27,28). An AP generated by an excitable cell, is stable in physical size and duration and signals are coded through frequency and pattern (22). For example, the number of AP's increases in higher depolarizing stimuli and is described as a relationship between strength of stimulus and AP-frequency (26). Furthermore, the AP is subjects to the "all-or-none" principle, which means that an AP is only generated after a certain threshold of depolarization is reached (22,26,27). For cortical neurons, this threshold is variable (29). Depolarization is defined as an increase of the negative RP (22,27). Different mechanisms exist of how an AP is generated, depending on the localization and type of cell, respectively the neuron (22). While the mechanisms are variable, phases of the AP are stable; six phases can be described, starting with depolarization, rising phase, peak phase/overshoot, falling phase/repolarization, undershoot phase and refractory period (26). The AP is characterized by a shifting of electric charges upon the membrane, which can be described as a stereotypical sequence of ionic shifting (26). First described in 1950 by Hodgkin and Huxley, the model of the mechanisms behind the AP is still relevant nowadays (26,30). According to the model, the AP can be described as a time dependent program of change in conductivity for the previously mentioned ions (26). The depolarization is caused by an influx of Na^+ ions into the cell, while the repolarization is based on the efflux of K^+ (22). In detail, after reaching the threshold, voltage activated Na^+ channel open and cause a rapid depolarization, caused by Na^+ influx and driven by the negative intracellular membrane potential (26). This phase is called the rising phase, which is followed by the so-called overshoot; the overshoot is characterized by an increased permeability for Na^+ ions, which is approximately 40 times higher than in the RP (26). During this phase, the membrane potential is closer to the potential equilibrium of Na^+ than K^+ (26). The result is a reversion of the potentials up to +20 to +40 mV (26). In the falling phase, Na^+ channels close, while K^+ channels open (26). The open K^+ channels cause an efflux of K^+ and a decrease of the membrane potential to negative values (26). The undershoot phase is a consequence of a convergence to the potential equilibrium of K^+, due to a decrease in the permeability for Na^+ ion within this phase; the undershoot phase is terminated by the closing of K^+ channels (26).

Until the closing of these channels, the membrane potential is hyperpolarized, which means it is more negative than in RP; this phase is called the relative refractory phase, because a higher impulse of depolarization is required for generating an AP (22,26). While the potential decreases in the falling phase, a reopening of Na^+ channels is not possible, this phase is called the absolute refractory phase because an AP cannot be generated at this time (22,26). Figure 5 shows the previously described processes as a course of the potentials among the axonal membrane.

In the model of Hodgkin and Huxley, AP's are generated in the axon initial segment (AIS) (22,30). However, the model does not fit for all types of neurons. Different types of neurons are characterized by various electrical features (22). Some key features of cortical neurons are outside the behaviour described by the Hodgkin-Huxley model (31). Within the cerebral cortex, different types of neurons exist, as mentioned in chapter 2.1. Therefore, a general statement regarding characteristics cannot be made. For example, in pyramidal cells, the frequency of AP's decreases over the duration of a constant stimulus, while other types of non-pyramidal cells maintain the frequency (22). The type of stimulus which causes the depolarization and elicits an AP is variable as well. For example, in peripheral nerves it can be the event of stretching the end of the nerve which causes Na^+ channels to open (22) and can be described as a transduction of an external stimulus in sensory neurons (28). In cortical cells, it mainly depends on temporal or spatial summation of synaptic inputs onto the dendritic tree of the neuron (30). Spontaneous depolarization is another way of eliciting an AP (26). The input is influenced by various factors like: types of synapses, neurotransmitters and number of dendritic spines (22). The current which elicits the AP is normally generated by the action of neurotransmitters released by other neurons but can also be applied artificially by DCS or TMS (28).

In case of DCS, the after-discharges are an important phenomenon of changes in the membrane potential (5). After-discharges are defined as “the proportion of the response to a stimulation in a nerve which persists after the stimulus has ceased and consists of rhythmic, high-voltage, high frequency spikes, sharp waves, or spike-wave complexes which occur at the region stimulated and are distinctly different from background activity” (5,32).
After-discharges are measured by electrocorticography in awake surgeries to determine the maximum range of stimulation intensity (5). The importance and assessment will be explained in chapter 2.6.

In summary, the neurophysiological processes of the cerebral cortex consist of a complex interplay between structural and physiological factors. This chapter however only discusses the insight into different parts of the cortical network. Methods like DCS and TMS can influence physiological processes depending on site, intensity and frequency (5). Therefore, the examiner needs to consider interindividual anatomical and electrophysiological features to assess the functions of the outlined networks. Especially in patients suffering from neoplastic diseases, neurophysiological processes may deviate from the usual assumptions of normal CNS function (22).

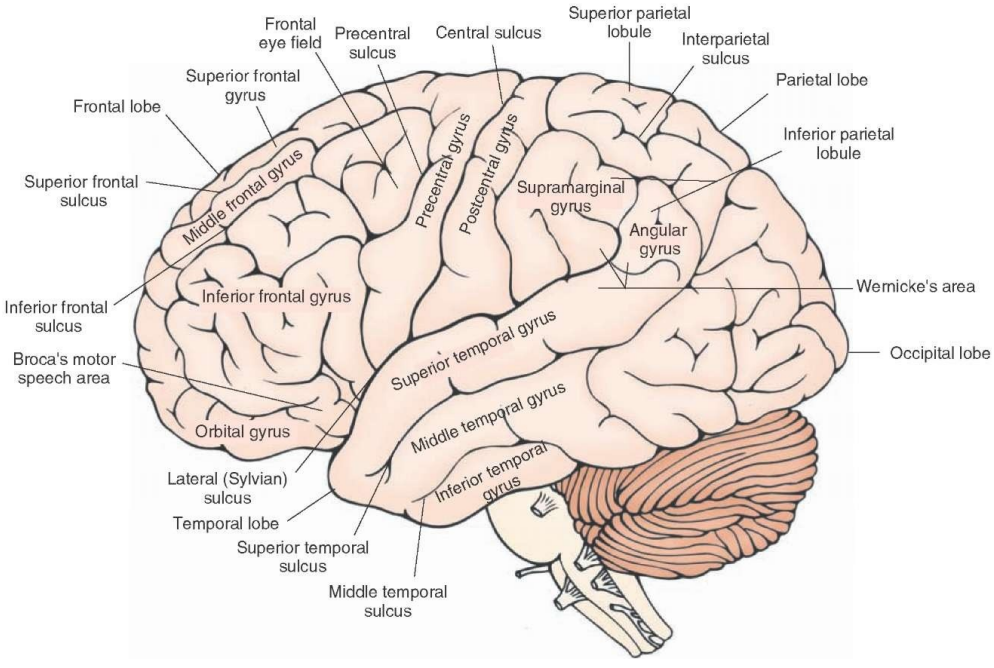

Figure 1 Cortical brain surface (33)

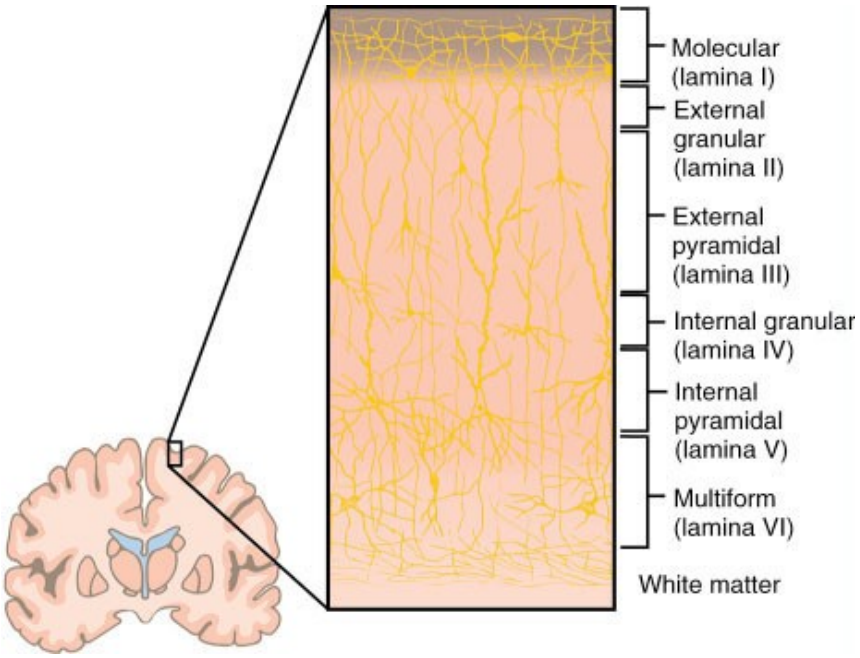

Figure 2 Cellular layers of the cerebral cortex (34)

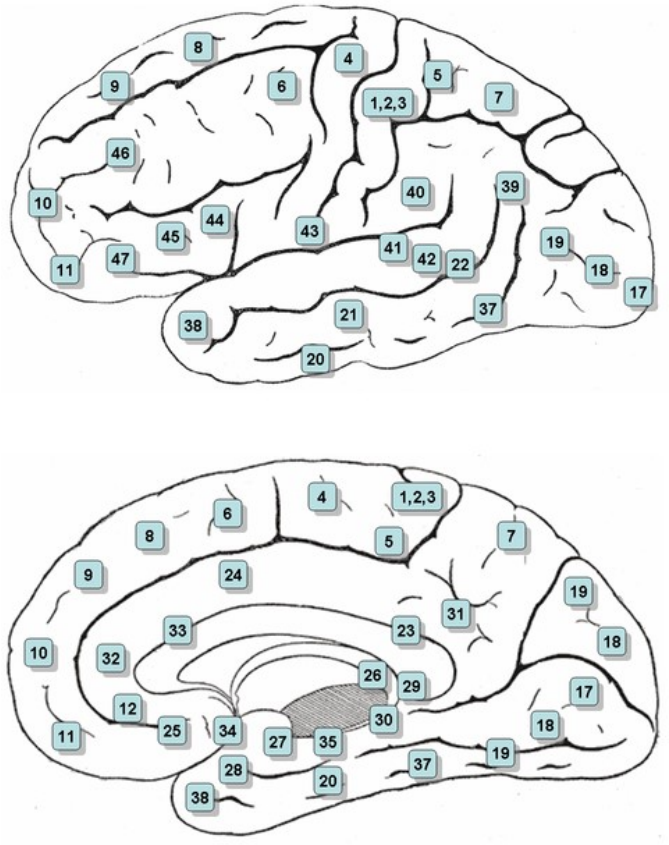

Figure 3 Brodmann areas (35,36)

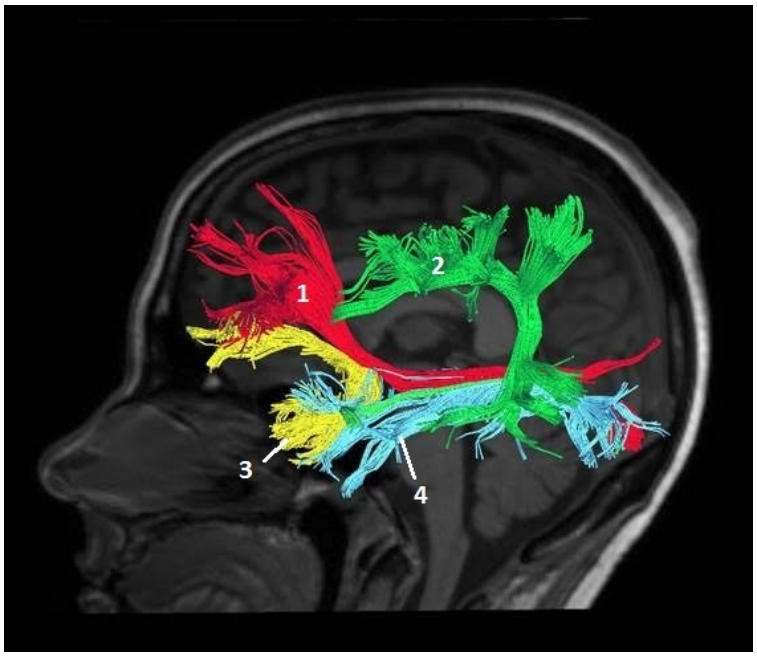

Figure 4 Important white matter tracts (by courtesy of Margit Jehna, MUG) 1: IFOF; 2: Arcuate fasciculus; 3: Uncinate fasciculus; 4: Inferior longitudinal fasciculus

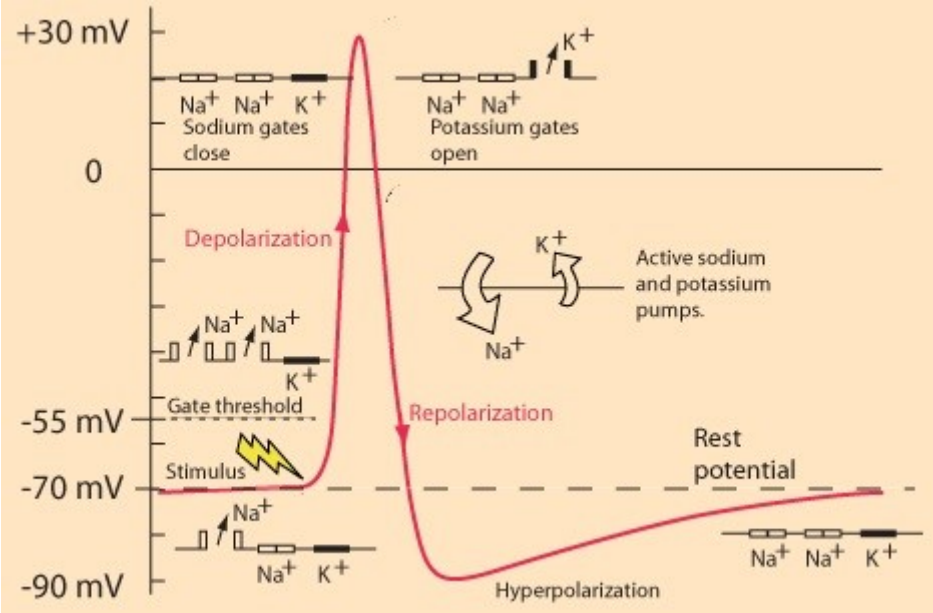

Figure 5 Action potential of a neuron (37)

2.2 Structural organization of language production and processing

Language in general is an umbrella term for all different kinds of communication; it includes verbal and non-verbal languages and is made up of a complex system of sounds, symbols and gestures (27). Defining the different portions of the processes involved in that system will help us understand the complexity of how the brain creates this unique cognitive function. Language involves many different organ systems which serve perception, comprehension, speech planning and production (5,22). Beside the central nervous system, six cranial nerves (trigeminal, facial, glossopharyngeal, hypoglossal, vagal and accessory), the respiratory system, the laryngeal system, the articulatory system as well as sensory receptors in joints, muscles and tendons take part in the previously mentioned aspects of language (5). The close coordination of all systems is required to ensure proper language function. Accordingly, this thesis will focus on the anatomical and functional correlates of language.

Nowadays, a wide gap exists between the basic “text book” knowledge and that of recent research regarding language function. Here, both the classical and modern models of language organization will be discussed. For a better understanding of the complexity in language associated areas of the CNS, the classical model will be described first.

The classical model of language organization is based on the findings of Paul Broca and Carl Wernicke (17,38). Based on lesion studies, first published in 1861, Broca described the loss of the ability to speak in patients with lesions in the inferior frontal gyrus, more precisely in the pars opercularis and triangularis (38).

Subsequently, in 1874, the German neurologist Carl Wernicke described paraphasic errors like impaired naming and comprehension caused by a lesion within the posterior superior temporal gyrus while speech fluency and the ability of producing speech were still intact (38,39). Due to that finding, Wernicke postulated the theory of two language associated areas (38,39).

His theory was based on the assumption that in relation to the Rolandic fissure, anterior regions process motor functions and posterior regions process sensory functions (39). Therefore, an anterior as well as a posterior language area, both connected by white matter tracts, were described (38). In 1965 Norman Geschwind assigned this connection to the arcuate fasciculus, a white matter tract which connects the superior temporal lobe to the inferior frontal lobe (38). This is why the classical language model is called the Wernicke-Geschwind-Model (38).

The key elements of the Wernicke-Geschwind language model are: Broca's area, Wernicke's area and the arcuate fasciculus. Often, the angular gyrus as well as sensory and motor areas are also considered part of the language model (22). Broca's area is located in the inferior frontal gyrus, corresponding to Brodmann's area 44 (40); it is described as the motor language area which activates the motor program for speech (19). Wernicke's area, located in the superior temporal gyrus, corresponding to Brodmann area 22, is the sensory region of language organization and is crucial for language comprehension (19). Both primary language areas can also be described as receptive area and expressive area (5). The angular gyrus, as a part of the association cortex, connects the visual cortex to Wernicke's area (19). The arcuate fasciculus, a subcortical white matter tract and part of the superior longitudinal fasciculus, connects the frontal lobe to the temporal lobe with a course superior to the insula (17,19). In over 95% of right handed people and 60- to 70% of left handed people, the primary language areas are located in the left hemisphere (19). However, the non-dominant hemisphere can also be involved in language processing too; for example, the right hemisphere participates in the processing of voice tones with regards to emotions (19).

Beside the key elements mentioned above, many other regions are involved in the complex language organization. A widespread network of connections from Broca's area to other frontal lobe regions exists such as the connection to the supplementary motor area, the prefrontal cortex and the premotor cortex, in order to create higher motor function, including the aspects of speech formulation and planning (17,19,22).

Similar to these frontal lobe networks, there are connections existing between Wernicke's area and the supramarginal gyrus, the angular gyrus as well as particular regions of the temporal lobe like Brodmann's area 37 (17,19). These areas and networks are important especially in language comprehension (17).

The classical model of language organization is defined by specific cortical areas and subcortical pathways. These structures build up complex networks that enable the different parts of language processing to function. The principle of cortical and subcortical structures forming a complex network can be adapted to the modern language theories (38).

In the Wernicke-Geschwind-model, connective networks play a very important role. However, the fundamental idea of distinct regions for language production and comprehension remains a central part of the classical theory. Modern theories tend to propose the distribution of language function into large scale cortical and subcortical networks and an increased relevance of hodology; it pertains or describes specific connections between neurons (41).

One of the modern theories is the dual stream model of language organization (39,42). It is based on the existence of a ventral and dorsal stream, which both can be seen as a connective network between specific language-associated regions (figure 6) (38,42). Therefore, language function is not attached to a single area, but associated with a defined stream and network (39). The course of the ventral stream includes the posterior superior temporal gyrus (STG) and the superior temporal sulcus (STS), which can be seen as the Wernicke's area, as well as the anterior and middle temporal lobe (38). Its key functions are: auditory processing, speech recognition and lexical concepts (38). The dorsal pathway involves the dorsal STG, the temporo-parietal junction, the sylvian parieto-temporal region and the posterior frontal lobe (38). The key feature of the dorsal stream, which includes two divisions, is the sensorimotor integration (38). While the ventral stream is supposed to be located bilaterally, the dorsal stream is mostly found in the dominant hemisphere (38).

The dual stream model is a widely accepted theory for language organization, although different variations of this model exist. For example, the Hickok-Poeppel model postulates a ventral stream which is located bilaterally, as mentioned above (38). In contrast, the Rauschecker-Scott model argues that both streams are located only in the dominant hemisphere (38).

When taking a closer look at the process of language production, more detailed theories can be found. Riecker et al. (43) described another dual system particularly for language production. It includes a preparatory loop, consisting of the supplementary motor area (SMA), insula, superior cerebellum, dorsolateral frontal cortex, and an executive loop represented in the primary motor cortex, thalamus, basal ganglia and inferior cerebellum (5,43)

Taking into account recent research and classical theories, it can be shown that language comprehension and production are still dissociable. Despite the lack of a more precise model, which of the areas are involved, the basic idea of function and language associated regions remains the same, extended by the highlighted role of hodological aspects.

It is important to mention that specific pathophysiological findings explain particular theories. For example, it is a common assumption that a lesion to the classical Broca's area could cause Broca's aphasia characterized by impairment of expression and fluency while language comprehension is intact (19,20). Modern diagnostic approaches like functional MRI show the differences between the truly involved areas and associated areas; it was shown by Ardila et al. in 1990 (40), that the classical Broca's aphasia is caused by a lesion that expands beyond the original Broca area. A damage to the insula, lower motor cortex and several white matter pathways is also required and can cause the same symptoms (40).

The impact of modern technology like fMRI, DTI, TMS, Magnetencephalography (MEG) and DCS on the idea of how language is organized and processed is tremendous. Beside the range of diagnostic approaches, the variety of language tests also has an important influence on assessing language organization. Specific tests can be used for different parts of the language associated regions. To evaluate parts of the language serving system, awareness must be given to diagnostic and imaging techniques as well as to appropriate language tests (5).

Although comprehensible and promising models exist, an overall clarification of language organization has not yet been made. Due to the complexity and individual variability of language organization within the CNS, specific diagnostic approaches are necessary to assess language in healthy volunteers as well as in patients suffering from CNS disorders.

2.3 Language testing and language disorders

Mapping of language eloquent areas during brain tumour surgery is crucial for procedures in areas likely to hold associated functions (10). The mapping of those areas is complex, including different kinds of neurophysiological and neuropsychological methods. Beside technical requirements, the type of language task is an essential part of the protocol.

The previous chapter already outlined the understanding on how language is processed in the CNS. In the following, principles of neuropsychological assessment for language testing will be discussed. Special remarks refer to the applications used in pre- and intraoperative settings. To better understand the assessment, basic principles of language disorders will be briefly outlined.

