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MOTOR AREAS IN PATIENTS WITH MOTOR ELOQUENT BRAIN TUMORS, INSIGHTS DERIVED FROM NAVIGATED TRANSCRANIAL MAGNETIC STIMULATION MAPPING

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For my dad

And my grandmother

TABLE OF CONTENTS

1. ABBREVIATIONS

3D	3-dimensional
ADM	abductor digiti minimi muscle
APB	abductor pollicis brevis muscle
BCS	biceps muscle
BMRC	British Medical Research Council
CMCT	central motor conduction time
CST	corticospinal tract
DES	direct electrical stimulation
DH	motor dominant hemisphere
EEG	electroencephalography
EMG	electromyography
fMRI	functional magnetic resonance imaging
FCR	flexor carpi radialis muscle
GABA	gamma-aminobutyric acid
GCN	gastrocnemius muscle
LGG	low-grade gliomas
M1	primary motor cortex
MEG	magnetoencephalography
MEP	motor evoked potential
MFG	middle frontal gyrus
MNI	Montreal Neurological Institute
MRI	magnetic resonance imaging
NDH	non-dominant hemisphere
nTMS	navigated transcranial magnetic stimulation
PET	positron emission tomography
PoG	postcentral gyrus
PrG	precentral gyrus
PMd	dorsal premotor area
PMv	ventral premotor area
PMC	premotor cortex
rMT	resting motor threshold
SD	standard deviation
SFG	superior frontal gyrus
SMA	supplementary motor area
TA	tibialis anterior muscle

TES	transcranial electrical stimulation
TMS	transcranial magnetic stimulation

2. INTRODUCTION

2.1. Development of motor cortex definition throughout history

The organization of functional areas in human brain has been investigated extensively in the past. Brodmann was one of the first neuroscientists to describe areas of the human brain with similar cytoarchitecture indicating that these areas participate in execution of the same function (Fig. 1) (Brodmann, 1909).