The functional architecture of language processing can be explained as a 3 level-model (44). First: the emotional and cognitive level; second: the language system processing, including decoding and encoding; third: the in- and output systems. Different language disorders can affect different levels (44).

The differentiation between aphasia and dysarthria is another basis of language disorder assessment (44). Aphasia is defined as an acquired language disorder caused by lesions within the CNS (27). In reference to the 3-level-model, different types of aphasia are grouped into multimodal or supra-modal disorders (44). In contrast, dysarthria is an impairment of speech control, respectively of the peripheral neuromuscular unit (27). Within the 3-level-model, dysarthria belongs to the output-system level (44).

This chapter will focus on the causes of aphasia and the diagnostic approaches. As different kinds of aphasia exist, various forms of tests are required to assess specific parts of language processing. Therefore, determining the suitable test mainly depends on the indication of language testing (45).

Language testing is necessary in patients suffering from CNS diseases (44). The most common cause for aphasia is apoplexy followed by traumatic brain injuries, neoplastic diseases, encephalitis and atrophic processes (44,45). Testing for aphasia is a complex procedure including differentiation between aphasic syndromes, determination of severity levels and identification of non-classified aphasia (45). The standard aphasic syndromes include: global aphasia, Wernicke's aphasia, Broca's aphasia and amnesic aphasia. Beside the general assessment method, more specific neurolinguistic procedures exist. Neurolinguistic testing is the key feature in real-time assessment of language in combination with other diagnostic approaches like fMRI, TMS or DCS (5). The analysis includes the definition of aphasic impairments within the different aspects of language, e.g.: spontaneous speech, repeating, reading, writing as well as auditory language comprehension (45).

Many standard protocols exist to assess aphasia. They allow to classify the severity and type of language disorders (45). Well-established tests include the Minnesota Test for Differential Diagnosis of Aphasia (MTDDA), the Porch Index of Communicative ability (PICA), the Neurosensory Center Comprehensive Examination for aphasia (NCCEA), the Boston Diagnostic Aphasia Examination (BDAE), the Aachner-Aphasie-Test (AAT) as well as the Western Aphasia Battery (WAB) (22,44). Each test protocol contains several tasks to assess the different portions of language processing. These tests were specifically designed to generally assess aphasia and its sub classifications (44). However, specialized or additional tests are required for neurolinguistic approaches. Especially for real time assessment, complex battery tests are not practicable due to their long duration (46). Therefore, specialized neurolinguistic tests, like the Boston Naming Test or supplements to the AAT, are preferred for language mapping and were shown to be most sensitive for the identification of aphasia linked to neoplastic disorders (45,47).

Since this thesis focuses on brain tumour patients, this chapter aims to give an overview on the common language disorders associated with this disease. It will further outline the diagnostic modalities used to assess these disorders. Due to the various locations of the lesion, every type of aphasia can be observed in a brain tumour patients (44). The main aphasic syndromes are associated with typical involved areas; for instance, amnesic and semantic aphasia are basically caused by a small lesion within the temporo-parietal cortex and subcortical structures like the inferior fronto-occipital fasciculus (IFOF) (45,48).

Common symptoms are semantic paraphasia and a word finding disorder, characterized by difficulties to find the correct meanings of words (27,44,45). Lesions within the inferior frontal gyrus, the lower motor cortex and several white matter pathways cause the classic Broca's aphasia, or expressive aphasia (17,40,45); disorders like agrammatism, apraxia as well as phonematic paraphasia also occur within this syndrome (44,45). Conversion of syllables, indistinct articulation and missing grammatical structures are clinical presentations (44,45).

Wernicke's aphasia, also called posterior aphasia, is caused by a lesion within the posterior part of the left posterior superior temporal gyrus and left inferior parietal region (19,45), including the angular and supramarginal gyrus (17). Semantic and phonological paraphasias with severe mistakes and impaired language comprehension are key features of Wernicke's aphasia (44,45).

Global, or expressive-receptive aphasia is the most severe form of the basic aphasia syndromes (45). Almost all eloquent areas are involved in creating the characteristic appearance of severe impairment of language production and comprehension (44,45). Automatisms, neologisms and perseverations are typical features of the residual language function (45).

Beside the four basic syndromes, special types of aphasia also exist. These include conduction aphasia and transcortical aphasia (44). The classic definition of conduction aphasia is based on the Wernicke-Geschwind language model, stating that a lesion of the arcuate fasciculus (AF) causes this particular type of aphasia (45). The key feature of conduction aphasia is an impaired ability of repetition (44,45,49). However, recent studies suggest, that lesions within the AF are not essentially responsible for conduction aphasia; moreover, it was stated that conduction aphasia can be a consequence of cortical damage only (49). Furthermore, conduction aphasia is suggested to be rather a transient phenomenon than a permanent neurological deficit (49). On the other hand, transcortical aphasia is subdivided into transcortical-motor aphasia, transcortical-sensory aphasia and mixed-transcortical aphasia (45). The common features of these types are a maintained ability of repetition (45). Furthermore, transcortical aphasia, also called the “isolation of speech area” (45,50), is characterized by a maintained formal language function and impaired connection of semantic processing (45). Patients suffering from transcortical motor aphasia can repeat and read fluently but lack spontaneous speech (45). The lesion which causes transcortical motor aphasia is within the inferior frontal gyrus or in the vicinity to the supplementary motor area (45,51).

Transcortical sensory aphasia is often caused by a lesion within the temporo-occipital white matter tracts; it is characterized by a fluent language production but impaired language comprehension (44,45,51). This manifests as semantic paraphasia and echolalia (45). Impaired connections of the perisylvian region to the association areas is the reason for mixed transcortical aphasia which presents as comprehension and production disorder characterized by automatism, echolalia and stereotypic responses (45).

Knowledge of classical syndromes and types of aphasia is based on studies made in the 20th century. Since then, a tremendous technological development in imaging and analysis of functional brain areas has occurred. For instance, recent findings suggest a different approach for categorizing aphasia. The recent assumption is that a symptom based approach might be more suitable for classifying aphasia (52,53). The reason is the complexity of language processing, which in turn requires a more refined classification than just the syndromes described above (52). Following the symptom-based approach, an association between deficit and the involved site must be established; it allows a more precise correlation of results regarding symptoms and lesion (52,53).

Figure 7 illustrates the schematic overview of functions that can be affected by a syndrome. Particular deficits are associated with specific sites; therefore it is reasonable to define precise symptoms to assess or classify the elicited deficits. Most commonly used categories are: No response/speech arrest; hesitation, neologism, semantic paraphasia, phonological paraphasia, circumlocution and perseveration (54–57). In DCS-mapping, the most frequently elicited symptom is no response (38). Semantic errors can be induced by stimulating the posterior middle temporal gyrus, anterior supramarginal gyrus and inferior frontal gyrus (38). Circumlocutions and neologisms are elicited in the superior temporal sulcus, phonological errors or even speech arrest in the posterior superior temporal gyrus, in the inferior frontal gyrus and in the lower precentral gyrus (38). By stimulating subcortical pathways, specific symptoms can occur as well. Articulatory disturbances can be caused by a transient lesion of the superior longitudinal fasciculus, phonological paraphasia and repetition disorders are associated with impairment of the arcuate fasciculus, and the inferior fronto-occipital fasciculus is linked to semantic deficits (9,58).

The combination of aphasia-assessing tests and other methods like TMS or DCS require specific paradigms (5,54). It is crucial that the chosen test involves the regions of interest in order to elicit deficits during the examination. For this purpose, specific test paradigms exist beside the previously mentioned test batteries. The most commonly used are: object naming- (ONT), pseudo-word reading-, verb-generation-, action naming-, word reading- and counting tasks.

Examples of other test modalities include sentence completion, reading sentences aloud, translating paragraphs, naming of famous faces and writing sentences. Furthermore, BOLD fMRI (described in detail below), allows an assessment of activated areas and involved parts of language processing (5).

In fMRI-based studies, mapping of functional areas and assignment to different aspects of language according to the applied test paradigm can be made by hotspot analysis. The ONT for example activates the posterior middle temporal gyrus for semantic and phonological proportions, the opercular inferior frontal gyrus for motor processing and the precentral gyrus for motor initiation and articulation (54). Pseudo-word reading shows activation within the left hemisphere in visual areas, in the anterior temporal region for lexical and semantic processing, in the inferior frontal gyrus for phonological output and in the inferior parietal cortex for spelling-sound conversion (54). Similar results exist for each task which explains errors occurring in particular areas. Therefore, tests which activate many sites simultaneously appear to be the most suitable to detect all associated areas. The test should also be easy to perform to ensure feasibility during the stimulation by TMS or DCS (54). The application of different tests depending on the stimulated site could reveal higher details of function while adversely complicate the test procedure. Prolonged test duration and difficult tasks can increase loss of attention in the patient and therefore lead to false positive results.

The Object Naming Task (ONT) is a form of the Boston Naming test which requires the patient to name simple pictures of common objects; it shows a large-scale distribution within cortical language areas (5,54,59) and is the most common task for language mapping (38). It involves numerous cortical and sub-cortical functions (54) like visual object recognition, memory recall, semantic processing, lexical retrieval, phonological encoding, articulatory planning and execution (38). Furthermore, it can reproduce the whole process of word production and incorporates all language eloquent brain regions (56,57). Therefore, the ONT is postulated to be the most discriminative task for assessing areas involved in language processing (54).

In summary of this section, language testing plays a fundamental role in the assessment of speech disorders and mapping of language eloquent areas. Furthermore, mapping results are significantly influenced by the chosen test modality. Therefore, a careful consideration of the goal, practicability and anticipated results needs to be taken beforehand. Moreover, the task results also depend on the location of the lesion, cognitive abilities, age, education and preoperative deficits of the patient (46). It is more reasonable to group elicited language disorders into deficit or symptom-based categories, than using the classical basic syndromes of language disorders. The newly developed language testing modalities should consider the current knowledge of cognitive neuroscience regarding language processing (46). Within the setting of TMS and DCS, standardized and adjusted tests are required (46). Sensitivity, specificity and predictive value are the main parameters to assess the feasibility of these test modalities. Beside these factors, it has to beinvestigated , whether the applied tasks are capable of truly improving mapping results and identification of language eloquent areas (46).

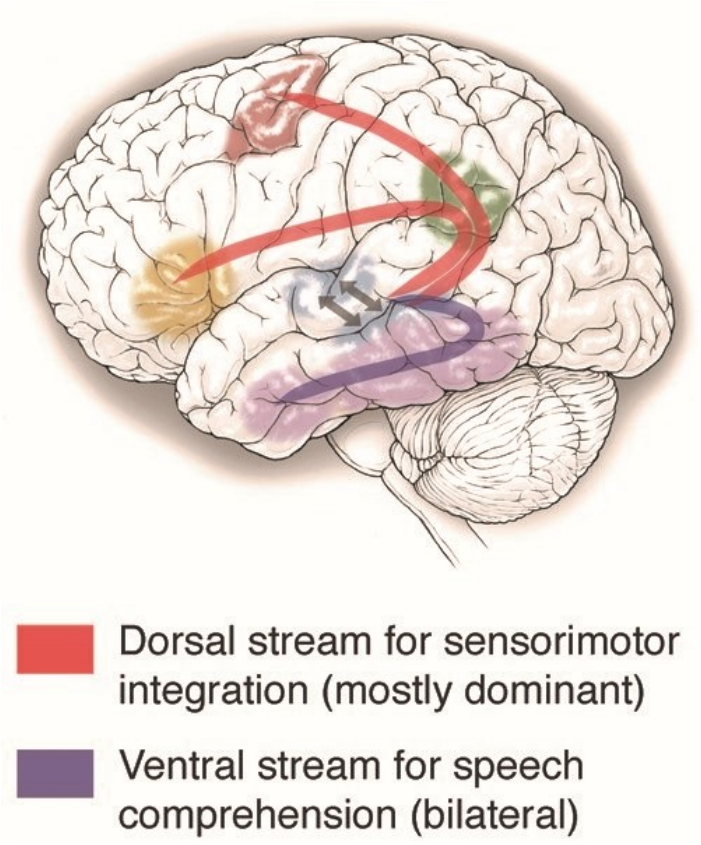

Figure 6 Ventral and dorsal streams of language function (38)

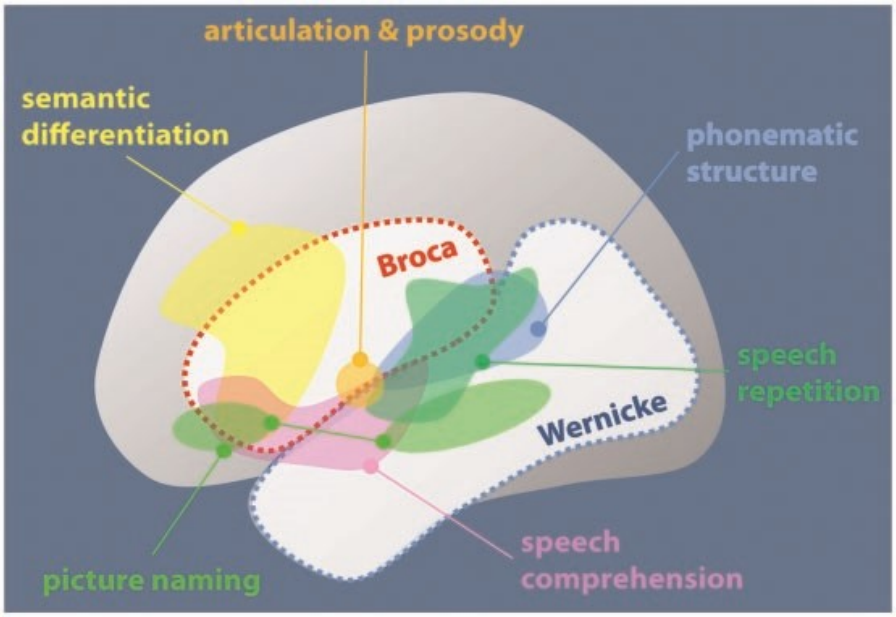

Figure 7 Cortical language areas (52)

2.4 Brain tumours in general and gliomas in particular

2.4.1 General information

A tumour is defined as an outlined increase of tissue volume; it is a neoplastic and autonomous growth caused by an impairment of regulations on cellular level (e.g. proliferation, apoptosis, differentiation) (27,60). The subsequent degeneration of cells is caused by failures in gene expression for tumour suppressor genes and proto-oncogenes including p16, p21, p52, BAX, Riboprotein, RAS and the G-protein gene (60,61). Altered gene expression is due to various types of mutation (e.g. point mutation, deletion, duplication) leading to a loss of function in tumour suppressor genes and an increased expression of oncogenes (60,61). The classification of tumours is based on the cells or tissue which are the origin of the neoplasm. The World Health Organization (WHO) classification and grading of tumours of the CNS is also based on cells of origin, their developmental differentiation and histological similarities (62–64). For instance, histogenesis is a concept related to light microscopic features of tumours in haematoxylin and eosin-stained sections (63).

Brain tumours can be described as neoplasms within the CNS originating from cells assigned to this system (60). The recent classification of brain tumours is based on the revised 4th version of the WHO classification and grading of tumours of the CNS from 2007. In comparison to the former classification, there are new features added to the 2016 version, which will be explained in this chapter (62). To understand the changes, an overview of the basic classification will be given. As previously mentioned, CNS tumours are classified according to their cells of origin: aberrant glial cells for example lead to tumours called gliomas, including different subtypes like astrocytomas, oligodendrogliomas and many more (60).

The recent 2016 revision of the 4th WHO classification of CNS tumours is based on the microscopic findings and cells of origins, but for the first time also incorporates molecular parameters into the classification (62,63).

It can therefore be described as an integrated classification, combining histological/phenotypic and molecular/genotypic features (63,65). Due to the basis of histopathological findings, the nomenclature consists of a histological name followed by the genetic feature (63). The term NOS stands for “not otherwise specified” and is used for all entities which have not been or could not be genetically examined (63). Based on the molecular characterization, an assumption regarding prognosis and clinical behaviour can be made (66). In contrast to other types of tumours, gliomas are not staged (66), because they rarely metastasize outside the CNS (64).

Approximately 2% of all tumour entities are primary brain tumours (64), with a global age-standardized incidence of approximately 3,7 per 100 000 people (67). The incidence is higher in men than in women, and also higher in more developed than in less developed countries (67). Within the umbrella term of brain tumours, the majority of tumours originating from CNS cells are gliomas (~81%) (65,68–70). The latter can be divided into glioblastomas (~45-49%), unspecified or other astrocytomas (NOS) (~18%), oligodendrogliomas (~9%), ependymomas (~1,5%) and embryonal tumours (~1%) (69,71). Each entity is associated with a certain age cohort (72).

Gliomas arise most frequently in the cerebral hemispheres (4,73) and are most likely located in the frontal lobe as can be seen in figure 8 (74). These locations explain why gliomas have an inherent risk of invading functional regions like language associated areas. Depending on size and location gliomas can cause general neurological symptoms such as headache, nausea, vomiting, fatigue and dizziness and/or specific neurological symptoms such as motor deficit, sensory deficit, language impairment, visual impairment or seizures (4).

Even in the 4th revised edition of the WHO classification, gliomas are still classified according to their cells of origin (table 1). 26 different subpopulations of gliomas are defined and divided into two main groups: diffuse astrocytic and oligodendroglial tumours and other astrocytic tumours (60,62,63). Beside this division, different mutations and chromosomal aberrations are taken into account for the new classification (62,63). They include characteristic mutations and

chromosomal aberrations such as the IDH (isocitrate dehydrogenase) mutation (63).
IDH isoforms are located on chromosome 2 and 15 and called IDH1 and IDH2. Mutations in both genes are linked to the oncogenesis of gliomas (68). Beside these two driver mutations, TP53, TERT, ATRX mutations and chromosomal aberrations like the 1p/19q co-deletion are additional molecular markers which allow further characterization (63,75). Figure 9 shows a classification for diffuse gliomas according to IDH status and other genetic parameters (63).

Beside origin and genetic features, gliomas are also distinguished by tumour grading; four grades are defined by the WHO, ranging from mostly benign-looking to highly malignant according to histopathological features (e.g. atypia, mitotic activity, anaplasia, occurrence of necrosis) (60,76,77). The former classification of low grade gliomas (LGG) included WHO Ⅰ and Ⅱ gliomas, whereas the 2016 classification groups diffuse LGG together regardless of their origin or grade (astrocytomas and oligodendrogliomas in one group) because of their genetic similarities (65,76). Subdivision is now done by analyzing the IDH mutation status (IDH-mutant, IDH-wildtype, NOS) and chromosomal aberrations (63,65,76). The recent classification addresses challenges regarding different behaviours of tumour entities (e.g. growth pattern, genetic mutations, prognosis, clinical outcome) (65,76,77).

Especially for adjuvant therapy, a correct diagnosis is crucial to ensure a successful treatment. Despite many studies, the results of additional treatments like radio- or chemotherapy remain modest in terms of benefits for PFS and overall survival (76). An assumption regarding prognosis can be made based on the tumour grade but seems to be more accurate by genotype-analysis. Hence the prognosis of an IDH-mutant astrocytoma seems to be more favourable than that of a IDH-wildtype one (62,63). Personalized and evidence-based therapy can be enhanced by a more detailed diagnosis, ensured by the new classification (76,78).

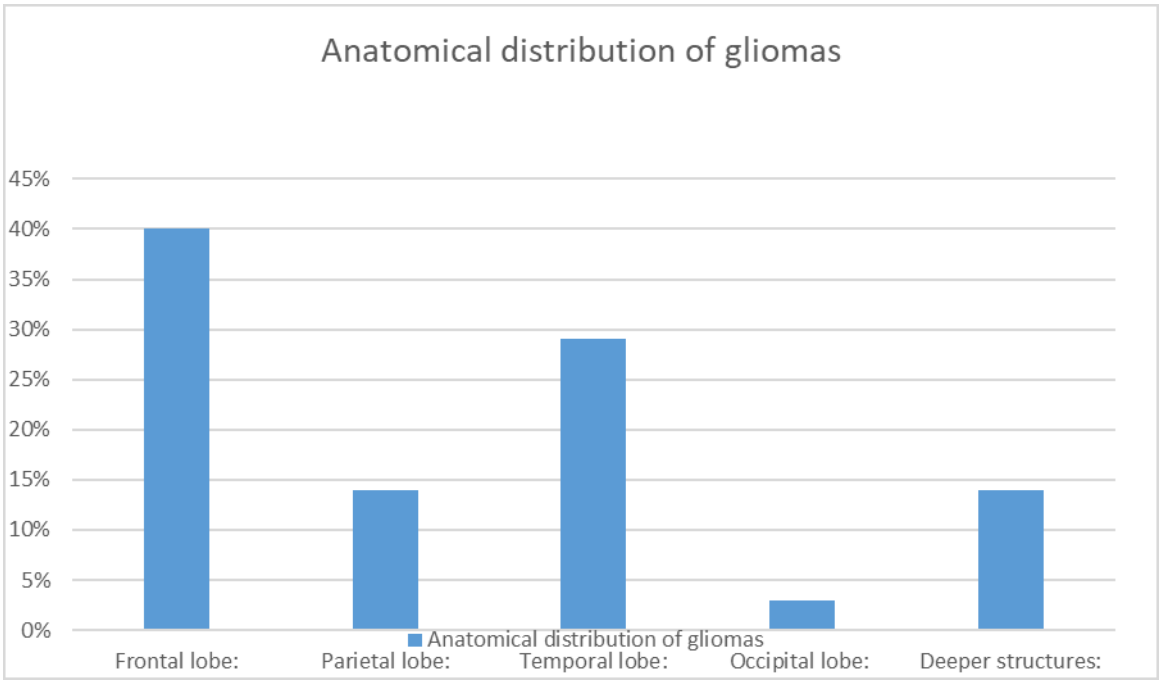

Figure 8 anatomical distribution of gliomas (74)

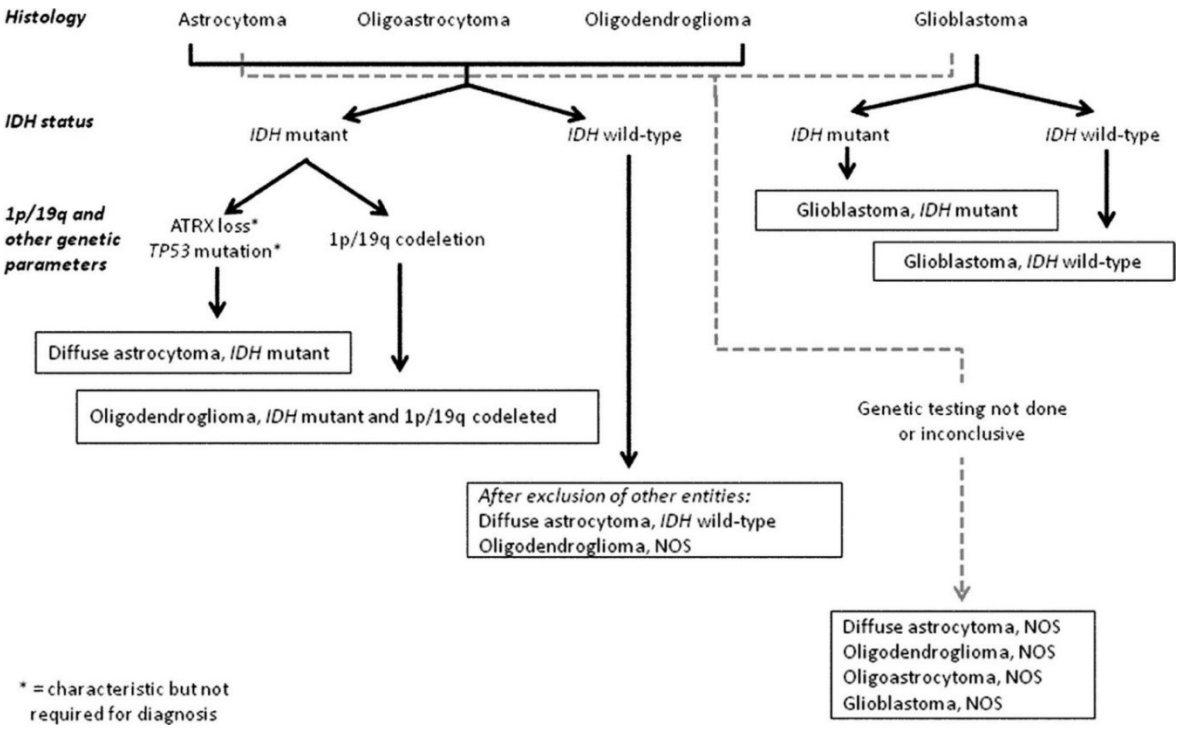

Figure 9 Overview of genetic pathways of Gliomas (63)

Table 1 WHO classification of CNS tumours I (63)

Diffuse astrocytic and oligodendroglial tumours	
Diffuse astrocytoma, IDH-mutant	9400/3
Gemistocytic astrocytoma, IDH-mutant	9411/3
Diffuse astrocytoma, IDH-wildtype	*9400/3*
Diffuse astrocytoma, NOS	9400/3
Anaplastic astrocytoma, IDH-mutant	9401/3
Anaplastic astrocytoma, IDH-wildtype	*9401/3*
Anaplastic astrocytoma, NOS	9401/3
Glioblastoma, IDH-wildtype	9440/3
Giant cell glioblastoma	9441/3
Gliosarcoma	9442/3
Epithelioid glioblastoma	*9440/3*
Glioblastoma, IDH-mutant	9445/3*
Glioblastoma, NOS	9440/3
Diffuse midline glioma, H3 K27M–mutant	9385/3*
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	9450/3
Oligodendroglioma, NOS	9450/3
Anaplastic oligodendroglioma, IDH-mutant and 1p/19q-codeleted	9451/3
Anaplastic oligodendroglioma, NOS	*9451/3*
Oligoastrocytoma, NOS	*9382/3*
Anaplastic oligoastrocytoma, NOS	*9382/3*
Other astrocytic tumours	
Pilocytic astrocytoma	9421/1
Pilomyxoid astrocytoma	9425/3
Subependymal giant cell astrocytoma	9384/1
Pleomorphic xanthoastrocytoma	9424/3
Anaplastic pleomorphic xanthoastrocytoma	9424/3
Ependymal tumours	
Subependymoma	9383/1
Myxopapillary ependymoma	9394/1
Ependymoma	9391/3
Papillary ependymoma	9393/3
Clear cell ependymoma	9391/3
Tanycytic ependymoma	9391/3
Ependymoma, *RELA* fusion–positive	9396/3*
Anaplastic ependymoma	9392/3
Other gliomas	
Chordoid glioma of the third ventricle	9444/1
Angiocentric glioma	9431/1
Astroblastoma	9430/3
Choroid plexus tumours	
Choroid plexus papilloma	9390/0
Atypical choroid plexus papilloma	9390/1
Choroid plexus carcinoma	9390/3
Neuronal and mixed neuronal-glial tumours	
Dysembryoplastic neuroepithelial tumour	9413/0
Gangliocytoma	9492/0
Ganglioglioma	9505/1
Anaplastic ganglioglioma	9505/3
Dysplastic cerebellar gangliocytoma (Lhermitte–Duclos disease)	9493/0
Desmoplastic infantile astrocytoma and ganglioglioma	9412/1
Papillary glioneuronal tumour	9509/1
Rosette-forming glioneuronal tumour	9509/1
Diffuse leptomeningeal glioneuronal tumour	
Central neurocytoma	9506/1
Extraventricular neurocytoma	9506/1
Cerebellar liponeurocytoma	9506/1
Paraganglioma	8693/1
Tumours of the pineal region	
Pineocytoma	9361/1
Pineal parenchymal tumour of intermediate differentiation	9362/3
Pineoblastoma	9362/3
Papillary tumour of the pineal region	9395/3
Embryonal tumours	
Medulloblastomas, genetically defined	
Medulloblastoma, WNT-activated	9475/3*
Medulloblastoma, SHH-activated and *TP53*-mutant	9476/3*
Medulloblastoma, SHH-activated and *TP53*-wildtype	9471/3
Medulloblastoma, non-WNT/non-SHH	9477/3*
Medulloblastoma, group 3	
Medulloblastoma, group 4	
Medulloblastomas, histologically defined	
Medulloblastoma, classic	9470/3
Medulloblastoma, desmoplastic/nodular	9471/3
Medulloblastoma with extensive nodularity	9471/3
Medulloblastoma, large cell / anaplastic	9474/3
Medulloblastoma, NOS	9470/3
Embryonal tumour with multilayered rosettes, C19MC-altered	9478/3*
Embryonal tumour with multilayered rosettes, NOS	*9478/3*
Medulloepithelioma	9501/3
CNS neuroblastoma	9500/3
CNS ganglioneuroblastoma	9490/3
CNS embryonal tumour, NOS	9473/3
Atypical teratoid/rhabdoid tumour	9508/3
CNS embryonal tumour with rhabdoid features	*9508/3*
Tumours of the cranial and paraspinal nerves	
Schwannoma	9560/0
Cellular schwannoma	9560/0
Plexiform schwannoma	9560/0

Table 2 WHO classification of CNS tumors II (63)

Melanotic schwannoma	9560/1
Neurofibroma	9540/0
Atypical neurofibroma	9540/0
Plexiform neurofibroma	9550/0
Perineurioma	9571/0
Hybrid nerve sheath tumours	
Malignant peripheral nerve sheath tumour	9540/3
Epithelioid MPNST	9540/3
MPNST with perineurial differentiation	9540/3
Meningiomas	
Meningioma	9530/0
Meningothelial meningioma	9531/0
Fibrous meningioma	9532/0
Transitional meningioma	9537/0
Psammomatous meningioma	9533/0
Angiomatous meningioma	9534/0
Microcystic meningioma	9530/0
Secretory meningioma	9530/0
Lymphoplasmacyte-rich meningioma	9530/0
Metaplastic meningioma	9530/0
Chordoid meningioma	9538/1
Clear cell meningioma	9538/1
Atypical meningioma	9539/1
Papillary meningioma	9538/3
Rhabdoid meningioma	9538/3
Anaplastic (malignant) meningioma	9530/3
Mesenchymal, non-meningothelial tumours	
Solitary fibrous tumour / haemangiopericytoma**	
Grade 1	8815/0
Grade 2	8815/1
Grade 3	8815/3
Haemangioblastoma	9161/1
Haemangioma	9120/0
Epithelioid haemangioendothelioma	9133/3
Angiosarcoma	9120/3
Kaposi sarcoma	9140/3
Ewing sarcoma / PNET	9364/3
Lipoma	8850/0
Angiolipoma	8861/0
Hibernoma	8880/0
Liposarcoma	8850/3
Desmoid-type fibromatosis	8821/1
Myofibroblastoma	8825/0
Inflammatory myofibroblastic tumour	8825/1
Benign fibrous histiocytoma	8830/0
Fibrosarcoma	8810/3
Undifferentiated pleomorphic sarcoma / malignant fibrous histiocytoma	8802/3
Leiomyoma	8890/0
Leiomyosarcoma	8890/3
Rhabdomyoma	8900/0
Rhabdomyosarcoma	8900/3
Chondroma	9220/0
Chondrosarcoma	9220/3
Osteoma	9180/0
Osteochondroma	9210/0
Osteosarcoma	9180/3
Melanocytic tumours	
Meningeal melanocytosis	8728/0
Meningeal melanocytoma	8728/1
Meningeal melanoma	8720/3
Meningeal melanomatosis	8728/3
Lymphomas	
Diffuse large B-cell lymphoma of the CNS	9680/3
Immunodeficiency-associated CNS lymphomas	
AIDS-related diffuse large B-cell lymphoma	
EBV-positive diffuse large B-cell lymphoma, NOS	
Lymphomatoid granulomatosis	9766/1
Intravascular large B-cell lymphoma	9712/3
Low-grade B-cell lymphomas of the CNS	
T-cell and NK/T-cell lymphomas of the CNS	
Anaplastic large cell lymphoma, ALK-positive	9714/3
Anaplastic large cell lymphoma, ALK-negative	9702/3
MALT lymphoma of the dura	9699/3
Histiocytic tumours	
Langerhans cell histiocytosis	9751/3
Erdheim–Chester disease	9750/1
Rosai–Dorfman disease	
Juvenile xanthogranuloma	
Histiocytic sarcoma	9755/3
Germ cell tumours	
Germinoma	9064/3
Embryonal carcinoma	9070/3
Yolk sac tumour	9071/3
Choriocarcinoma	9100/3
Teratoma	9080/1
Mature teratoma	9080/0
Immature teratoma	9080/3
Teratoma with malignant transformation	9084/3
Mixed germ cell tumour	9085/3
Tumours of the sellar region	
Craniopharyngioma	9350/1
Adamantinomatous craniopharyngioma	9351/1
Papillary craniopharyngioma	9352/1
Granular cell tumour of the sellar region	9582/0
Pituicytoma	9432/1
Spindle cell oncocytoma	8290/0
Metastatic tumours	

The morphology codes are from the International Classification of Diseases for Oncology (ICD-O) (742A). Behaviour is coded /0 for benign tumours; /1 for unspecified, borderline, or uncertain behaviour; /2 for carcinoma in situ and grade III intraepithelial neoplasia; and /3 for malignant tumours. The classification is modified from the previous WHO classification, taking into account changes in our understanding of these lesions.
*These new codes were approved by the IARC/WHO Committee for ICD-O.
Italics: Provisional tumour entities. **Grading according to the 2013 *WHO Classification of Tumours of Soft Tissue and Bone*.

2.4.2 Diagnostic procedures in glioma patients

Prior to surgery many diagnostic procedures can be done in brain tumour patients. Apart from the compulsory neurological examination, imaging has become an extremely important part of the preoperative diagnostic assessment. With the emergence of magnetic resonance imaging (MRI) the detection of brain tumours, especially gliomas, has become significantly easier. For certain entities like gliomas, additional advanced procedures (e.g. fMRI and neuropsychological assessment, awake surgery) need to be performed. FLAIR and T2 are the most suitable MRI sequences to investigate gliomas and BOLD fMRI is used to detect functional areas (5,79). Neuropsychological protocols depend on tumour location and symptoms and include a wide range of investigated functions like: attention, learning and memory, processing speed, executive function, language, visuospatial function and motor function (80).

The basic entities of functional brain mapping are: functional magnetic resonance imaging (fMRI), magnetencephalography (MEG), transcranial magnetic stimulation (TMS) and direct cortical stimulation (DCS) (5). DCS is considered the gold standard (7,12,57,81) and is mandatory during awake surgery to guarantee a reliable and secure assessment of functional areas. However, the other methods mentioned before are valuable non-invasive and preoperative diagnostic tools, allowing a more precise preoperative risk assessment. Which methods are used depends mainly on the availability and experience of the techniques at the facility.

To preoperatively detect functional sites within the brain, the standard method in clinical practice and cognitive neuroscience is fMRI (5). As a reliable imaging technique, fMRI is able to provide indirect functional neuroimaging, including both anatomical and functional information (5). Mapping functional areas is basically conducted by blood oxygenation level dependent (BOLD) MRI and diffusion tensor imaging (DTI).

The underlying theory of the BOLD MRI is that there is an increase in metabolism in certain cortical areas that are activated during a certain task (e.g. finger movement, speaking, reading, etc.) and therefore a higher consumption of oxygen leading to a higher concentration of deoxygenated haemoglobin within erythrocytes and a change in perfusion (5). These changes can be visualized and are used as an indirect marker of neuronal activity in certain cortical areas during certain tasks (5).
Due to that principle, pathophysiological changes found in brain lesions and their vicinity might cause distorted results, thus impairing the accuracy of fMRI (7,11). This can be attributed to factors like: vascular changes within the lesion, malfunctions of the blood brain barrier (BBB) and oedema (7). Beside the mapping of functional cortical areas, the localization and course of white matter pathways is also important for preoperative diagnostic assessment and surgical planning. Diffusion tensor imaging is a non-invasive MRI technique to visualize white matter tracts by analysing the translational movement of water molecules (5). DTI is based on the detection of water molecules that move and line up with axonal bundles; reconstruction of this spatial information allows a tractography by combining the DTI data with structural images (5). While this technique provides unique information, limitations must be considered, especially in cases of impairment of normal structure and function. For glioma surgery, brain shift during resection, oedema and altered spatial organization (e.g. kissing or merging fibres) can induce a significant bias into DTI results (5).

Based on other physical principles, magnetencephalography (MEG) is a different method to assess the distribution of functional areas in the brain. MEG measures ionic currents created by action potentials along axons and postsynaptic potentials through detecting changes in a magnetic field (5). As a result, it offers time resolved image sequences of brain functions (5).

All the methods mentioned above have in common, that they are indirect, non-invasive imaging techniques (5). As they are incapable of modulating brain function, a functional assessment of potentially eloquent areas is not possible (5).
Apart from DCS there are three methods capable of modulating neuronal function.

Transcranial electric stimulation (TES) applies electric currents to the brain through electrodes on the scalp. It affects the neurons by de- or hyperpolarizing their resting membrane potential. Within the literature, the use of TES for neurosurgical purposes is not widespread, this might be due to relatively imprecise localization of the stimulated area.

Extra-operative electrical mapping is an invasive approach of mapping functional areas by implanting an electrode-covered grid on the cortical surface (5). The cortical function is evaluated by three measurements: recording of spontaneous activity, recording of cortical evoked potentials, and electric stimulation (5). As an invasive intracranial monitoring it is often used in patients suffering from epilepsy (5). While methodically closely related to DCS, a critical risk evaluation is crucial prior to the use of extra-operative electrical mapping because it increases the risk of infection and other surgical complications such as haemorrhage (5,82) .

It is important to mention that every method relies on specific neuropsychological tests to assess sensory-motor and cognitive functions and practical feasibility can be a limiting factor. For example, language testing in a MRI scanner is limited by the movements of the head. Diagnostic approaches for mapping functional areas within the cerebral cortex can be grouped into invasive and non-invasive methods as well as direct and indirect techniques. Imaging of functional areas and techniques that directly influence cognitive function can also be grouped into the previously mentioned categories.
TMS can be described as a non-invasive method which can modulate cortical function (83) and appears to be a promising approach to fill the gap between fMRI and DCS.

2.4.3 Therapy of gliomas

2.4.3.1 Surgery

The treatment of gliomas faces special challenges in comparison to other neoplastic diseases. The confined space of the cranium, the complex anatomy combined with essential and interindividual variable functional areas, the lack of clear tumour margins and frequently observed invasion of essential functional areas (84), as well as the blood-brain-barrier as an obstacle for therapeutic agents are some of the challenges faced when treating glioma patients (84).

The key issue in glioma therapy is the balance between extent of resection (EOR) and maintenance of function and therefore quality of life. The difference between a functionally and oncological good and bad result is often a matter of millimetres. Postoperative deficits, residual volume (RV), progression-free-survival (PFS), time to malignant transformation, quality of life and overall survival are the main variables that represent these key issues (79,85,86).

Postulations regarding the tumour entity and possible malignancy can be made by radiological appearance, positron emission tomography (PET) scans, contrast medium uptake, location of the tumour and age of the patient (84). However, the final diagnosis can only be made after histopathological and genetic examination (4).

To achieve the best possible therapeutic result, various pre- and intraoperative diagnostic modalities are available. This chapter aims to give an insight into different modalities, as well as the principles of therapy in general and more specifically.

For the treatment of gliomas, three conventional treatment modalities exist: surgical resection, radiotherapy and chemotherapy (4). All of them can be applied while the use and effectiveness depend on the entity, grading, tumour location, age of the patient, as well as pre-existing neurological deficits (4).

In addition to the classical modalities, there are also immunotherapies and retroviral therapies which target special properties of tumour cells (78). However, cytoreductive surgery is still the first line of treatment if possible (87).

The resection of tumour tissue within the area of radiological changes is the present principle of brain tumour surgery (78). However, tumours frequently extend beyond the margins depicted in MRI scans (85,88). A supra-total resection might therefore improve the outcome of glioma patients (79,85). A supra-total resection is described as a resection beyond MRI-defined margins (79,85). The determination of resection margins is crucial to achieve a maximum EOR. Strategies for determining the tumour margins are variable and depend on the imaging modality. Glioma surgery is governed by a maximal safe resection using various intra-operative diagnostic methods (e.g. neuro-navigation, intraoperative MRI, 5-ALA, ultrasound) to enable a maximal EOR without causing permanent functional deficits (4,88,89).

In high grade gliomas, the pro-drug 5-aminolevulinic acid (5-ALA) is capable of visualizing tumour tissue. As a product of haemoglobin metabolism, 5-ALA is taken up by glioma cells and metabolized to protoporphyrin IX (PpIX). Due to a specific defect of the iron-binding enzyme ferrochelatase, PpIX accumulates within glioma cells and can be detected by a pink-red fluorescence under blue light (84). It has been shown that the application of 5-ALA improves the EOR, PFS and overall survival (81). In the case of low grade gliomas, the fluorescence effect is too low to be macroscopically detectable intraoperatively (81,84), therefore, other modalities must be used for a better intraoperative assessment of tumour margins in these cases. Besides 5-ALA, Raman spectroscopy is another real-time application for the differentiation of tumour cells and normal tissue. Raman spectroscopy, also called Raman scattering microscopy, is a physical application which relies on vibrational qualities of intracellular structures and can thus visualize the cytoarchitecture of the tissues (81). By analysing the cytoarchitecture, Raman spectroscopy detects pathological spectroscopic signals of brain tissue and can define tumour margins (81).

The classical method of histological sectioning and pathological assessment of tumour cells can then be done in the sample. In comparison to the classical method which is an in-vitro application, one of the advantages of the previously mentioned methods is that their application is in-vivo (81).

A complete tumour removal should be the goal of surgery, if it does not induce a permanent neurological deficit (88). Extent of resection therefore also depends on the location of the tumour and the distribution of eloquent areas. In the treatment of low grade gliomas, it was shown that the overall survival is increased from 61,1 to 90,5 months with a greater EOR (85).

The question whether a surgical resection is reasonable at all often arises in glioma treatment. The discussion of advantages and disadvantages of biopsy only vs. tumour resection is constantly discussed. Jakola et al. (90) have shown that the overall survival of patients in a surgical resection group was significantly higher than in a biopsy group. Additionally, recent studies suggest a benefit of resective surgery regarding overall survival (76). Furthermore, the risk of intervention is similar for both biopsy and surgical resection (85). Recent recommendations therefore suggest an individual approach for weighing risks and benefits in each patient individually (79).

The preservation of neurological function is another essential parameter for glioma treatment. Various pre- and intraoperative modalities are available to increase patient safety and decrease postoperative deficits. As previously mentioned, the preoperative assessment of functional areas is made by fMRI, DTI, MEG and TMS. The intraoperative gold standard for mapping functional areas is DCS during awake surgery (12,57,81). Awake surgery has been proven to decrease the risk of postoperative deficits while maximizing the extent of resection (58,91). In the vicinity of functional areas such as the language, motor or somatosensory areas, awake surgery is a reliable method to avoid surgically induced deficits (58,91). Awake surgery is indicated for supratentorial tumours near functional areas (81). The main contraindications for awake surgery are uncontrolled persistent cough, severe dysphasia and failure to cooperate (81).