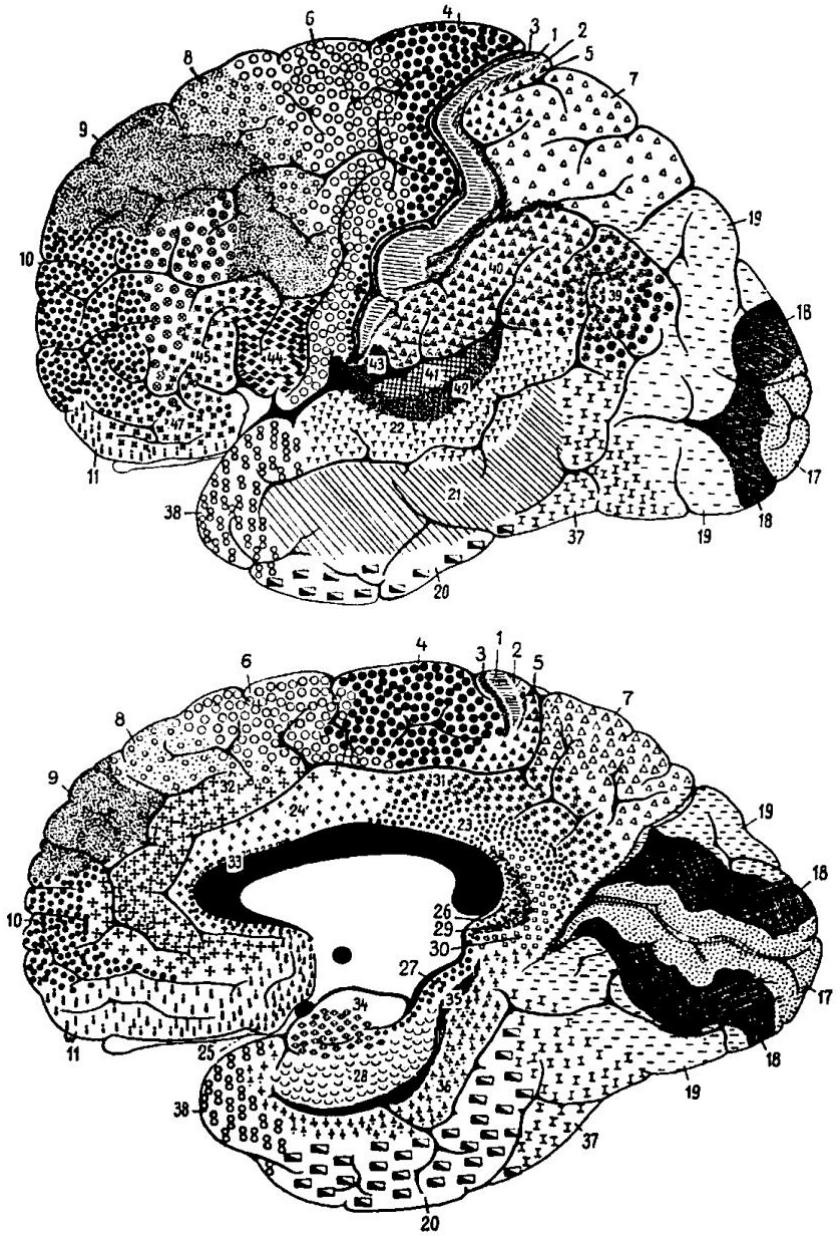

Fig. 1: Brodmann areas of the human cerebral cortex; upper row: lateral view, lower row: medial view; reprinted from the digital copy of Brodmann (1909) provided by ZB MED – Informationszentrum Lebenswissenschaften

Brodmann areas are still commonly used in scientific settings, although the full impact of this cytoarchitecture-based distinction is still unclear. Brodmann's theories have undergone many adaptations until today to attribute these areas to those of specified cortical functions.

In the early mid-twentieth century, Fulton used Brodmann areas to define the location of the human motor cortex. Studying the cytoarchitecture and performing stimulation studies in primates, he and his group hypothesized that Brodmann area 4, the posterior part of the precentral gyrus (PrG), is, in part, the origin of the pyramidal tract, referred to as the primary motor cortex (M1). He referred to Brodmann area 6, which consists of the anterior part of the PrG and the posterior part of the frontal lobe, as the "extrapyramidal" motor cortex and used the term "premotor area" (Fulton, 1935). Other researchers agreed to Fulton's theories and made their own amendments. Foerster, for example, widened the definition of the extrapyramidal motor cortex accordingly to whether the electrical stimulation led to movement, adding areas in the anterior frontal lobe, in the parietal lobe, and in the temporal lobe (Fig. 2) (Foerster, 1936). The consent at that time was that area 4 was superior to area 6 considering motor control, with area 6 playing rather a modulatory role.

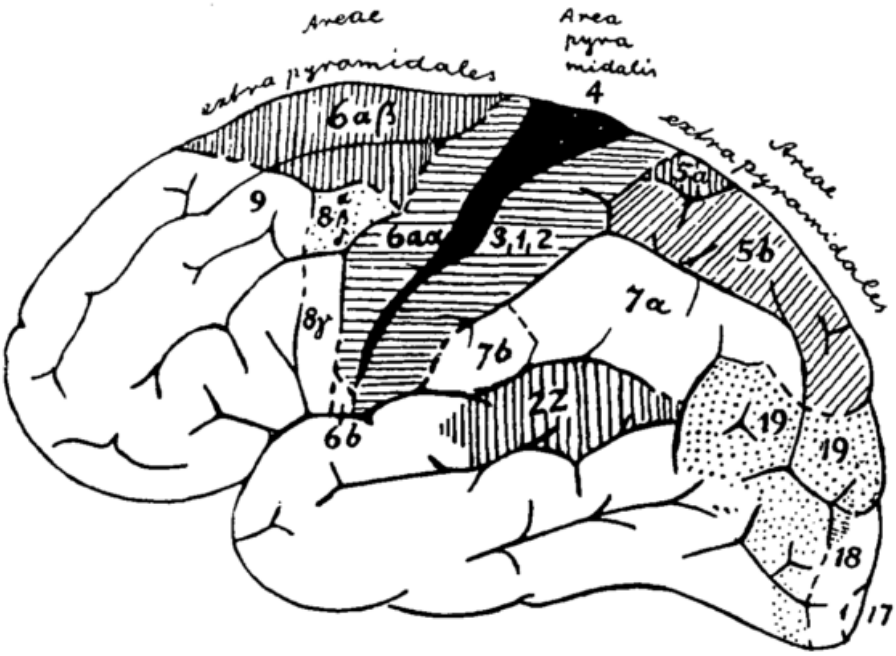

Fig. 2: Description of the motor cortex as areas where stimulation leads to limb movement; according to Brodmann's cytoarchitectural studies, the authors differentiate pyramidal and extrapyramidal areas; Foerster, O. (1936). The motor cortex in man in the light of Hughlings Jackson's doctrines. Brain, 59(2), 135-159. doi:10.1093/brain/59.2.135; reproduced with permission of Oxford University Press on behalf of the Guarantors of Brain

Later, Penfield systematically investigated the location of the human motor cortex by intraoperative direct electrical stimulation (DES). He didn't agree to the strict cytoarchitectural

boundaries differentiating 2 motor areas in the PrG (Penfield & Boldrey, 1937). He found motor responses to DES only when stimulating the PrG and the postcentral gyrus (PoG), hereby adding PoG to the definition of the motor cortex (Penfield & Boldrey, 1937). In his experience, stimulation of areas outside the sensorimotor cortex did not induce real motor responses, rather “convulsive” movements due to “epileptiform discharge”. Furthermore, he found a somatotopic organization of the M1, pointing out that upper limb representations are located in the middle, and lower limb representations are in the superior part of PrG, each body part represented by areas at different size. He captured his theories in the form of the human homunculus (Fig. 3) (Penfield & Boldrey, 1937). Later, in 1951, he was one of the first researchers to describe the medial area 6 as the supplementary motor area (SMA) and to point to its implications for execution of movement (Penfield, 1950; Penfield & Welch, 1951).

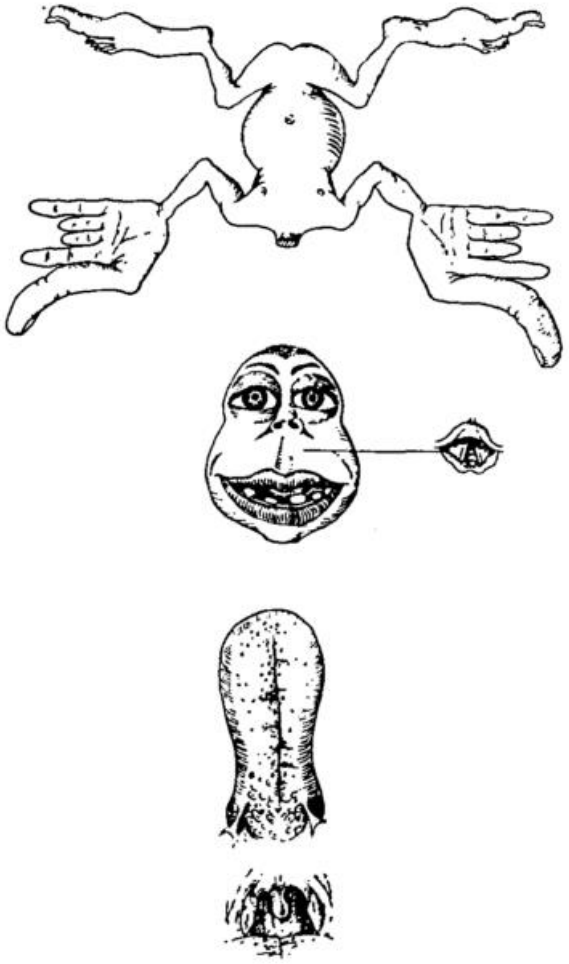

Fig. 3: Sensory and motor homunculus; this graphic depicts differences in size and location of motor and sensory representations of different body parts as they appear from above down upon the PrG; Penfield, W., & Boldrey, E. (1937). Somatic Motor And Sensory Representation In The Cerebral Cortex Of Man As Studied By Electrical Stimulation. Brain, 60(4), 389-443. doi:10.1093/brain/60.4.389; reproduced with permission of Oxford University Press on behalf of the Guarantors of Brain

Starting in late 80's, Dum and Strick published a large series of studies, introducing a more detailed depiction of the premotor cortex (PMC) in the frontal lobe of primates. Using retrograde tracer injection, they were able to distinguish 6 premotor areas with direct cortico-cortical projections to the PrG as well as corticospinal projections, namely the ventral premotor area (PMv), dorsal premotor are (PMd), the SMA, and 3 cingulate motor areas (Dum & Strick, 1991, 2002) (Fig. 4). They showed that not only does low current stimulation of these areas evoke limb movement, moreover, the movement might be evoked through corticospinal projections, as these areas interact directly with motor neurons of the forelimb (Dum & Strick, 2002). In addition, their modulatory role on the PrG through cortico-cortical projections should be noticed (Dum & Strick, 2002)