2.4.3.2 Radiotherapy

Radiotherapy is based on five fundamental principles of radiobiology: repair of sublethal damage, re-oxygenation of the tumour, re-assortment of redistribution within the cell cycle, repopulation or regeneration of surviving normal and malignant cells and radio-sensitivity (92,93). Based on these principles, radiotherapy is capable of inducing apoptosis in the irradiated area (92). According to the previously mentioned principles, re-assortment, re-oxygenation and radio-sensitivity can increase tumour cell death (93). In contrast, a critical disadvantage is the potential damage to healthy brain parenchyma through the application of conventional radiotherapy (4). Based on the same principles, stereotactic radiosurgery (SRS) is a special form of radiotherapy which allows to focus the effects on a certain area and can deliver a high dose of radiation to a target area (93). Even though radiobiological differences exist between conventional radiotherapy and SRS (93), SRS is an effective treatment for brain tumours, e.g. in single or multiple brain metastases (94). Furthermore, SRS is stated to be an important modality for additional treatment of gliomas, especially in critical locations like deep brain structures or brain stem, or for residual and recurrent tumours (93). Whilst surgical resection is the primary treatment of choice for gliomas, radiotherapy is appropriate for both adjuvant treatment and application in relapse situations (81,89,95). Optimal timing for radiotherapy depends on various factors like EOR, age, Karnofsky index and tumour location (89). Reviews have shown that delayed radiotherapy can improve PFS, but not overall survival; however it also contributes to preservation of function and seizure control (89,95).

2.4.3.3. Chemotherapy

Chemotherapy and more recent targeted therapies like antibody treatments face specific challenges associated with special characteristics of the CNS. Probably the most important factor is overcoming the BBB. Even the best medication is useless if it cannot reach its target tissue. Therefore, the BBB can be a limiting factor for medical tumour therapy. Defined as a selective permeable barrier between blood and brain parenchyma (27), the BBB is a cellular barrier of high organization and complexity (96). Processes like efflux transporter and interstitial pressure gradients complicate the passage for many substances (97).

Depending on the tumour entity and grading, the blood-brain-tumour-barrier (BBTB) is considered dysfunctional (96). It is widely assumed that, especially in high grade gliomas, the BBTB is dysfunctional (96). However, the “leaky” part of the BBTB is limited to the central tumour proportions (96); therefore, chemotherapeutic agents fail to overcome the BBTB in peripheral parts of the tumour (96).

Substances which pass the BBB naturally are suitable for the treatment of brain tumours (96,97). Nitrosoureas, procarbazine, temozolomide and methotrexate are such agents (97,98). However, the concentration reached in the target tissue might be very low and therefore requires high systemic doses of the agent (e.g. the concentration of temozolomide in the brain is just 1/5 of the concentration in the blood) (97,99). To increase the effectivity and to enable the use of other cytostatic agents and targeted therapies, strategies for overcoming the BBB are required.

Two basic strategies for overcoming the BBB can be followed: direct placement of substances inside the BBB or enhancement of delivery of the substances to the brain (97). Several options exist for both strategies. Direct placement is possible by intrathecal application, direct application into the brain parenchyma, convection-enhanced delivery by a catheter, and implantation of chemotherapy-containing polymers into the surgical cavity (96,97).

Increase of delivery can be achieved by high dose systemic chemotherapy, intra-arterial chemotherapy, temporary disruption of the BBB or biochemical modulation of the substances (96,97,100).

Chemotherapy in general is defined as an oncological treatment by substances which cause damage to tumour cells by blocking cellular processes (27). Specifically, the administration of cytostatic agents is a fundamental part of every chemotherapy (97). Cytogenic agents are a heterogenous group of substances, which inhibit or slow down cell division; they are classified based on their effect (27). Temozolomide is an alkylating agent which is commonly used in glioma treatment (71). Its mechanism of action is relies on the methylation of DNA and RNA (101). Furthermore, temozolomide acts as a radiosensitizer and is a key component of combined radio-chemotherapy (99).

Beside the classical cytostatic agents, new generations of targeted therapies also exist. The use of antibodies is a new approach in the treatment of neoplastic disorders, including brain tumours (98). These specifically designed proteins are capable of binding to tumour cell receptors (98). Different types of antibodies exist, e.g. for carrying cytostatic agents or radioactive material or for interfering with cellular proteins for growth control (98).

Since 1992 gene therapy strategies have been developed to treat gliomas (102–104). The aim of antineoplastic gene therapy is the death of cancer cells or the enhancement of the immune response through the application of therapeutic genetic material (105). Five fundamental principles of gene therapy exist: delivery of suicide genes, delivery of genes which attract immune cells against the tumour (also called immune gene therapy), delivery of tumour-suppressor genes to induce apoptosis, delivery of oncolytic viruses, and antisense therapy (102,104,105). Delivery of the previously mentioned agents is done by viral and non-viral vectors (106). Viral methods include virus producing cells (VPC), that can be implanted into the tumour cavity: depending on the target, VPC can be specifically designed as adenoviral or retroviral VPC (104).

Even though many strategies exist in the field of antineoplastic gene therapy, they are still under investigation in clinical trials. Although studies have proven their clinical safety, they failed to show a significant benefit regarding PFS and overall survival (102).

Another adjuvant approach is metabolic therapy. Its principle is based on the “Wartburg-effect”, which describes an alteration in the metabolism of cancer cells regarding an increase of aerobic glycolysis (107). Glioma cells have a high demand for glucose (107,108). Therefore, the beneficial effect of metabolic therapy is an enhancement of other treatments and a slower growth-rate by a reduction of the availability of glucose (107,108). Low carbohydrate ketogenic diet (KD) is a therapeutic approach to improve life expectancy of patients suffering from high grade gliomas. KD can reduce tumour growth, angiogenesis, inflammation, peritumoral oedema, as well as enhancing the effects of radio- and chemotherapy (108). Furthermore, a neuroprotective effect is described for KD. Ketone bodies have antineoplastic effects themselves and can reverse tumour mediated immune suppression (108). The beneficial effects of KD can be enhanced by combination with glycolysis targeting drugs like 2-deoxyglucose or 3-bromopyruvate (107).

In summary an individualized and personalized therapy for each patient is important to maximize the clinical outcome and the patient’s quality of life.

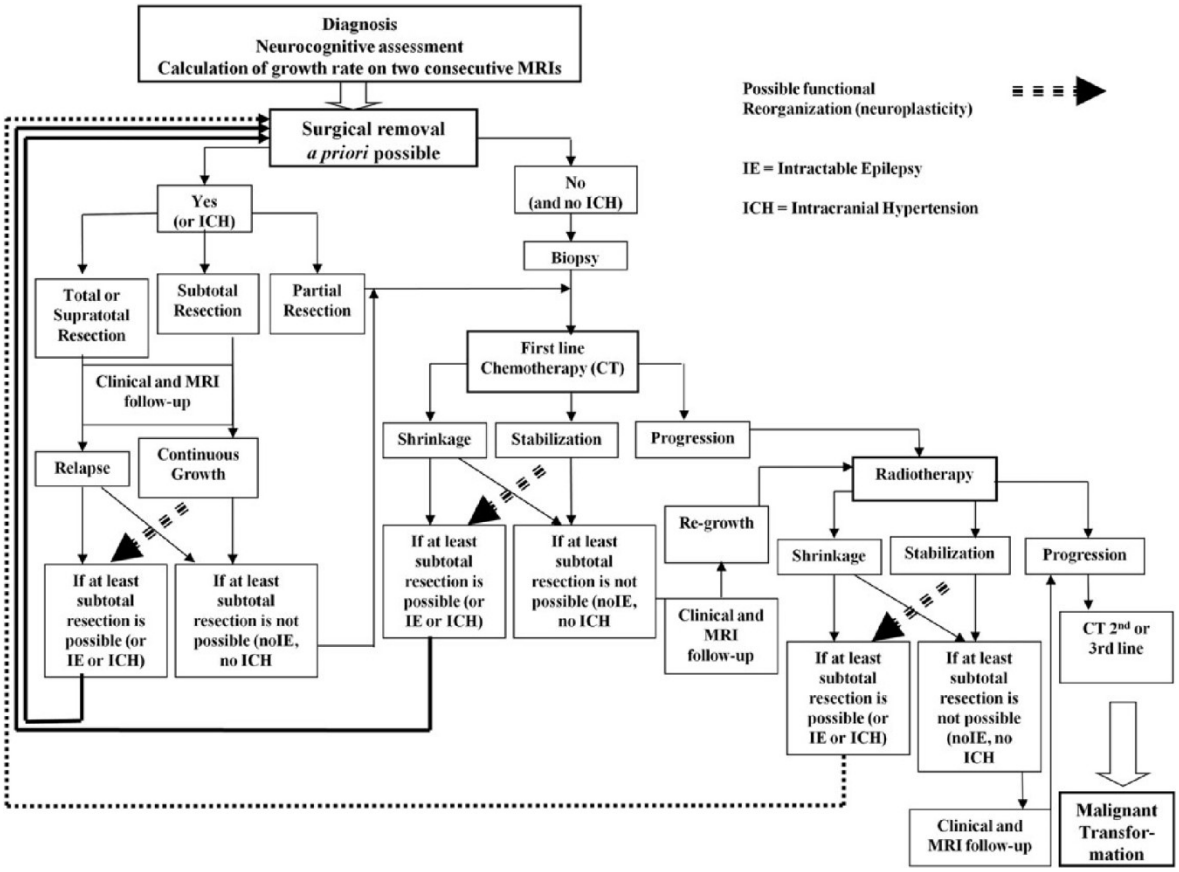

Figure 10 Overview of therapeutic strategies for LGG (85)

2.5 Direct cortical stimulation

„Which elements are excited in electrical stimulation of mammalian central nervous system?" (109)

With this quote, James B. Ranck, an American professor of physiology, posed a fundamental question of direct cortical stimulation (DCS) in 1975. Also called direct electrical stimulation (DES), DCS is an invasive method to influence excitability of cortical neurons, its principle being based on the application of an artificial non-physiological signal into the brain (5). The signal is an electric current which affects the voltage gradient of neuronal membranes (110). Its effect causes a temporary functional lesion either by inhibiting a function or by enhancing a function.

While different types of electric stimulation exist, (e.g. intracellular deep brain stimulation) the discussion will be limited to the extracellular application on the cortical surface and subcortical structures. It was first used in 1870 by Fritsch and Hitzig for stimulating the motor cortex in dogs (111). Today the method constitutes a crucial part in neuroscience and clinical use. Its most common form is the Penfield Stimulation (PS), which was introduced by Wilder Penfield in the 1940s (110) and refined by George Ojemann in the 1970s. The main parameters of the technique will be explained with regard to PS. DCS is usually applied as a constant bipolar stimulation at 60 Hz with a 1 ms biphasic current. However, DCS also depends on the amperage which is individually determined in a range of 1-10 mA. Amperage, also called electric current, is defined as an electric charge across a surface at the rate of one Coulomb per second (112). As the amplitude of the electric current is an individual parameter, it must be defined before sufficient stimulation can be performed. Therefore, the patient is asked about occurring sensations while increasing the amperage simultaneously (113). Another way to determine the intensity is by defining the threshold according to the appearance of after-discharges (5). The reported sensation depends on the stimulated area: usually the motor area is used to define the threshold (5).

For mapping of higher cognitive functions, the stimulation is applied for approximately 3-4 seconds in cortical areas and 4-10 seconds subcortically (114). For motor mapping the so called “train technique” is used beside PS (84,114). The train technique was developed to sufficiently elicit MEP’s under anaesthesia for intraoperative monitoring purposes (115–117). Cortico-motoneuronal transmission is altered by effects of general anaesthesia. To enable supratentorial provocation of MEP’s, several research groups conducted studies to find a suitable paradigm in the 1990’s (118–120). Consisting of a train of 3-9 pulses with a 0.4-0.75 millisecond pulse width, the “train technique” elicits motor evoked potentials MEP (114).

The higher the stimulation intensity the more cells are activated (110). Regarding the quoted question at the beginning, the term “cell” is not precise enough because the effect is directed to a certain area. The stimulated area is considered as a sphere, containing activated neurons (110,121). Within that sphere, the activated cells are distributed in sparse patterns (110). Therefore, the temporary lesion is highly focused and located in a small region (122). As mentioned, the target cell population are neurons (110). Moreover, the activation is basically transmitted through axons (5,110) in both directions, towards the cell body as well as to the synaptic terminals (123). Two major types of activation patterns are described. First, the direct induction of an action potential caused by passive current spread; second, the responses to postsynaptic effects. Direct depolarization seems to be the dominating mechanism for the activation of cells (110). Astrocytes are not significantly influenced by DCS (110). Beside the defined populations of neurons and glial cells like astrocytes, the neuropil is another portion of the CNS. The neuropil is defined as an area of the CNS in between the cell bodies, consisting of dendrites, axons, and glial processes (27). Containing components of surrounding cells, the neuropil responds to a stimulation like an averaged version of these cells with a slow decrease of activation at increasing distance (110).

After discussing the effects of DCS on the cellular level, the effects on higher cognitive functions will be illustrated. Depending on indication and investigated area, the monitored effects are various. Used in awake surgery, DCS allows mapping of functional areas and axonal pathways (5). Therefore, intraoperative electrostimulation mapping (IEM) can be performed for motor, somatosensory-, visual, auditory and for language function (5). Furthermore, IEM is capable of mapping areas of higher cognitive function like calculation, memory, spatial cognition, cross modal judgement and emotional processing (5). Another indication for direct electrical stimulation is the deep brain stimulation in movement disorders (5). The purpose of using DCS in awake surgery with IEM is to identify and maintain neurological function during surgery. Therefore, it is widely used in epilepsy and oncological neurosurgery.

Regarding the mapping of language associated areas, three main categories of effects can be outlined, including vocalizations (as a part of dysarthria), speech arrest and aphasia-like language impairments (22). A more detailed subdivision contains speech arrest, anarthria, speech apraxia, phonological disturbances, semantic paraphasia, perseveration anomia and syntactic errors (5). Each documented impairment allows the surgical team to determine if a cortical or subcortical area is crucial to maintain a certain function or not. There are several phenomena associated with specific brain areas. For example, speech arrest can be induced by DCS in the pars opercularis of the posterior inferior frontal gyrus, in the operculo-insular connection (5) as well as in the ventral premotor cortex (124). These phenomena and the classification of language errors are presented in chapter 2.3. While the OS stimulation inhibits language associated areas, it is important to mention that DCS can also increase excitability (5). For example, applying OS on sensory or motor areas can induce a positive effect by mimicking sensory-motor behaviour (5).

DCS is considered the gold standard in mapping functional areas of the brain (12,57,81). However, due to the phenomena mentioned above, it is important to be aware of potential limitations of DCS. There are impairments caused by the method or its application. For example, sub-threshold stimulation can lead to false negative results (5). The reasons for sub-threshold stimulation can be stimulation with an intensity lower than the threshold for eliciting an AP and cerebrospinal fluid (CSF) shunting (5). The CSF affects the DCS by shunting the current through a lower resistant area than the grey matter (5). The anatomical location of the electrode pair is involved in the conduction of a CSF-shunt (122). Another bias leading to false negative results is the inappropriate selection of the testing task (5). In fact, DCS and the surgical removal of tissue are not based on the same principle. Therefore, effects of both, temporary lesion caused by DCS and irreversible removal by resection can be different. This fact leads to a decreased specificity because of the functional compensation by long term plasticity (125).

The major side effect of DCS is the possibility of triggering a seizure. The incidence of seizures varies between 1,2% for the “train technique” and 9,5% for OS (126). Seizures can impair awake surgery by making further examination impossible. Observation of after-discharges can decrease the incidence of seizures (5). In this regard, DCS is limited by its invasive character.

Even though DCS is capable of mapping motor functions under full anaesthesia by recording MEP’s, the examination of higher cognitive functions requires an awake patient. DCS is also affected by the ability of the patient to concentrate. An empirical value of 1 to 2 hours can be defined for the awake phase in which the patient should be able to fully concentrate on and participate in the testing. If the awake phase extends beyond that duration, interpretation of DCS results can be difficult due to lack of concentration and exhaustion of the patient.

2.6. TMS

2.6.1. Development and History of TMS

Electric brain stimulation became public in 1937 with the introduction of electroconvulsive therapy (ECT) and was widely used to treat psychiatric disorders (127). In the 1970s, the application of electricity to the brain was primarily used for diagnostic purposes, e.g. the measurement of motor conduction time by transcranial electric stimulation (127). The first study using transcranial magnetic stimulation was published in 1985 by Anthony Barker. He showed that application of brief magnetic pulses to the motor cortex area of an intact skull can evoke motor responses on the targeted muscle (128). Later on, TMS was also used to induce speech arrest and counting errors (129). These studies are nowadays regarded as pioneering studies in TMS language mapping.

Beside the diagnostic application, TMS was also used as a method to treat major depression (130). However, as the popularity of TMS began to rise and the method became more established, concerns regarding safety of the procedure emerged and led to the publication of guidelines published in 1998 (130). At a consensus conference in 2008, these guidelines were reviewed and are to be followed as standard in every application and protocol (131). In these guidelines, intensity, frequency, duration and stimulation pattern were defined as key parameters of TMS (131).

TMS can influence neuronal function by disrupting or enhancing neural computation (14) and modulating cortical excitability (127). It has a large field of application such as therapeutic management of several neurological and psychiatric disorders including depression, acute mania, bipolar disorders, panic disorders, Parkinson’s disease, Alzheimer’s disease, hallucinations, catatonia, post-traumatic stress disorders, drug craving, chronic pain and migraine as well as enhancing recovery in stroke patients (131). It also is applied in basic research focusing on neuronal function and diagnostics in clinical practice (13).

In general, an increase in preoperative functional information can reduce the risk of surgical treatment in glioma patients and is a general clinical benefit. Although TMS was shown to be safe for mapping language eloquent areas in tumour patients, further work is needed to improve its precision and reliability (8,132). This leads to an enhanced preoperative evaluation and an accurate risk assessment. A more precise knowledge of the functional cortical areas prior to surgical treatment allows a smaller craniotomy and a reduction of operation time (8). Furthermore, a recent study suggests a lower percentage of speech deficits in postoperative process and a higher Karnofsky index (performance score for oncological patients) (8).

Due to the numerous possible applications of TMS, it is of high interest for research purposes. A wide variety of studies and literature exists today dealing with TMS. Several studies used TMS to investigate and map functional regions in the cerebral cortex such as the motor cortex (128) and the visual cortex (133,134). However, mapping of language eloquent areas is not yet fully established.

For this purpose, the current state of knowledge will be further explained with regards to studies on healthy participants as well as brain tumour patients. Currently, there is still a gap in knowledge about the basic physiological mechanisms of TMS (14,127). A universally valid assumption is that TMS evokes a transient virtual inhibitory lesion of cortical cells by disrupting information processing of specific cortical regions (12,14,15). It was also shown that depending on the parameters and stimulated area, TMS is able to cause facilitation or increased excitability as well (25,135,136). In clinical use, this enables nTMS to be a useful tool in mapping functional regions like language eloquent areas; however the accuracy of these maps is still below the level of DCS (5,12,81). Areas are considered language eloquent if a lesion within this area causes a permanent speech deficit (57). Several studies have confirmed the safety of TMS as a diagnostic approach in brain tumour patients (8,132). As previously mentioned, there is also evidence of the clinical benefit for these patients (8). However, there is still a need for further research to establish nTMS as a standard method in mapping language eloquent areas in the clinical setting.

For instance, an issue about the accuracy in different nTMS-protocols was discussed by Krieg et al. (55). Therefore, there is a need to further clarify the procedure used in mapping particularly of the areas at the vicinity of cerebral lesions. Additionally, a standardized protocol to improve the mapping results of language eloquent areas must be established (8). Brückner et al. (15) suggested that cTBS could replace the conventional TMS paradigm for this purpose.

2.6.2. Theoretical basis of TMS

TMS is a non-invasive method of stimulating neural tissue. The technique can influence sensory-motor and cognitive functions by modifying neural excitability via depolarization of neurons (13). The physical principle of TMS is based on Faraday's law of electromagnetic induction (13,137,138).

In TMS an electric current in a conducting material is induced by a changing magnetic field. The secondary magnetic field is produced by fast changing electric fields in fast discharging capacitors (135). The electric field produced by TMS is applied to the brain using a coil and conducting materials (131,135). In case of TMS, the conducting materials are the neural elements (127), especially the cerebral cortex, spinal roots, as well as cranial- and peripheral nerves (13). The stimulated cortical neurons cover an area of approximately 3 cm^2 and a depth of 2 cm (135). It can be assumed that the size of the influenced area depends on the intensity and changing rate of the current in the coil (83,131). This contrasts with DCS, where the influenced area is comparatively robust in comparison to TMS. The higher the TMS intensity, the bigger the influenced area and the higher the degree of cortical function disruption (13). In DCS, the area remains roughly the same, while the number of influenced neurons within this area increases with higher intensities (5).

Another factor which influences the extent of the stimulated area is the shape of the coil. There are three main shapes: a figure-of-eight coil, a double-cone coil and a circular coil. The figure-of-eight shape provides a more focused magnetic field and is therefore more focal than circular coils (83,135). The shape of the electric field for a circular as well as for a figure-of-eight coil can be seen in figure 11. The double-cone coil is used to stimulate deeper cortical areas (131).

The current can pass the tissue in between the surface and the cerebral cortex without significant loss (13,25,139). Yet, studies are missing which evaluate possible influences of bone density and thickness. Beside the shape of the coil, there are four fundamental parameters of TMS: intensity, frequency, train duration and inter-train interval (13). These parameters determine the effects caused by TMS. On the cellular level, the immediate effects can be depolarization or hyperpolarization (131). These effects also depend on the stimulated site, so to ensure precise anatomical localization of these sites, a frameless stereotactic system can be used (13). Furthermore, the position and angle of the coil in a spatial link to the cortical surface is another important factor. Therefore, according to MEP results, the effect is most effective when the current is directed in a posterior- anterior direction and perpendicular orientation to the leading sulcus (83). The first application of TMS mapping was mostly done in motor areas. Figure 12 shows theoretical mechanism of TMS in the production of a MEP. The precise physiological mechanisms are however unknown (127). Presumably the same mechanisms are applicable to other parts of the brain (83), but further studies are still needed.

To determine the correct intensity, the motor threshold is used (25). The motor threshold is the lowest stimulus threshold needed to trigger MEPs in a muscle (13). It is defined as the intensity which elicits MEPs in 50% out of 10 to 20 stimuli (139). The motor threshold represents the membrane excitability of corticospinal neurons and interneurons (13,83).

In reference to the threshold two main categories of stimulation can be defined. First, sub-threshold stimulation, which only excites interneurons and therefore does not elicit a MEP; second, supra-threshold stimulation which directly effects cortical neurons (13). In particular, TMS preferentially affects excitatory populations of neurons (140,141). The depth of the affected area is limited by the TMS intensity in comparison to the threshold limits. Therefore, a stimulation below 120% threshold is not able to directly stimulate neurons deeper than 2 cm beneath the scalp (131,142). In clinical practice, two types of motor thresholds are important: Resting Motor Threshold (RMT) and Active Motor Threshold (AMT). The RMT is measured in a fully relaxed target muscle, while AMT is determined by an isometric muscle contraction of about 20% of the maximal strength (25,139). The RMT is the basic unit for defining the intensity of TMS (143). The AMT is suspected to be approximately 25% lower than the RMT (83). This phenomenon appears because motor neurons work on a higher level of activity and therefore less intensity is needed to provoke an increase of activation (83). It is recommended to always measure both to properly assess the excitability (139). Furthermore, the thresholds are physiologically variable (13,139) and therefore should be determined at the beginning of any TMS-session.

Two methods are available to monitor the muscle response. First the use of an electromyogram (EMG), second the visual observation of muscle twitch (5,144). As the risk of seizure and discomfort rises with increased TMS intensity, the EMG method should be preferred, because it provides a more precise assessment of the muscle response and therefore leads to a lower TMS intensity (144). Using the visual method to assess muscle responses, the measured threshold will be approximately 11,3% higher than the threshold determined by EMG monitoring (144).

TMS studies are most likely to use intensities determined by the measurement of motor thresholds (7,15,25,56,131). The threshold for motor neurons cannot be necessarily adopted to non-motor areas, especially to areas serving higher cognitive functions (121,145–147). The method for measuring motor thresholds might be widely used because of its objectivity and practicability.

An alternative for determining the intensity according to the motor threshold is using the phosphene threshold (PT). The PT is defined as the minimal TMS intensity to elicit phosphenes by stimulating the visual cortex (13,131,147). Phosphenes are visual light phenomena occurring without an adequate stimulus of the visual organ (27). As most of the studies on higher cognitive function like language use motor thresholds to define the TMS intensity, lack of knowledge about the applicability of the PT exists. It is assumed that a correlation between motor and phosphene threshold exists (147). Further studies are needed to investigate the reliability of PT in the use on TMS for influencing higher cognitive functions. Furthermore, in using the same intensity in different cortical areas, the varying distance between the coil and brain is neglected (131).

Beside the intensity, the TMS paradigm is another factor for determining stimulation effects. There is a high variability to define TMS paradigms due to the high number of parameters. The primary classification is based on the pattern of applied pulses/stimuli. The main TMS paradigm includes single, paired and trains of repetitive pulses (83,130,146). For paired pulse and repetitive TMS (rTMS), the inter-pulse interval, frequency, number of pulses or trains as well as inter-train interval must be defined.

Another parameter is the pulse waveform which can either be monophasic or biphasic. Biphasic TMS pulse is more effective due to a double current direction which causes an increase of the initial current (25). In contrast, a monophasic TMS pulse produces only one initial current which is longer than the biphasic pulse (135), as can be seen in figure 13. The use of either monophasic or biphasic TMS influences the effects and results of the examination (136). In single pulse TMS, a biphasic waveform is more effective (136) and powerful (25) because of the second phase current enhancement. Nonetheless, biphasic waveforms are often used in repetitive TMS paradigms (25,131,136). For biphasic waveform TMS, the RMT is lower than for monophasic TMS (136). Therefore, side effects are more likely to appear in monophasic stimulation because of higher intensity (131,136). Monophasic rTMS is more appropriate for producing cortical effects (131,136).

Monophasic TMS stimulates one population of neurons, whereas biphasic TMS also stimulates inhibitory neurons simultaneously and the neural pattern influenced by biphasic waveform is more complex than the pattern of monophasic TMS (144).

In summary, monophasic TMS might be more effective in eliciting changes of cortical excitability in rTMS (131,136). However, most of the rTMS studies use biphasic waveforms. At present discrepancy in research still exists between theoretical knowledge and clinical application. The more complex activation pattern of biphasic waveforms might be an advantage for stimulating areas of higher cognitive functions because these networks are more complex in comparison to motor areas. However, most of the findings are based on studies using MEP measurement. As mentioned previously, the findings cannot be applied directly to non-motor areas. Therefore, there is a need to assess the influence of different waveforms on non-motor areas (131,136).

Regarding frequency, TMS can be applied with low and high frequency stimulation. Low frequencies are defined as 1 Hz or less (131,135). Impairment of cortical function increases with higher frequencies (13,25). Frequencies higher than 5 Hz are also able to cause facilitation and an increase of cortical excitability (13,83). When comparing low and high frequency rTMS, immediate and outlasting effects in cortical excitability must be differentiated (13). As previously mentioned, the disruption of cortical function as an immediate effect increases with higher frequencies. Therefore, high frequency stimulation can produce a transient cortical functional lesion in the targeted area (135). For the long lasting effects, low frequencies cause inhibitory effects while high frequencies also influence excitatory circuits (134). Though the mechanisms behind long lasting effects are unclear, they might be due to gene induction and modulation of neurotransmitters (13). Regarding safety concerns it is noteworthy that the risk of seizures increases with higher frequencies of rTMS (83,131).

The indication of TMS determines the paradigm used, as both diagnostic and therapeutic applications exist. Furthermore, TMS also provides prognostic possibilities, for example in functional motor recovery of traumatic brain injury-, multiple sclerosis- and stroke patients (13). TMS is also used in basic research to investigate functional brain areas and their connections. As a diagnostic method, TMS is a useful tool for mapping functional cortical areas in the context of pre-surgical planning (14). Additionally, TMS is usable for diagnostic analysis of multiple sclerosis, Bell's palsy, psychogenic paresis and plexus neuropathy (13). Furthermore, TMS can be used for seizure suppression in pharmacologically intractable epilepsy patients (148).

As mentioned before, repeated sessions of rTMS lead to cognitive changes and cumulative effects (131), as well as long lasting influences on synaptic strength (83,146). Changes in synaptic strength can lead to secondary anatomical changes, for example alterations in dendritic spines (83). Therefore, TMS can induce plasticity in cortical areas which explains the beneficial effects of TMS in recovery of stroke patients (13,25,83,131). TMS can also influence the pathophysiological increase or decrease in cortical activity (13) which is another explanation for its effectiveness in the treatment of psychiatric and neurological disorders. These effects lead to measurable behavioural changes (13) and influence disorders like depression, acute mania, bipolar disorders and panic disorders (25,57). As an example, TMS can increase inhibition in the primary motor cortex in patients suffering from dystonia and therefore cause an improvement in movement performance (83). Furthermore, TMS is also used in the treatment of Parkinson's disease, chronic neurogenic pain and recovery after stroke (13,57,83).

Despite being considered a safe method, a critical risk assessment is crucial for the use of TMS as it may be associated with side effects and risks. These include seizure induction, hypomania induction, syncope, headache, local pain, transient hearing changes, transient cognitive and neuropsychological changes, scalp burns, induced currents in electrical circuits, structural brain changes and histotoxicity (131). The intensity of side effects depends on the TMS paradigm (131). For each paradigm, the risk of the previously mentioned side effects can be seen in table 3. The most common side effect is headache, reported in 5% (135) to 8,9% of patients (148). It is caused by local muscle contraction and stimulation of nerves, like the trigeminal nerve (131,135). The risk also depends on the stimulated area. Frontal and temporal regions are more likely to be associated with headache and discomfort than parietal and occipital regions (131). The risk of inducing seizures is mainly attributed to the rTMS frequency with high frequency rTMS more likely to induce seizure (135). The probability to induce a seizure event varies from 1,4% (131) to 2,9% (148). Whether patients with a seizure disorder are at a higher risk or not is debatable (135,148). Some studies suggest a combination of electroencephalogram (EEG) and TMS for monitoring early seizure activity to minimize the risk (135,146). This is not described in the guidelines for TMS application, because epileptiform EEG activity is a rare event in conventional rTMS (131). However, a simultaneously applied EEG monitoring can help to increase safety in new paradigms. Contraindications are rare, the only absolute contraindication is the presence of metal (iron-containing) hardware like implanted devices (131).

Summing up and giving an outlook on future applications of TMS, it can be said that TMS provides a unique opportunity to investigate cognitive functions and their anatomical relation. TMS gives a direct insight into cortical excitability, inhibition, plasticity and connectivity (135). It is to be expected, that the use of TMS in its wide range of applications will increase in the next years.

2.6.3. Hypothesis of primed cTBS TMS

The focus of this thesis was the detection and mapping of language eloquent areas by navigated transcranial magnetic stimulation (TMS) in patients suffering from brain tumours. The primary aim was to find a high correlation of results from two mapping techniques: TMS before surgery and DCS mapping during awake surgery. Our team explored the reliability and accuracy of a specific TMS paradigm called continuous theta burst stimulation (cTBS), particularly its clinical potential as a routine diagnostic tool. For cTBS, a “priming” paradigm (application of another stimulation, prior to the real stimulation condition) was applied in one of the sessions. Comparisons were made on the findings of the primed and unprimed cTBS sessions. The hypotheses are as follows:

1. Both paradigms (primed vs. unprimed cTBS session) result in clear detection of language-associated areas. However, primed TMS induces more inhibition of language-associated areas than unprimed cTBS.

2. The results from the unprimed cTBS show higher correlation to the DCS results than primed cTBS.

These hypotheses are based on the concept of neuronal plasticity (as induced by cTBS). Plasticity can be defined as the ability of the nervous system to adapt functionally and structurally to changing environmental or intrinsic demands (137). This process is involved not only during the normal brain development, but also in compensating the effect of an injury to the brain and the loss of functional areas by recruiting other pathways and neuronal circuits (137). The foundation of plasticity mechanisms is based on Hebb’s rule: it describes the mechanisms by which neurons adapt to changing activity states (149,150). The mechanisms of plasticity are diverse and might depend on the system being investigated. It is a complex process observed from cellular to neuronal, synaptic, systemic and cortical levels (140). For each level, various physiological processes influence induction of plasticity, e.g. post synaptic potential amplitudes (151), calcium level dependent plasticity (CaDP) and the effects of N-methyl-D-aspartate-receptor (NMDAR) activity (140,152).

These physiological processes influence plasticity and lead to functional changes like strengthening or weakening of already existing synaptic connections, as well as structural changes such as pruning or formation of new synapses (137).

For functional synaptic plasticity, the two most studied types are long-term potentiation (LTP) and long-term depression (LTD) (153). LTP was described as a persistent increase in synaptic strength, while LTD is a long-lasting decrease of synaptic efficacy (154). Both mechanisms influence synaptic strength from a period of seconds to hours and even days, depending on the number of stimuli (140). The plasticity direction is determined by the rTMS frequency (140). With regard to rTMS, less than 20 pulses induce a short-term plasticity that lasts only seconds, while significantly more pulses will cause LTP or LDP for minutes or hours (140). The after-effect mainly depends on the pattern of stimulation during plasticity induction (140). Recent research discovered the effects of applying magnetic stimulation in burst at theta frequency; two paradigms were established: continuous Theta Burst Stimulation (cTBS) and intermittent Theta Burst stimulation (iTBS). Even though the allocation of a specific paradigm to a certain effect is controversial, cTBS is more likely to cause LTD while iTBS effects are more likely to lead to LTP (137,140,155–157). However, some studies also observed a reversal of the plasticity direction when the duration of the stimulation was doubled (140). In fact, they suggested that cTBS initially induces a potentiation effect, which turns into LTD after 80 seconds (140). Furthermore, the after-effects of rTMS are found to be NMDAR dependent, since blockage of this receptors with an antagonist abolished the LTP and LTD-like effect (152). The NMDAR plays an important role in regulating the Ca^{2+} homeostasis of neurons and directly influences plasticity by Ca^{2+} influx (140,152).

Another important aspect of plasticity, which is crucial in understanding the underlying principle of brain stimulation, is homeostasis. Homeostatic plasticity refers to the ability of the brain to limit plasticity to just an optimal level; this is achieved through the capacity of the neuronal networks to adjust or rescale the threshold of plasticity induction (137). This is important in balancing effects of increasing and decreasing synaptic transmission and crucial for a normal function of neuronal systems (137).

Several homeostatic effects exist to stabilize plasticity which serve as negative feedback mechanisms (140). Aberrant homeostasis can be observed in brain tumours where too many and uncontrollable anatomic and functional changes take place. Additionally, brain states characterized by hyper-excitability such as in epilepsy also show uncontrolled unbalanced homeostasis (140).

The concept of homeostasis is partly related to the Bienenstock-Cooper-Munro theory (BCM-theory) developed in 1982. The theory describes a change in synaptic transmission due to the different patterns of stimuli (158). Additionally, recent studies have shown that the prior history of synaptic activity also influences the synapse's susceptibility to LTP or LTD induction (159). In other words, plasticity induction is dependent on the activity-dependent synaptic state, a property that is called meta-plasticity or plasticity of plasticity (159). Therefore, the concepts of homeostasis and meta-plasticity are partly explored in the present study. By performing a priming paradigm (homeostasis), we tested whether the BCM theory still holds true for neuronal structures affected by tumours. Theoretically, priming can also reset the neuronal network to the upcoming real stimulation condition, thereby testing meta-plasticity at the same time.

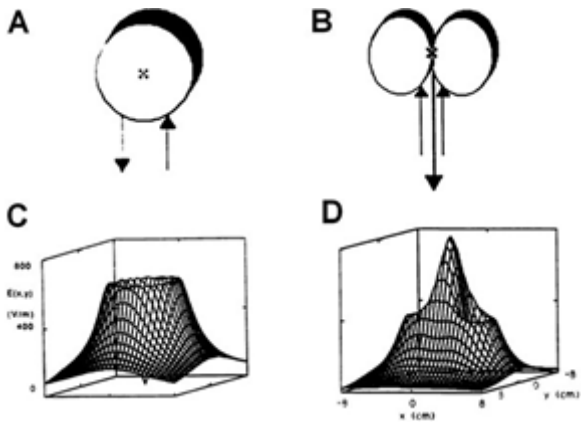

Figure 11 Electric field of circular (C) and figure-of-eight coil (D) (83)

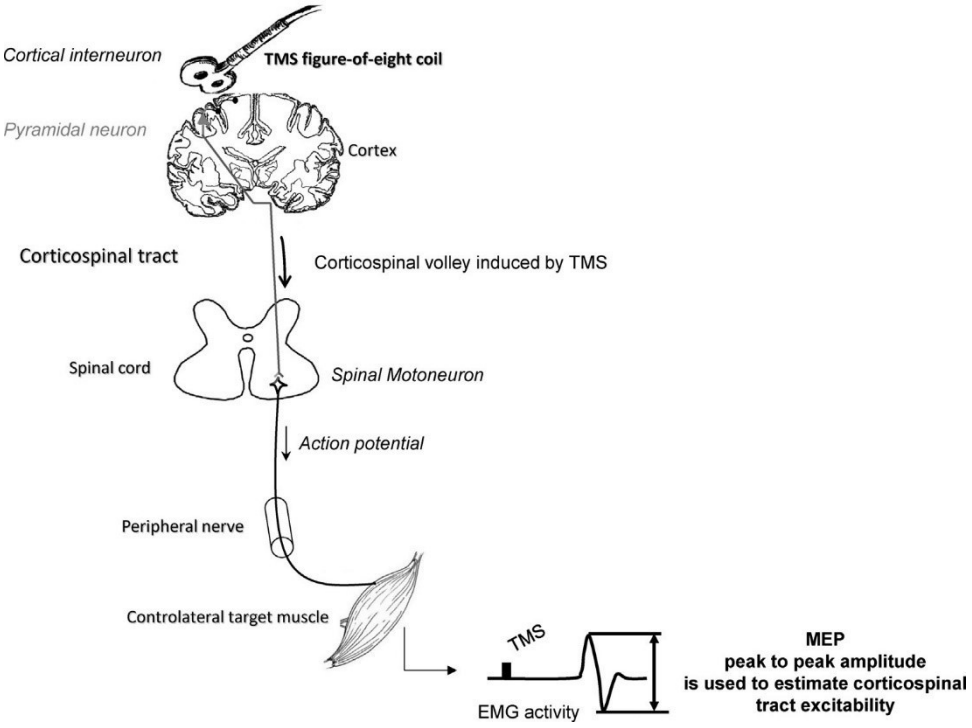

Figure 12 Simplified diagram of the effect of TMS on the motor cortex (146)

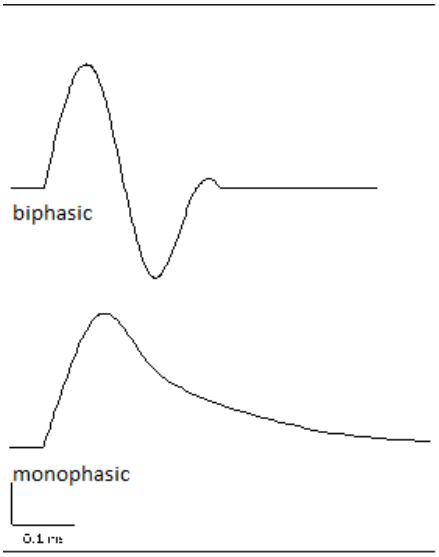

Figure 13 Time course of electrical current in bi- and monophasic wave forms (135)

Side effect	Single-pulse TMS	Paired-pulse TMS	Low frequency rTMS	High frequency rTMS	Theta burst
Seizure induction	Rare	Not reported	Rare (usually protective effect)	Possible (1.4% crude risk estimate in epileptic patients; less than 1% in normals)	Possible (one seizure in a normal subject during cTBS) (see para 3.3.3)
Transient acute hypomania induction	No	No	Rare	Possible following left prefrontal stimulation	Not reported
Syncope	Possible as epiphenomenon (i.e., not related to direct brain effect)				Possible
Transient headache, local pain, neck pain, toothache, paresthesia	Possible	Likely possible, but not reported/ addressed	Frequent (see para. 3.3)	Frequent (see para. 3.3)	Possible
Transient hearing changes	Possible	Likely possible, but not reported	Possible	Possible	Not reported
Transient cognitive/ neuropsychologial changes	Not reported	No reported	Overall negligible (see Section 4.6)	Overall negligible (see Section 4.6)	Transient impairment of working memory
Burns from scalp electrodes	No	No	Not reported	Occasionally reported	Not reported, but likely possible
Induced currents in electrical circuits	Theoretically possible, but described malfunction only if TMS is delivered in close proximity with the elec brain				tric device (pace-makers,
Structural brain changes	Not reported	Nor reported	Inconsistent	Inconsistent	Not reported
Histotoxicity	No	No	Inconsistent	Inconsistent	Not reported

Table 3 Side effects observed in various TMS paradigms (131)

3. Methods

3.1. Transcranial magnetic Stimulation

For TMS, a MagPro X100 magnetic stimulator (MagVenture, Denmark) was used with a figure-of-eight coil (MCF B65). The stimulator was combined with a Localite TMS Navigator (Localite GmbH, Germany), connected to a Polaris Spectra infrared tracking camera (NDI, Canada), to provide a precise localization of the coil within the targeted areas. A structural MRI was used as the basis for the navigation (as described in section 3.3).

The TMS targets were determined by anatomical landmarks and areas deemed to be relevant for language. These areas include the opercular region including the inferior frontal, angular and supramarginal gyrus. Furthermore, the hotspots detected by fMRI were also considered as targets for TMS. In addition, targets were put in the vicinity to the tumour to cover the area which was then mapped by DCS.

The coil was placed tangentially to the scalp at an angle of 45 degrees to the midsagittal plane. The angle was defined previously, orienting the target in a perpendicular direction to the referring sulcus.

Motor thresholds were determined by observing the response of the abductor pollicis brevis (APB) muscle on a MEP monitor. For that purpose, the electrodes were placed as follows: the active electrode over the APB muscle, the ground electrode on the dorsal side of the wrist and the reference electrode on the thumb base joint. The MEP monitor was set to a sensitivity of 200µ/div (scaling of the MEP screen). For determining the motor threshold, the default mode of the stimulator with single pulse application was used. A target area which elicited the biggest MEP response within the precentral gyrus was used to determine both the RMT and AMT. The patient was asked to relax his hand and arm completely, while determining RMT. For defining the AMT, the patient pressed the index finger against the thumb with moderate intensity.