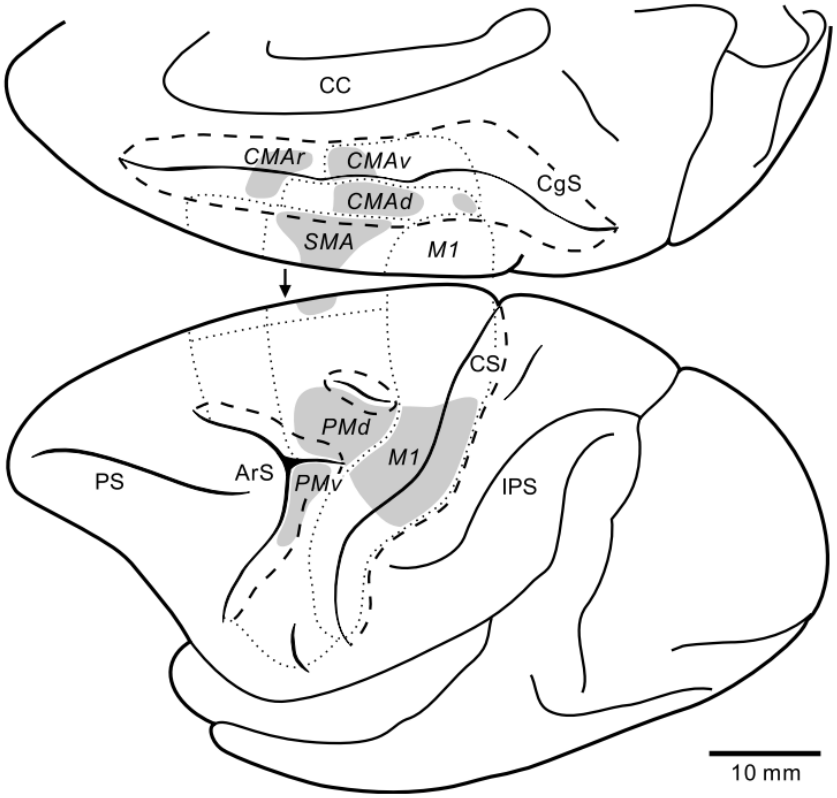

Fig. 4: *Motor areas in the frontal lobe of primates; shaded regions show regions with corticospinal projections; reprinted from Dum, R. P., & Strick, P. L. (2002). Motor areas in the frontal lobe of the primate. Physiol Behav, 77(4-5), 677-682, with permission from Elsevier*

2.2. Brain plasticity - a mechanism behind functional reorganization

Starting in late 20th century, researchers and clinicians moved from a mere view at the location of functional areas to the search for mechanisms influencing their location. Rather than a static composition of functional areas, whose impairment would lead to irreversible deficits, the brain

was more and more regarded as a dynamic structure capable of functional reorganization, also referred to as neuroplasticity (Duffau, 2006; Kong et al., 2016; Pascual-Leone et al., 2005). Reorganization of functional areas in response to brain lesion or injury was visualized by studies using different stimulation or neuroimaging modalities.

The group of Hugues Duffau from France contributed numerous works concerning plasticity of the brain in patients with brain tumors, investigated with DES. They were able to detect short-term plasticity by displaying changes in organization of the motor cortex during the same surgery, directly before and after tumor resection (Duffau, 2001). Later, they depicted reorganization of functional areas between 2 tumor resection surgeries several years apart (Duffau et al., 2002; Robles et al., 2008) and provided many manuscripts and reviews addressing the field of plasticity (among others, consider Desmurget et al., 2007; Duffau, 2006).

Based on this development, literature proposes several mechanisms for neuroplasticity (for reviews, see Duffau, 2006; Kong et al., 2016; Nudo, 2013; Rossini & Pauri, 2000; Sanes & Donoghue, 2000). Synaptic plasticity for example, the main mechanism behind memory and learning, is mediated through glutamate receptors and is based on long-term potentiation or depression of postsynaptic potentials (Bliss & Collingridge, 1993; Bliss & Lomo, 1973; Buonomano & Merzenich, 1998). Horizontal fibers, which interconnect adjacent and distant functional units in the brain, employ this principle (Hess & Donoghue, 1994). With their excitatory as well as inhibitory effects, mediated through for example gamma-aminobutyric acid (GABA)-ergic interneurons, they maintain the balance in activity of functional areas (Jones et al., 1978; Nudo, 2013). This balance is often disturbed in lesioned brains. It was shown that in brains with focal cortical malformations, there is a widespread imbalance of excitation and inhibition, probably mediated through downregulation of GABA receptors that results in hyperexcitability of brain areas (Nudo, 2013; Redecker et al., 2000). Moreover, changes in lesioned brains occur on gene expression level as well, for example in genes associated with branching of neurites and dendrites (Urban et al., 2012). It seems as neuroplasticity is not limited to preformed pathways, but allows for formation of new synapses and neurons as well (Duffau, 2006; Gross, 2000).