TMS intensity was set to 50% of the maximum stimulator output (MSO), then gradually increased when no motor response was observed. When a clear response was noticed, the intensity was slowly decreased until it reached a level when a MEP was observed in 5 out of 10 stimulations. The intensity that fits the prior definition was set as AMT/RMT. For a successful cTBS, 90% of the AMT intensity was used as the majority of TMS studies applied a similar percentage of the AMT as well (15,138,156,157,160,161). If the patient could not tolerate the intensity, it was decreased in 10% steps until an acceptable intensity was found.

After determining the motor threshold, the tongue area on the precentral gyrus was stimulated with cTBS while the patient performed a counting paradigm. Theta burst frequencies were changed from 5 Hz to 6 Hz and 7 Hz. The frequency which elicited the clearest effect (e.g. disruption or heavy feeling) on the tongue was used for the entire following procedure. The cTBS was set to the parameters noted in table 8 of the case report.

Each target was stimulated continuously while 3 pictures from the language task were shown. Adjusted to the frequency, each target was stimulated between 7 and 10 seconds during the task. After the procedure was applied to all targets, positive targets were stimulated again in non-perpendicular orientation.

In a second session, the procedure remained the same, but priming was added prior to the language task. Due to physiological daily variations, the motor threshold was defined again. Priming was defined as a stimulation using the same cTBS paradigm 15 minutes before the actual task was performed. However, the language task was not performed during the priming. The targets assumed to be accessible within the craniotomy were exported from the Localite Navigator and imported into the StealthStation neuronavigational system (Medtronic, USA) in the operating room.

3.2 Language task

A picture-naming task was used for both the TMS sessions and DCS mapping. The task consists of 54 pictures from the Boston Naming Test (162); exemplary pictures can be seen in figure 14. The pictures are presented in a random sequence using a presentation program (Neurobehavioral Systems, NBS) on a common 15” Laptop screen. The program was operated by an experienced neuropsychologist. The distance between the screen and the patient’s head was individually determined by considering comfort, as well as subjective feeling of having a good view. Additionally, it depended on the intraoperative setting with respect to the specific patient position on the operating table. A baseline naming task was done before the TMS examination or the DCS procedure during awake surgery. Objects that could not be named or identified correctly by the patient were discarded. Every part of the procedure, including the baseline naming task, was recorded on video for offline analysis. When naming the object, the patients had to use the phrase “this is a” before the object term (e.g. “this is a house”) in their native language, which was German in this case.

The picture presentation time (PPT) was set to three seconds. Pictures were presented in a randomized order to avoid a learning effects (12). The picture to trigger interval (PTI) was 0 ms because the specificity of TMS is higher at 0 ms than with a delayed PTI (55). Three pictures were shown in a sequence and after a sequence, the stimulation proceeded on the next target.

During surgery, DCS was performed by the surgeon who synchronized the stimulation with the language task through an audio signal presented simultaneously with the appearance of the picture. The display time was variable and depended on the patient’s response time and intraoperative modalities. Every stimulation site was tested three times by repeating stimulations over the exposed cortex in a grid-like manner. A site with positive responses in at least two out of three stimulations was considered positive.

For each stimulated site, the performance of the patient was documented as follows: A - no response (speech arrest); B - hesitation; C - circumlocution; D - semantic paraphasia; E - phonological paraphasia; F - neologism; G - normal response. For each response, which deviated from category G, a short note was taken for detailed information and offline analysis.

3.3 MRI

A T1 weighted 3D MPRAGE MRI sequence performed on a MAGNETOM Prisma 3.0 Tesla scanner (Siemens Healthcare, Germany) was used for TMS planning. The parameters of the T1 sequence can be seen in table 4. The images of this examination were also used as a reference for the fMRI. A single shot gradient-echo EPI sequence was used for the fMRI examination itself. During the fMRI sequence, several language tests were performed, including a silent object naming task (similar to the test conducted during TMS and DCS), a silent sentence generation task and simple motor tasks for tongue, hand and toes.

Table 4 Parameters of MRI T1 navigation sequence

Repetition time	1900 ms
Echo time	2.2 ms
Inversion time	900 ms
Flip angle	9 degrees
Number of slices	176
Acquisition time	3:25 minutes
Matrix size	256 x 256

3.4 Awake surgery

At the Department of Neurosurgery of the Medical University of Graz (MUG), a modified asleep-awake-asleep paradigm for awake surgery is used. An overview of the operating room setting can be seen in figures 13 and 14. At the beginning the patient is kept under light sedo-analgesia for initial positioning, placement of various catheters as well as the eletrodes for the neuromonitoring, and application of peripheral nerve blocks to the scalp areas of interest (incision, head holder pins). The patient is then woken up to fix the head in the Mayfield clamp and for optimal final positioning of the head and body. The patient was then kept again under sedoanalgesia until craniotomy is completed. Neuromonitoring consisted of a free running EMG of the following muscles: M. orbicularis oris, M. extensor digitorum, M. abductor pollicis brevis, M. tibialis anterior und M. abductor hallucis longus. After dural opening and exposing of the cerebral surface, DCS was started while the patient was still asleep. Stimulation thresholds were determined via after-discharges using a strip electrode and bipolar stimulationwith the ISIS IOM system (Inomed Medizintechnik, Germany). DCS was started at 2 mA and slowly increased until an after-discharge or a positive stimulation of the motor cortex were achieved. The constant current, applied through a bipolar electrode with a 5 mm interelectrode distance, was delivered at 1.0 msec monophasic square waves at 50 Hz using the predefined intensity.

The patient was then woken up again for the neuropsychological testing. Cortical mapping was performed using the object naming task described in chapter 3.2. Each site was stimulated 3 times for 3 sec maximum and marked positive when 2 out of 3 stimulations elicited significant language error. Whenever possible, TMS targets were verified by DCS directly. If intraoperative modalities did not allow a direct stimulation of the TMS target, both mappings were compared postoperatively.

3.5 Planned TMS study

The study aimed to examine a total of 12 patients. The case report presented here describes the first patient included in the study. The study was approved by the ethics committee of the MUG under the reference 28-144 ex 15/16. Every patient with a suspected left hemispheric tumour and who was scheduled for an awake surgery was a potential candidate for the study. For inclusion informed consent had to be obtained. Two sessions of TMS were added between the fMRI and the surgical procedure, starting at least 2 weeks prior to surgery. The inclusion criteria were: left hemispheric brain tumour in the vicinity of language associated areas (regardless of tumour grade and classification), right handedness, and voluntary participation. The exclusion criteria were as follows neurologic, psychologic or psychiatric disorders (e.g. epilepsy or schizophrenia), metal or electronic implants in the head or neck area, frequent headache or tinnitus, and pregnancy.

4. Case report

4.1 Patient history, planning and results

This case report is an exemplary review of the diagnostic and treatment modalities of low-grade glioma patients at the department of neurosurgery of the MUG. The main emphasis will be on the integration of TMS conducted language mappings into the diagnostic and surgical processes. TMS results regarding language eloquent areas were included in the established procedures for the first time. It enables the comparison of the different functional diagnostic methods fMRI, TMS and DCS regarding correlation, sensitivity and specificity of mapping language eloquent cortical regions.

The case report portrays a patient suffering from a left frontal and precentral cerebral lesion, verified as a diffuse astrocytoma, IDH-mutated. The report includes neurological, neuropsychological, radiological and histopathological results, collected prior to and after surgical treatment. Furthermore, a review about the surgical procedure and the postoperative process will be given. It should be mentioned that fMRI, TMS and DCS results were close enough to compare them regarding the accuracy of the language mapping.

The patient is a 52 years old caucasian right-handed female, working as a teacher. The cerebral lesion was an incidental finding during work-up for sudden deafness. There were no other symptoms or neurological complaints. There was no family history of brain tumours. Physical examination and neuropsychological assessment were normal. The chronological sequence of diagnostic and therapeutic events is shown in table 5.

After initial diagnosis at another institution, the patient came to the MUG requesting a second opinion. Figure 18 shows the structural MRI of the patient in sagittal and axial views. In the FLAIR sequence a hyperintense lesion in the left frontal lobe can be seen.

DTI revealed a medially displaced arcuate fasciculus (figure 19) and the fMRI depicted functional hotspots of the tongue area cranially to the lesion (figure 20). After fMRI assessment, the patient was offered an awake surgery for functional tumour resection.

Special diagnostic challenges can emerge in the work-up of brain tumour patients with lesions close to language eloquent or motor areas. A precise assessment of spatial distribution regarding functional regions is a crucial part in preoperative diagnostics. The standard procedure prior to the surgery consists of a structural MRI including T1 (with and without contrast medium), T2, FLAIR and a BOLD fMRI. During the fMRI examination, several tasks are performed, including various language activating paradigms like object naming, verb generation or letter tasks. This case report marks the first time TMS was used for preoperative examination at the neurosurgical department of the MUG. The histopathological findings showed a diffuse astrocytoma with IDH-mutation, WHO grade II.

The preoperative neuropsychological examination included the following tests: Oldfield handedness questionnaire, Edinburgh handedness questionnaire, Aachner Aphasie Test, trail making test (TMT), “Regensburger Wortflüssigkeitstest”, Visual Object and Space Perception (VOSP) and Hospital Anxiety and Depression Scale (HADS- D). The patient did not notice any reduction in daily performance, mood or disposition. The patient was clearly identified as a right-handed person, with a laterality index over 100 for the right side. The results of the TMT were above average. Standardized testing for aphasia showed no significant finding (Token Test PR=100; repeating PR= 100; naming PR= 100; auditory language comprehension PR=100; reading comprehension PR= 100; writing PR=100; spontaneous speech PR= 100). Based on the “Regensburger Wortflüssigkeitstest”, phonetic word fluency was average while semantic word fluency was above average (phonetic PR = 75; semantic PR > 90). The visual-perceptive function tested by using VOSP tests appears to be in the normal range. Figural productivity and flexibility as an indicator for cognitive performance within the frontal brain was also described as normal.

The following section outlines the TMS findings. For the TMS examination, the method described in section 3.1 was applied. Procedure and relevant patient related results will be outlined in the following. Ten targets were created within the structural T1 MRI. The definition of the targets was based on three major factors: first, anatomical landmarks (e.g. the hand-knob on the precentral gyrus for the motor hand area); second, the hotspots defined by BOLD fMRI; and third, the areas close to the lesion. A list of the targets can be seen in table 6 and figure 21. Due to various possible neuronal directions, each target was defined perpendicularly and parallel to the referring sulcus. Figure 22 shows both targets, prepared to determine the motor threshold. The targets were defined by using the fMRI hotspot and the anatomical hand-knob sign of the precentral gyrus. The fMRI hotspot was created by application of a repeated pincer grasp. Target 2 was used to define the AMT, while target 1 showed no optimal response in the targeted contralateral abductor pollicis brevis muscle. In the first session without priming, AMT was defined with 34% of the stimulator output correlating to a rate of chance of current of 52 A/ms. Succeeding measurements were performed with 90% of the AMT, therefore 31% of the stimulator output (47 A/ms). Targets 3, 4 and 5 referred to the tongue area and were used to define the stimulation frequency for the subsequent language testing: stimulation of target 3 showed no effect on the performed counting paradigm; on target 4, a contraction of the jaw was noticed; stimulation of target 5 led to speech arrest with jaw contraction. Both targets were tested with a frequency of 5 and 7 Hz. The effect was more significant during the stimulation using 5 Hz, therefore a frequency of 5 Hz was chosen. The list of findings during language testing can be seen in tables 6 and 7. A detailed image showing each target is depicted in figure 23 and figure 24.

A second TMS session was performed one week later. The AMT was 36% of the stimulator output during this session, corresponding to 56 A/ms. Again, 90% of the AMT (stimulator output: 32% / 49 A/ms) were used for the ensuing examination. The theta burst frequency of 5 Hz from the first session was used again without applying a counting paradigm at the tongue area. Before the language test was performed, each target was primed with the same stimulation as would be used for language testing. The results of the language testing of the primed targets can be seen in table 6. An overview of the stimulator parameters are shown in table 8.

Both TMS sessions were well tolerated by the patient, even though periods of pain were reported in the frontal regions. The pain was in the form of muscle and tooth ache, in a severity of 3-5 out of 10 on pain scale (VAS).
A few days after the second TMS session, the patient underwent awake surgery as outlined above. In the following section, the mapping of functional areas will be described.

DCS was applied using both bipolar and strip-electrodes. After-discharges were recorded at an amperage of 3.5 mA, which was defined as the stimulation-threshold. The motor area of the right hand was clearly identified posterior and superior to the lesion. Superior and anterior to the lesion, DCS elicited slowdown in speech production, grimacing and speech arrest in certain areas as seen in figure 25b. Language errors were elicited using DCS during language testing, as described in section 3.2.

Table 9 shows an overview of the effects of the DCS mapping, linked to the location on figure 25. Reaching the subcortical areas, the arcuate fasciculus was explored with bipolar stimulation starting at 10 mA. Resection was stopped when slowing of speech, word finding difficulties, dysarthria and speech arrest could be elicited at an intensity of 5 mA.
In the days after surgery, the patient showed symptoms of dysarthria, word finding difficulties which resolved within the following weeks. At 3- month follow-up, she reported slight residual word finding difficulties only in combination with fatigue or prolonged speaking. At 6-month follow-up she had resumed her job with a slightly reduced workload and stated that she has a very good quality of life.

In summary, this case report shows an example of diagnostic and therapeutic approach for the treatment of diffuse low-grade gliomas in direct vicinity of functional areas. Special attention was paid to the preoperative integration of TMS in the work-up, which can be considered successful. Within this diagnostic set up, TMS appears suitable in filling the methodical gap between fMRI and DCS. Furthermore, a more extensive preparation can help the patient cope with the uncommon experience of the upcoming stimulation during awake surgery.

Table 5 Chronical landmarks of the case report

Date	Event
April 2015	sudden deafness MRI→ incidental finding of left frontal cerebral lesion “wait and see”
July 2016	second opinion MUG fMRI awake surgery offered as possible treatment
November 2015- October 2016	increase in volume of the lesion (from 17 to 22 cm^3)
October 2016	TMS examination
November 2016	Awake surgery
Since November 2016	Rehabilitation

Table 6 Targets and effects of the first TMS session (without priming)

target	description	Result/effect
1	Hand area/ precentral gyrus	Muscle contraction hand
2	Hand area/ precentral gyrus	Muscle contraction hand, especially contralateral APB
3	Tongue area/ precentral gyrus	No effect
4	Tongue area/ precentral gyrus	Contraction jaw and facial muscles
5	Tongue area/ precentral gyrus	Contraction jaw and facial muscles + speech arrest
6	Language associated area/ pre-motor area	No effect
7	Language associated area/ pre-motor area	No effect
8	Language associated area/ middle frontal gyrus	Incapable of opening mouth + speech arrest
9	Language associated area/ middle frontal gyrus	Phonetic error/lisp
10	Language associated area/ pre-motor area	Contraction jaw

Table 7 Targets and effects of the second TMS session (with priming)

target	description	Result/effect
1	Hand area/ precentral gyrus	Muscle contraction hand
2	Hand area/ precentral gyrus	Muscle contraction hand, especially contralateral APB
3	Tongue area/ precentral gyrus	No effect
4	Tongue area/ precentral gyrus	Contraction jaw and facial muscles
5	Tongue area/ precentral gyrus	Contraction jaw and facial muscles
6	Language associated area/ pre-motor area	No effect
7	Language associated area/ pre-motor area	No effect
8	Language associated area/ middle frontal gyrus	Contraction jaw and tongue
9	Language associated area/ middle frontal gyrus	No effect
10	Language associated area/ pre-motor area	Contraction jaw + phonological error

Table 8 Stimulator Parameters

Paradigm	cTBS
Bursts	5
Waveform	Biphasic
Burst pulses	5
Inter-pulse interval	16.5 msec = 60 Hz
Repetition rate	5 Hz, 7 Hz
Pulses in train	5
Number of trains	10
Inter-train interval	0.2 sec

Table 9 Results of the intraoperative DCS mapping (see also figure 25)

target	amperage	Description/location	Result/effect
1	3.5 mA	Post central gyrus	Hand
2	3.5 mA	Precentral gyrus	(Tongue) hand
3	3.5 mA	Precentral gyrus	Face + grimacing
4	3.5 mA	Inferior frontal gyrus	Speech arrest
5	3.5 mA	Inferior frontal gyrus	Speech arrest
6	3.5 mA	Inferior frontal gyrus	(added later) Speech arrest
7	3.5 mA	Inferior frontal gyrus	Stutter

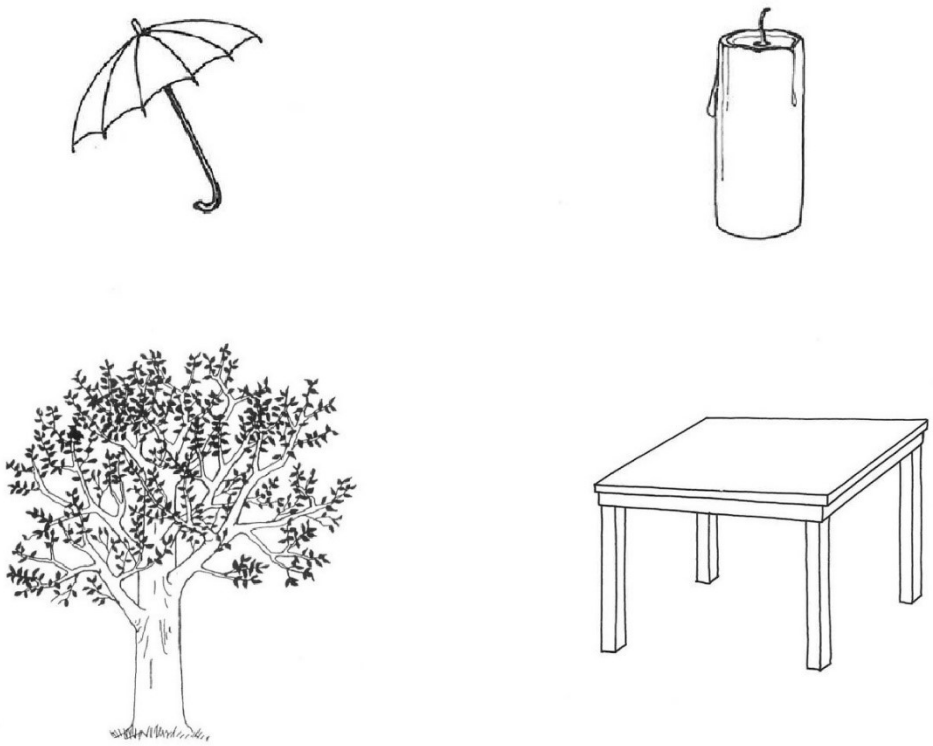

Figure 14 Picture samples used in the ONT

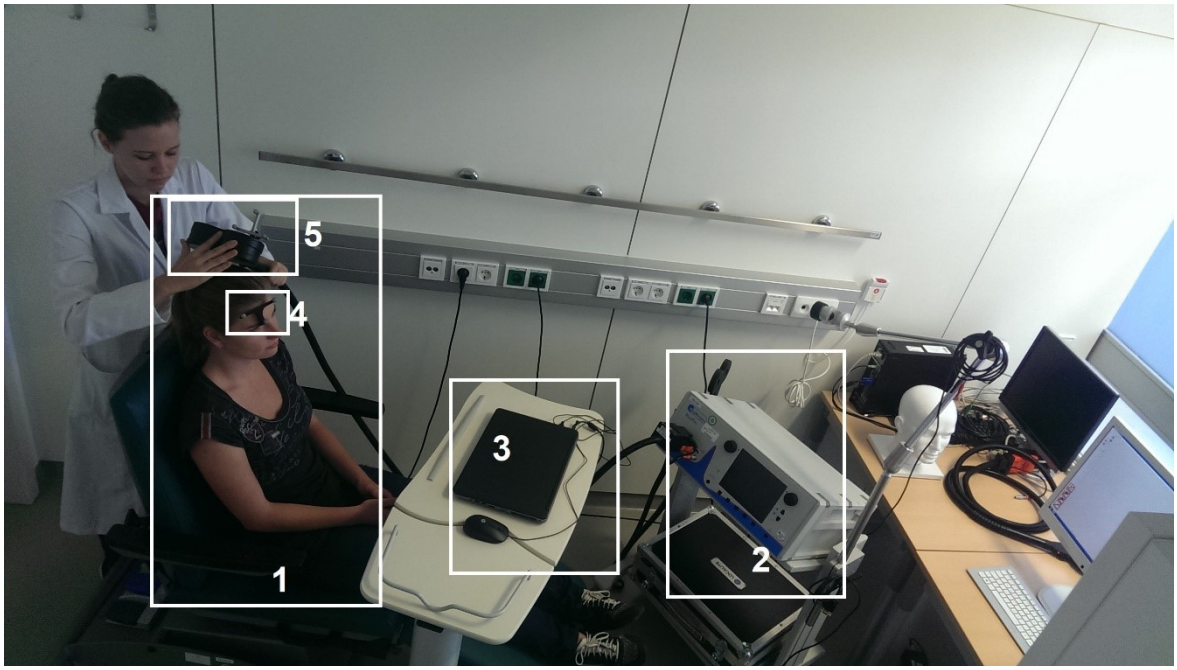

Figure 15 TMS-examination setup: 1: Patient/test subject; 2: Stimulator; 3: Laptop for language test; 4: Neuro-navigation tracking reference; 5: Figure-of-eight coil

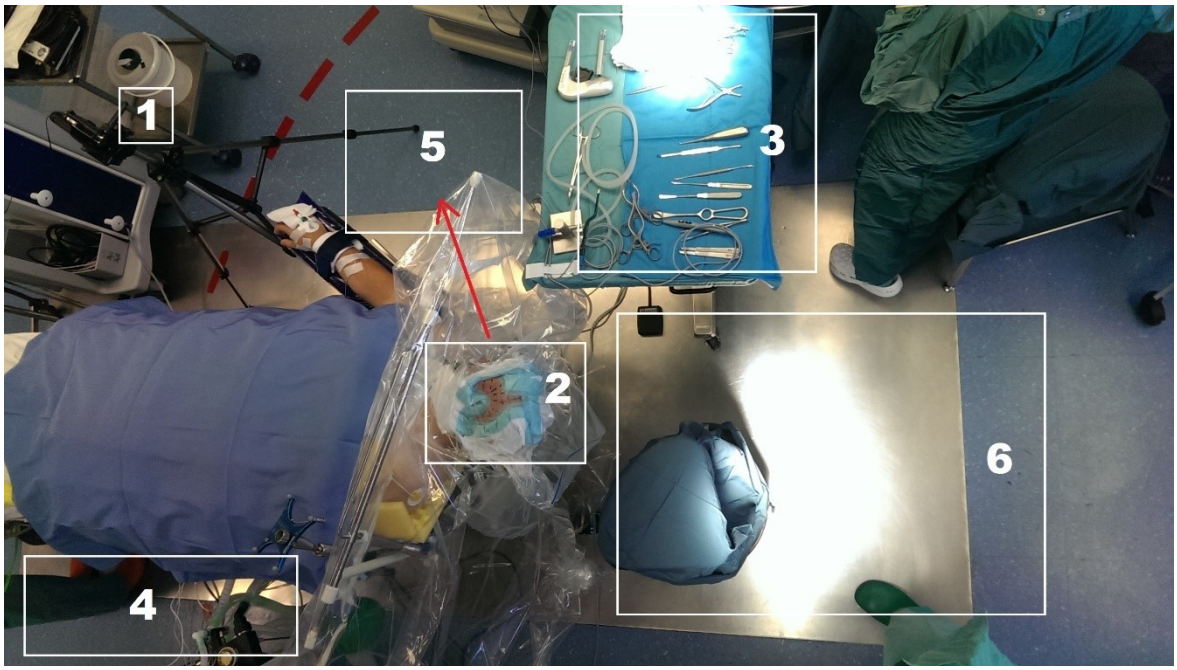

Figure 16 Operating room setup; 1: Video camera for offline analysis, 2: Patient's head/surgical area, 3: Surgical instruments, 4: Anaesthesia area, 5: Position of neuropsychologist during the awake phase, 6: Position of surgeons. Red arrow: patient's line of sight

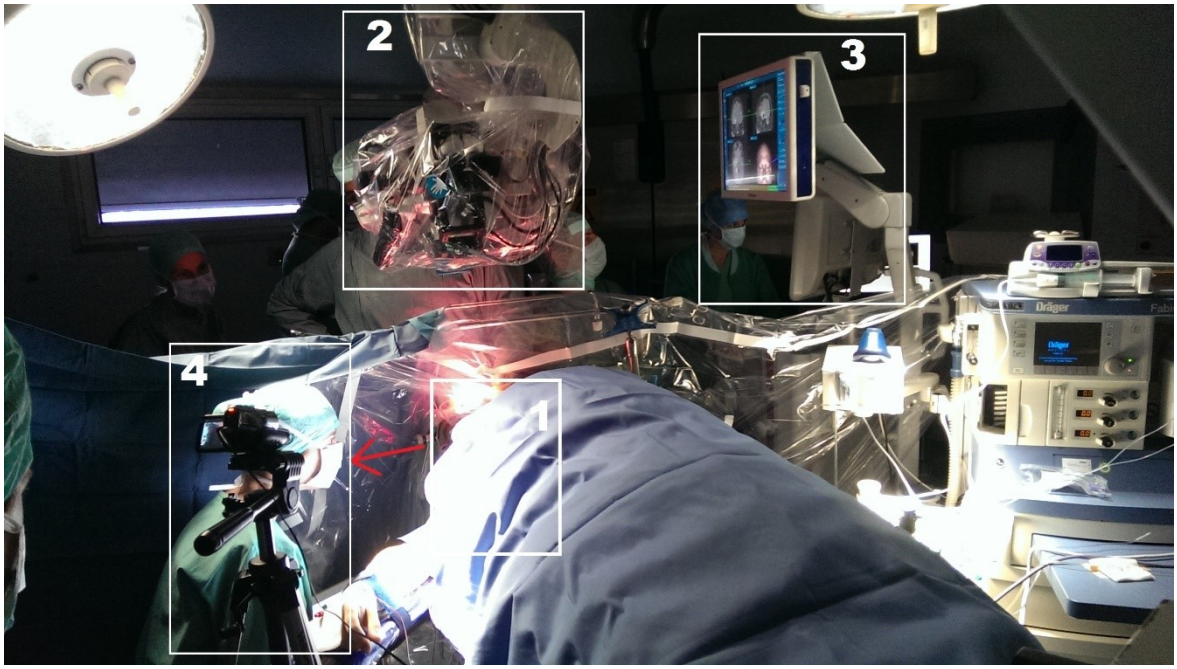

Figure 17 Operating room setup during the awake phase; 1: Patient, 2: Microscope, 3: Neuromonitoring and neuro-navigation area, 4: Neuropsychologist

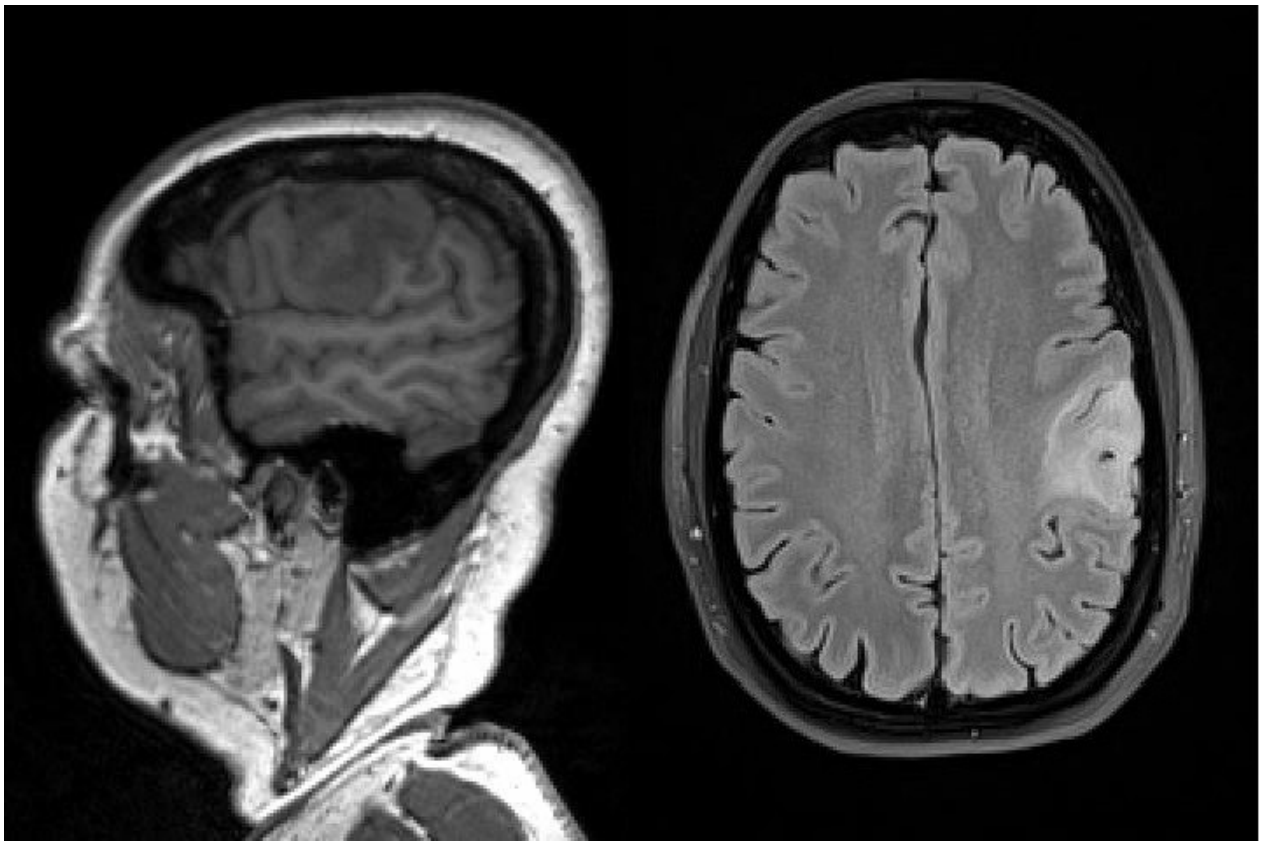

Figure 18 MRI tumour appearance, T1 & FLAIR sequences Left: Sagittal T1 sequence; Right: Axial FLAIR sequence

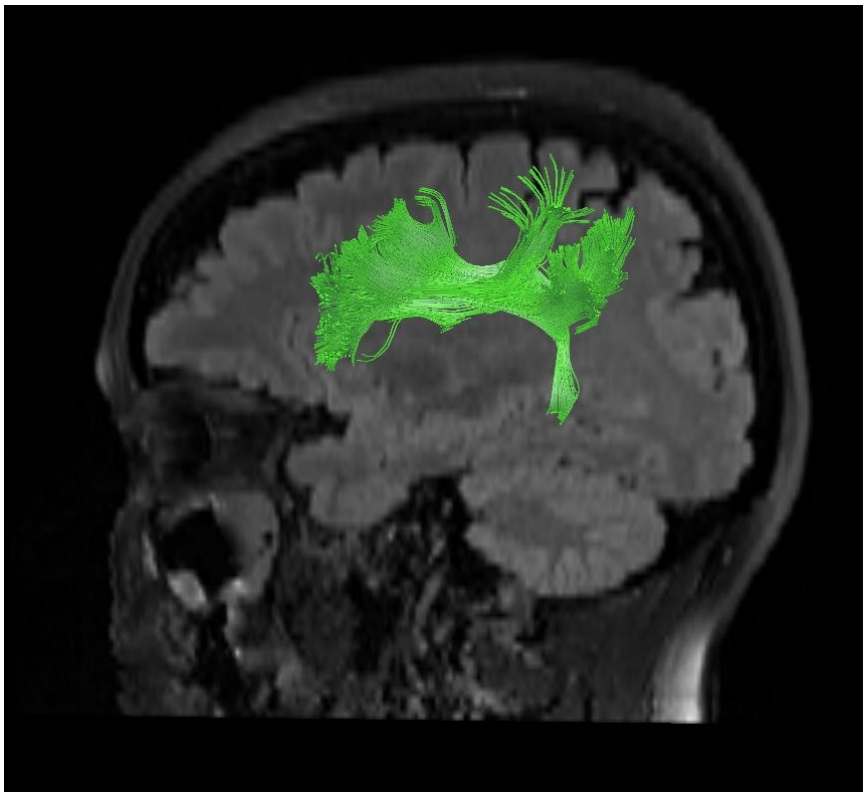

Figure 19 Sagittal T1 MRI sequence with overlaid arcuate fasciculus tractography (marked in green)

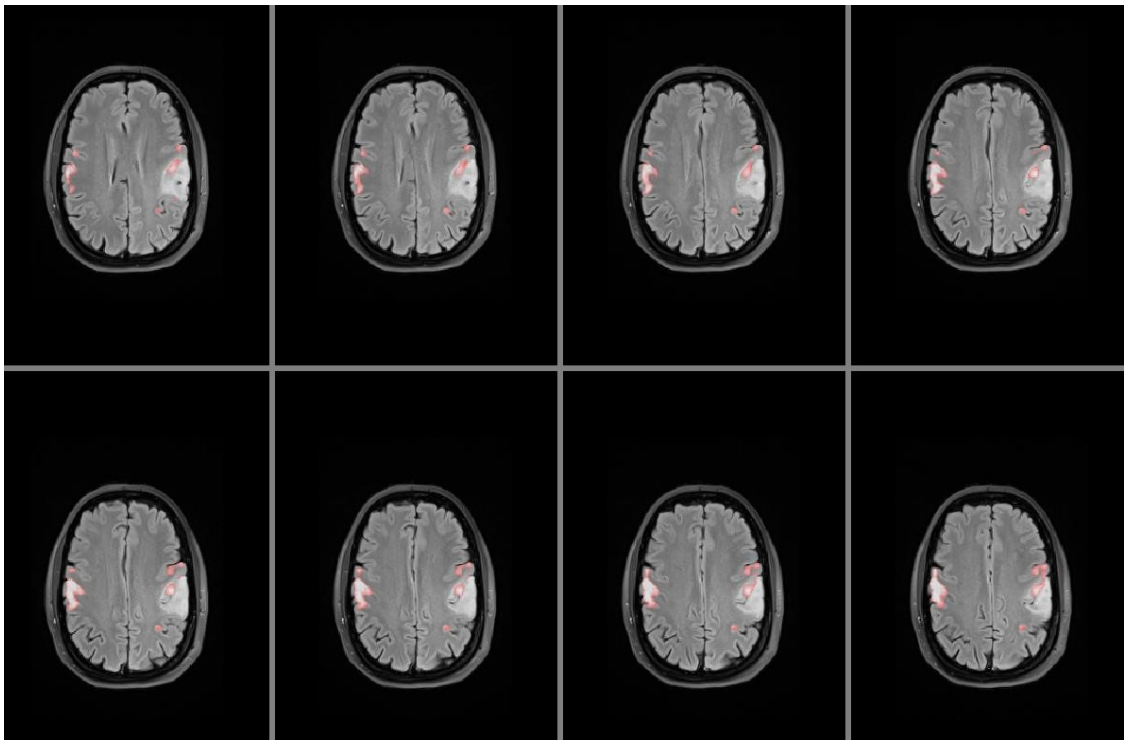

Figure 20 fMRI tongue hotspots created by tongue movement (marked in pink)

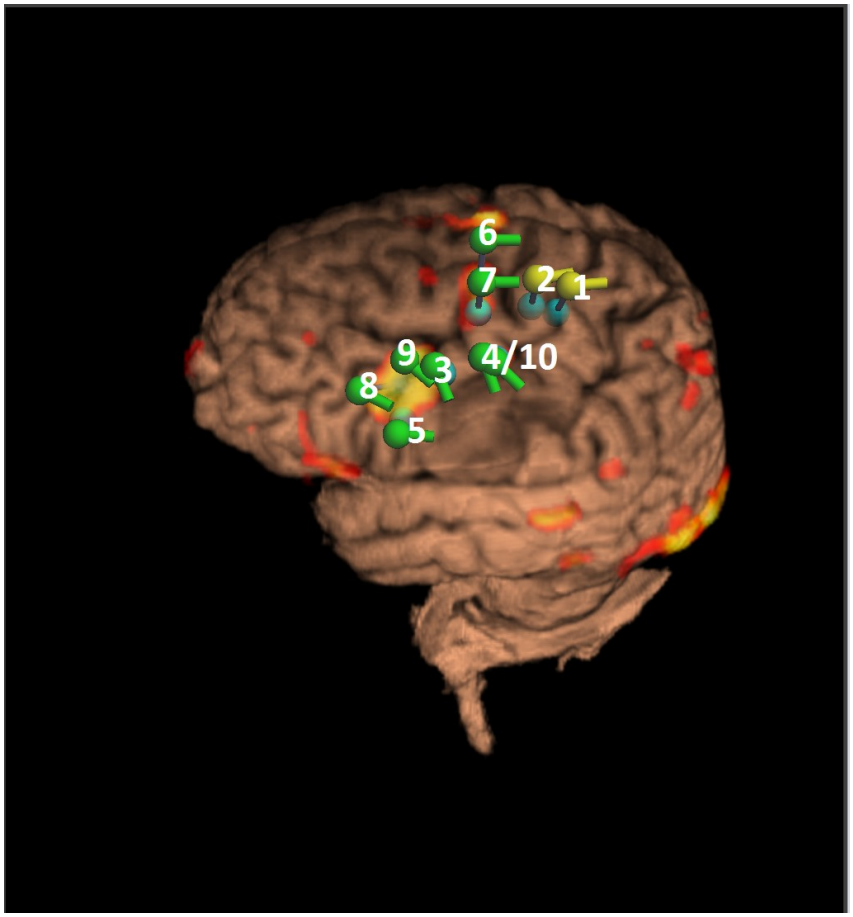

Figure 21 Overview of TMS targets (light blue) and entry points (green for language targets, yellowfor motor target). Each entry/target pair is given a number which correlates to the list in table 6. fMRI hotspots (red-orange) were created by using a verb generation task

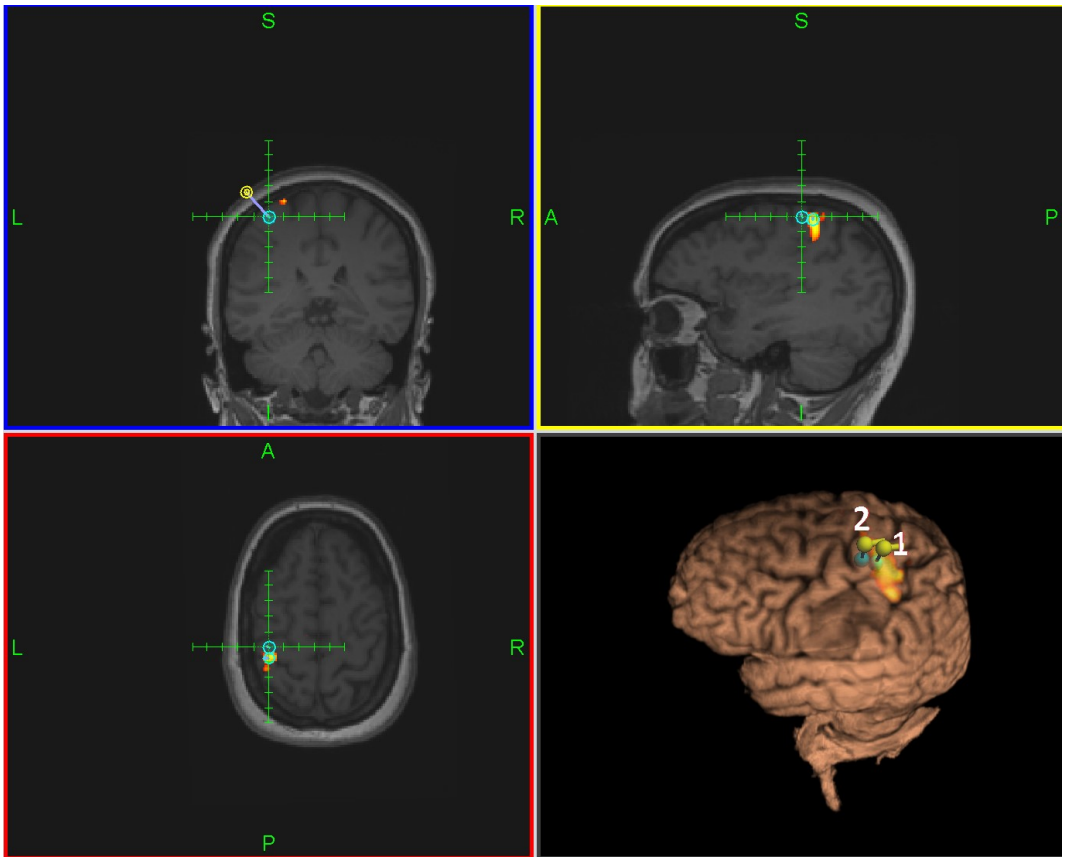

Figure 22 TMS target for AMT determination. The MRI images show target 2 on the PCG, anterior to the hotspot. The 3D reconstruction shows the spatial relationship of targets 1 and 2

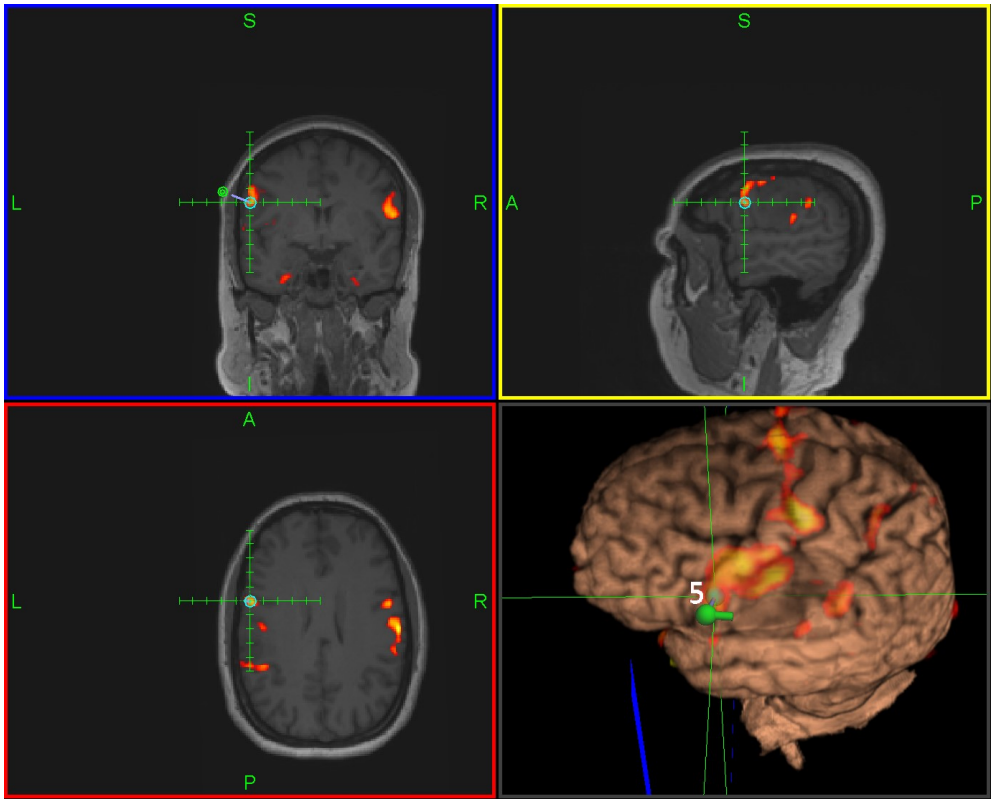

Figure 23 TMS target 5

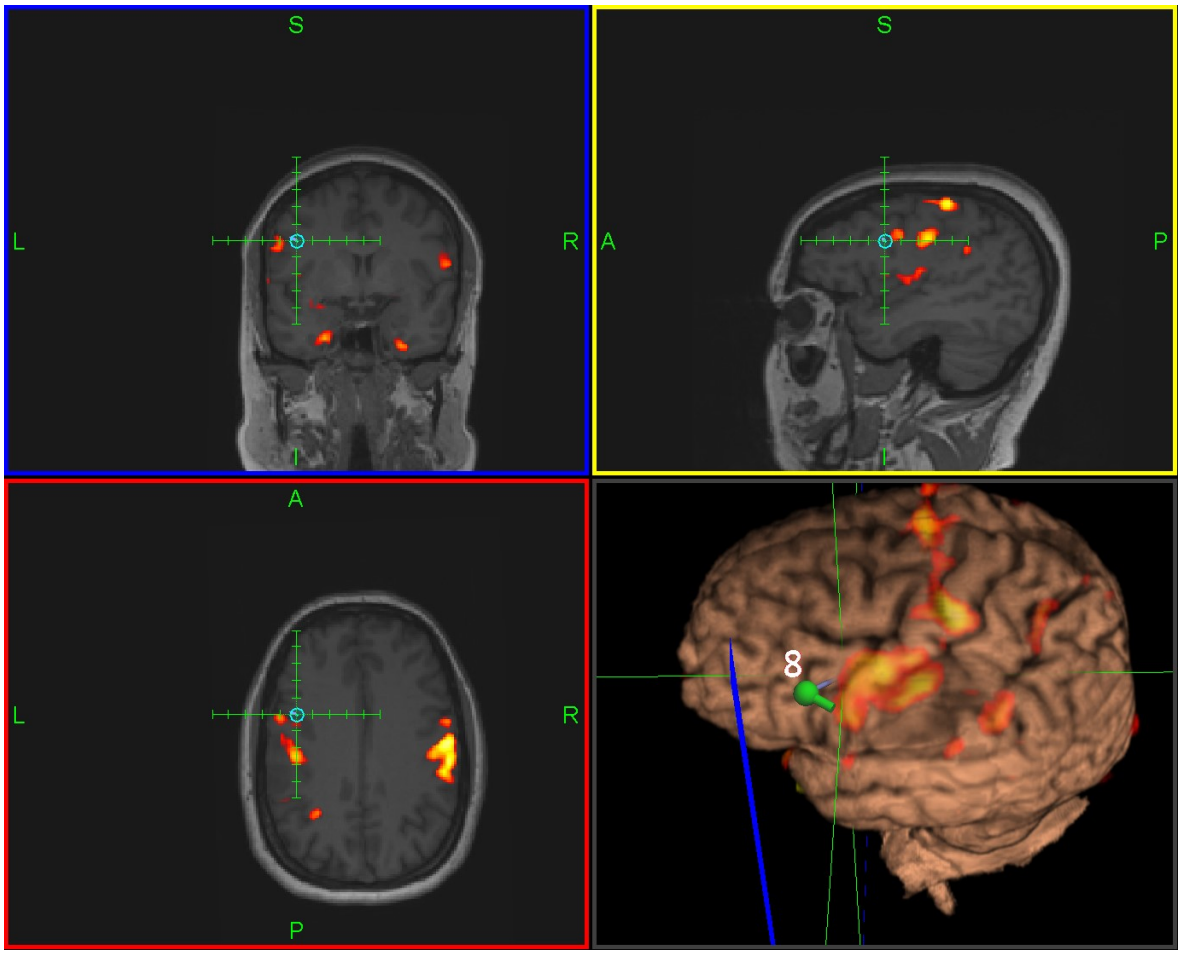

Figure 24 TMS target 8

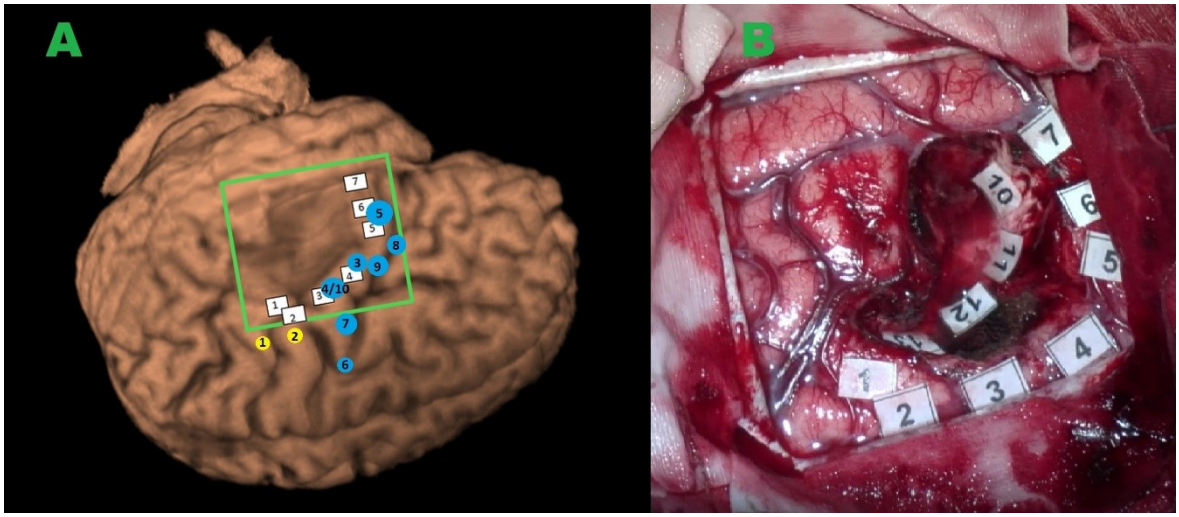

Figure 25 Overview and intraoperative image of DCS and TMS targets. A/left: The blue and yellow dots show the TMS targets, the numbers are equal to the ones in tables 6, 7, 9 and figure 21. The white squares correlate to the numbers shown in B and represent the areas_ identified by DCS. B/right: Surgical site after tumour resection (numbers as in table 9)

4.2 Induced speech deficits in total, error rate and review of anatomical distribution

Beside the hand targets, 8 language associated targets were defined. In the TMS session without priming, 3 errors of various types were induced. Therefore, 37.5% of the stimulated targets had a positive finding. In 2 targets, speech arrest was observed which correlates to a percentage of 25% of all targets. Both targets were marked in different regions (target 5: precentral gyrus and target 8: middle frontal gyrus). A minor error was induced by stimulating target 9, resulting in the patient having a lisp.
In the second TMS session, priming of each target was performed prior to the stimulation and the language task. During the task, no major error occurred. A minor phonological error was observed while stimulating target 10. The patient said “Mut” (german word for courage) instead of the correct term of the object “Hut” (german word for hat).

In comparison, TMS without priming elicited more language deficits. Without priming 37.5% of the stimulated targets lead to an error in total, divided into 25% as speech arrest or major error and 12.5% minor error. In the session with priming, the total error rate decreased to 12.5% with no major error.

4.3 correlation of both TMS paradigms to DCS and fMRI results

The correlation of TMS targets and DCS mapping can be seen in figure 25. TMS target 5 macroscopically correlates with DCS spots 5 and 6, with all three provoking speech arrest. TMS target 3 is in direct vicinity to DCS spot 4. In contrast to the intraoperative finding of speech arrest, TMS showed no significant effect on that target. TMS targets 4 and 10 are associated to the DCS finding labelled with 3, which showed a similar effect of contraction of facial muscles in both methods. TMS targets 1, 2, 6 and 7 were outside of the craniotomy, therefore a DCS verification was not possible. It appears that TMS target 2 correlates to DCS spot 2, which were both mapped as motor hand area.

Figure 21 shows fMRI hotspots, generated by performing a verb generation task during the examination. TMS targets 3, 5, 8 and 9 fit the hotspot within the anterior proportion of the precentral gyrus and the opercular proportion of the inferior frontal gyrus. As a result, TMS as well as DCS confirmed the fMRI finding of language eloquent areas in the above mentioned regions.

5. Summary and discussion

5.1 General results

Goal of the study was the TMS guided detection of language eloquent areas in brain tumour patients prior to awake surgery. Safety and potential of TMS in the preoperative management of tumours had been shown in several pioneer studies (8,132). In this thesis, we evaluated two specific TMS paradigms.

First, the high frequency cTBS, which is considered an inhibitory stimulation that can induce a virtual lesion (137,140,155,156). Therefore, high frequency stimulation is considered suitable for immediately disrupting higher cognitive functions like language (13,25). Second, a technique called priming, which modulates plasticity processes. Both paradigms were investigated with regards to the hypotheses formulated in section 2.4.2.3.

Awake surgery combined with DCS allows verification and validation of the TMS results. The distribution of language eloquent sites is variable, especially in brain tumour patients: as a result a need for a critical risk assessment, pre- and intraoperatively, arises (55).

The presented case report shows the promising potential and practicality of the protocols. The schedule of two TMS sessions was easily incorporated into the standard procedure of the preoperative work-up. Furthermore, the TMS results correlate with the findings of DCS and fMRI. Due to the limitations of one case report, definite statements of statistical significance cannot be made and further patients have to be studied to verify the primary results. Therefore, the following derivations will rely on the assumption, that future patients will have similar results.

In the examined patient, cTBS could elicit major language errors like speech arrest. These findings are important, as they demonstrate the capability of the chosen TMS paradigm to indeed inhibit language function. Beside the stimulation pattern, the effect of TMS also depends on the targeted area.

Positive findings such as muscle twitching or phosphene sensations can be elicited in the primary motor and occipital cortex, respectively. Additionally, disruptive effects on behaviour and cognitive functions are expected in many other regions like prefrontal, premotor and temporal cortices (151,163).

On the other hand, other studies suggest that negative mapping is sufficient for neurosurgical demands (57). However, potential biases of TMS were a major concern in negative mapping. These include: an understated threshold and the possibility of the requirement for different frequencies in different patients or even different areas. In fact, a working and sufficient stimulation should only be assumed when significant language errors are elicited.

Considering speech arrest and hesitation as the only major language deficits, the case report showed no lack in sensitivity. In fact, no false positive findings were observed. This stands in contrast with findings of other studies, which observed a high percentage of false positive targets in mapping language eloquent areas (56,57). Therefore, the assessment of language deficits might play a crucial role in the interpretation of the results. For instance, deficits that occurred due to pain or muscle contraction might lead to false positive results. In fact, the necessity to exclude non-specific effects in the results has been discussed before (151).

5.2 Comparison of the explorative approaches: fMRI, TMS, DCS

Modern neurosurgical assessment of brain tumours rests on MRI. Substantial surgical assumptions are made based on this diagnostic procedure, e.g. suspected tumour entity, chances/risks for a resection and postoperative outcome. The spatial distribution of functional areas is shown by fMRI, even though the use of fMRI can be unreliable in the vicinity of neoplastic lesions (7,56). It is often the first step to assess the distance of functional areas to the boundaries of the lesion. The reliability of fMRI in comparison to DCS in the assessment of language eloquent areas varies between 60% and 90% (164), therefore it should not be used alone for critical decision making (165).

Navigated TMS is also guided by structural MRI. However, due to the difference of the underlying methods, a direct comparison of fMRI to TMS and DCS is notsensible . While fMRI indirectly detects activated areas, TMS and DCS have the advantage of inducing a virtual lesion and therefore are capable of testing the actual function of the region of interest. Regardless of the differences the results of an fMRI examination are helpful to assess functional areas in a first step. Testing for the functional dependency of an area via TMS is the next step and closes the gap between fMRI and DCS. In fact, results of all examinations should not be taken independently, but rather be combined to have an overall view of the investigated functions and their spatial distribution.

When hotspot locations and TMS targets are compared to DCS findings several biases may occur. The most significant factor might be the brain shift, due to opening of the dura mater, swelling or loss of CSF. With regards to neuro-navigation, no reliable paradigms exist to correct pre-operative findings to compensate for the anatomical changes caused by brain shift during surgery. In tumour resection, studies suggest to maintain a security margin of 5-10 mm around the functional neuroimaging hotspots (164). For TMS however, reliable data for determining the minimal distance from a positive target does not exist yet.

If TMS is to be considered for use as a diagnostic tool in brain tumour patients, there is a need to improve the current security margins to increase the reliability and practicability in clinical use.

In general, a comparison of fMRI, TMS and DCS findings should be done carefully. For instance, the spatial boundary is not sharp and well defined in all of these methods. For DCS, the affected area is assumed to be roughly 10 mm around the electrode poles (166). In fMRI, it mainly depends on the thresholds chosen by the responsible examiner, and for TMS the size of the stimulated area depends on the shape of the coil and the intensity of the stimulation. Similar to DCS, an area of roughly 1 cm^2 can be assumed for TMS.

In the present case report, a high correlation of results from all methods was observed. The targets considered as language positive in fMRI and TMS were verified by DCS. However, DCS also detected additional language eloquent targets. With regards to TMS, the possible explanation could be the above-mentioned bias in the neuro-navigation or a change of the dimensional reference due to brain-shift during surgery. An incorrect handling of the TMS coil for the missed targets might be another possible reason. Furthermore, a different physiological state of the undetected area, like hyperpolarization or less involvement in the language task may also exist. Finally, another cause for varying results could be due to the intervening tissue (e.g. skin, muscle, bone, dura mater) between cortical surface and TMS coil.

Although both TMS and DCS induce a transient lesion, surgical removal of brain areas represents a different principle. The effects of irreversible removal of areas can be different. This is shown by a similar phenomenon called delayed onset of deficits (5). The removal of a specific area does not necessarily lead to the expected deficits but causes another area to overtake functions and therefore impair the original function of an initially unaffected area (5,164).

5.3 Assessment of cTBS and priming

One of the purposes of this thesis was to prove that cTBS is a practical and reliable paradigm for language mapping in tumour patients. Usually a positive mapping is required to be able to evaluate and trust the method. In TMS, the result of the examination is frequently determined by the chosen paradigm.

Beside the inherent characteristics of a paradigm, the efficiency to elicit language deficits also depends on the stimulation intensity which is chosen by assessing the motor-threshold. However, the assumption that 90% of the AMT is suitable for language mapping is only based on evidence obtained in former studies (15). One cannot completely out that a lower intensity might also be capable of causing language deficits. In fact, false negative results could be due to different threshold levels even within the same anatomical region (110).

To overcome the limitation of different thresholds and neuronal activity states, priming is a possible approach. Normally, synaptic transmission is not uniform and cortical neurons can be in different activity states as reflected by the variable threshold of activation. This phenomenon is called sliding threshold and is explained by the BCM-theory (167,168). Generally speaking, effects like LTD and LTP depend on the activation of postsynaptic neurons (151,168). LTD may occur when a presynaptic input failed to activate postsynaptic neurons (168). The induction of an AP in postsynaptic neurons depends on the history of postsynaptic activity (168). On the other hand, LTP is a result of strong post-synaptic depolarization secondary to a presynaptic activity (168). Both effects mainly depend on the Ca^{2+} level which leads to weakening or strengthening of synaptic transmission (169). As previously discussed, high frequency stimulation causes LTD by a NMDAR-dependent Ca^{2+} decrease (167). However, the theoretical concept of these mechanisms is based on physiological findings and often ignores the pathophysiological environment (e.g. tumour). Therefore, it has to be assessed whether the BCM-theory applies during neoplastic changes.

In our hypothesis, we expected priming to cause more inhibition due to LTD induction. Based on a lower Ca^{2+} level and decreased synaptic transmission, primed TMS would induce more inhibitory effects. Consequently, more language eloquent sites should be detected. Furthermore, it was assumed that priming creates a more homogeneous basis of excitability over the tested targets, which leads to more robust effects.

The cTBS paradigm itself causes inhibitory effects by inducing immediate LTD (137,140,155–157). Our results prove the applicability of the paradigm in tumour patients by successfully eliciting major language impairments like speech arrest and hesitation. However, elicited language errors decreased in primed TMS which contradicts our hypothesis. Based on our findings we can assume that although priming causes LTD, it did not improve the efficacy of the succeeding inhibitory stimulation. Primed TMS of targets which were considered language positive after unprimed TMS could not reproduce the impairments like speech arrest or hesitation. This might be due to the inherent effects of the cTBS priming: as priming uses the same paradigm and duration like the cTBS during the picture-naming task, it can be expected that the immediate effects are similar. Therefore, the reason why primed TMS is less effective in eliciting errors could be the physiologic processes underlying priming and language testing: the effects of priming immediately influence or modulate the effects of the second stimulation.

It is conceivable to suggest that an increase in synaptic strength causes resistance against the inhibitory effects of cTBS. Like the aphorism “neurons that fire together wire together” (140,150), a hypothesis is that priming causes LTP and improves connection to other regions by enhancing synaptic transmission temporarily. This can explain why the primed stimulation of a target does not show the same effect as an umprimed stimulation of the same target. For instance, a stimulus can elevate the Ca^{2+} level within “no man’s land” a zone, where neither LTP or LTD is induced (169), even though a change in the Ca^{2+} level is likely to occur after primed TMS and affect the second stimulation by plasticity mechanisms. Therefore, a clear prediction whether LTP or LTD will occur cannot be made, at least in our case (167). Indirectly, the result of the case report suggests that priming enhances neuronal function.

Another question was the correlation of primed and unprimed TMS to DCS. We assumed that unprimed TMS has a better correlation to DCS than primed TMS. In the case report, this assumption was correct. The effects of TMS and DCS in the targets where speech arrest was observed were comparable whereas primed TMS did not elicit these errors. The possible explanation is that unprimed TMS resulted in pure inhibitory effects and primed TMS was also affected by plasticity mechanisms (BCM-theory).

Primed TMS caused less inhibition leading to the assumption that plasticity mechanisms still play an important role in the assessment of language associated targets in tumour patients. Therefore, the BCM-theory still applies to cortical neurons in the vicinity of tumours.

5.4 Limitations of the study

Some potential limitations of the present study should be mentioned. Due to the small number of patients who participated in the pilot study definitive conclusions cannot be made at this stage and a complete statistical analysis from a high-powered study will be required to verifyour findings. Furthermore, TMS itself has its own limitations that include limited depth of penetration, confounding effects by direct muscle stimulation and the lack of precisely defined target areas. This is especially true when stimulating motor areas: activations of facial muscles, causing twitching and pain, make the assessment of a pure cortical effect difficult when targeting frontal regions. Additionally, non-responders to TMS do exist and these individuals may undergo the examination without yielding useful test results (170).

Another factor that makes language mapping more challenging in comparison to motor exploration is the fact that motor function is anatomically more confined than the widespread language associated areas. Furthermore, only 1/3 of the cortical neurons are found on the surface while 2/3 are located within the sulci. This makes the identification and definition of the targeted areas problematic. Finally, in language mapping an object naming task is performed during TMS. Using only one task for all language associated areas disregards the fact, that several areas are divided into different functional units as described in section 2.2. Hauck *et al.* (54) investigated the impact of different language tasks on rTMS mapping and concluded that in general ONT is most suitable to detect positive sites while action naming might be an advantage in mapping posterior regions; therefore, the selection of specific tests depending on the target area can be helpful in improving examination reliability (54).

6. Conclusion

This thesis discussed the application of nTMS as a diagnostic method for mapping functional areas of the cerebral cortex. It was shown that TMS results are comparable to intraoperative DCS results and can be used as an adjunct to existing preoperative diagnostic methods. Furthermore, nTMS is a unique method that allows a non-invasive modulation and function testing of cortical brain areas.

In this case report we could show that the cTBS paradigm is suitable for clinical evaluation of tumour patients. We were able to elicit major language deficits by stimulating cortical areas near the tumour. Cortical targets, which were considered language eloquent by TMS, could be verified by DCS mapping with a high correlation rate. However, due to the small number of subjects in the study general statistical conclusions cannot be made at this point. Assuming similar results in future patients, unprimed cTBS could be a reliable paradigm to detect language eloquent areas prior to awake surgery. Early data suggests that primed TMS is less suitable for language mapping, but this assumption has yet to be confirmed by the further studies.

Despite some limitations that make the comparison between TMS and the gold standard DCS difficult, TMS can be considered a suitable additional diagnostic method that can shorten the required awake phase during surgery or guide the surgeon when awake surgery cannot be performed (171).

With regard to our working hypotheses, we were able to show that unprimed cTBS might have a better correlation to DCS than primed cTBS. We suggest that the result of primed TMS is influenced by plasticity processes that seem to decrease the inhibitory effects of cTBS. However, as the results were only derived from one case report, more patients must be tested to support this claim. Future studies should also investigate other open questions, including the determination of the lowest stimulation threshold needed to reliably elicit language errors individually.

Language function is one of the most complex systems within the CNS and its multimodal assessment requires complementary approaches to enhance the safety of surgical procedures near it's vicinity. The combination of fMRI, TMS and DCS is a logical set up to serve this purpose.

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