There are multiple principles of how postlesional plasticity can enable a functional reorganization of motor pathways. Often, other neurons are recruited to compensate for impaired pathways. The recruited neurons can either derive from adjacent cortical areas, or from distant areas such as the contralateral cortex (Duffau, 2006; Kong et al., 2016; Nudo, 2013; Seitz et al., 1995; Weiller et al., 1993). Moreover, the reorganized neurons can consist of unmasked, so far inhibited projections (Duffau, 2006; Jacobs & Donoghue, 1991; Kong et al., 2016; Nudo, 2013; Sanes & Donoghue, 2000; Ziemann et al., 1998). During normal state of the brain, these latent connections are inhibited and get unmasked when the inhibition is

omitted. This is often regarded as an acute process, which takes place directly after lesion of the pathways, while other compensatory mechanisms might need more time to develop (Duffau, 2006; Jacobs & Donoghue, 1991; Kong et al., 2016; Ziemann et al., 1998).

In terms of functional reorganization of the motor pathways in patients with brain lesions, motor areas outside the PrG received increased attention. Here, studies mainly concentrated on 2 groups of patients, patients recovering from stroke, i.e. acute lesions, and patients with gliomas, i.e. rather slow-growing lesions. They pointed to different areas that have the ability to overtake motor function, if the original pathways are impaired; these were as well areas close to the lesion and the original pathways, such as the ipsilesional motor cortex, as well as more distant areas in the ipsilesional and the contralesional hemisphere (Carpentier et al., 2001; Duffau, 2006; Kong et al., 2016; Nudo, 2013; Rossini & Pauri, 2000; Sanes & Donoghue, 2000; Seitz et al., 1995; Weiller et al., 1993). Many of these findings were derived from stimulation studies, yet techniques for functional imaging, described in the following paragraphs, became more and more important over the last decades.

2.3. Intraoperative motor mapping

When resecting tumorous tissue from human brain, it is crucial to identify eloquent areas, i.e. areas that are essential for specific brain functions, because their resection leads to impairment of the respective function. In terms of motor function, the PrG is generally considered motor eloquent, with specific body parts being represented in different location along the PrG (according to the homunculus, Fig. 2). However, a complete tumor resection significantly influences the outcomes of patients with gliomas (Capelle et al., 2013; De Witt Hamer et al., 2012; Sanai & Berger, 2008; Stummer et al., 2008). Therefore it is necessary to distinguish between actually motor eloquent areas, and tumorous tissue which can be resected without motor function impairment.

The “gold standard” in neurosurgery for motor mapping, i.e. locating the motor areas in the brain, is the intraoperative DES (Berger et al., 1990; Cedzich et al., 1996; De Witt Hamer et al., 2012; Duffau et al., 2005; Kombos et al., 2000; Szelenyi et al., 2010; Taniguchi et al., 1993). The principles of DES are based on excitability of neurons through electric currents. After craniotomy and opening of dura mater, the surgeon stimulates specific areas of the brain cortex with an electrode (Fig. 5). In terms of motor mapping, stimulation of the motor cortex causes activation of muscles in the contralateral half of the patient’s body (Penfield & Boldrey, 1937). This can be measured in the form of motor evoked potentials (MEPs) in a continuous monitoring via electromyography (EMG). If repeated stimulation of a single site in the brain doesn’t evoke MEPs, the site is not considered essential for motor function.

Monitoring of MEPs is often applied during the whole tumor resection in the sense of neuromonitoring. Hence, repetitive stimulation of a motor site is performed and MEPs are being monitored; severe amplitude or latency alternations are an early sign for possibly permanent impairment of motor function and indicate the need for a thorough evaluation of further resection (Cedzich et al., 1996; Taniguchi et al., 1993). The patients are usually under general anesthesia. Studies showed that intraoperative motor mapping and neuromonitoring are beneficial for patients' outcomes (Berger et al., 1990; Duffau et al., 2005; Kombos et al., 2009; Krieg et al., 2012a).

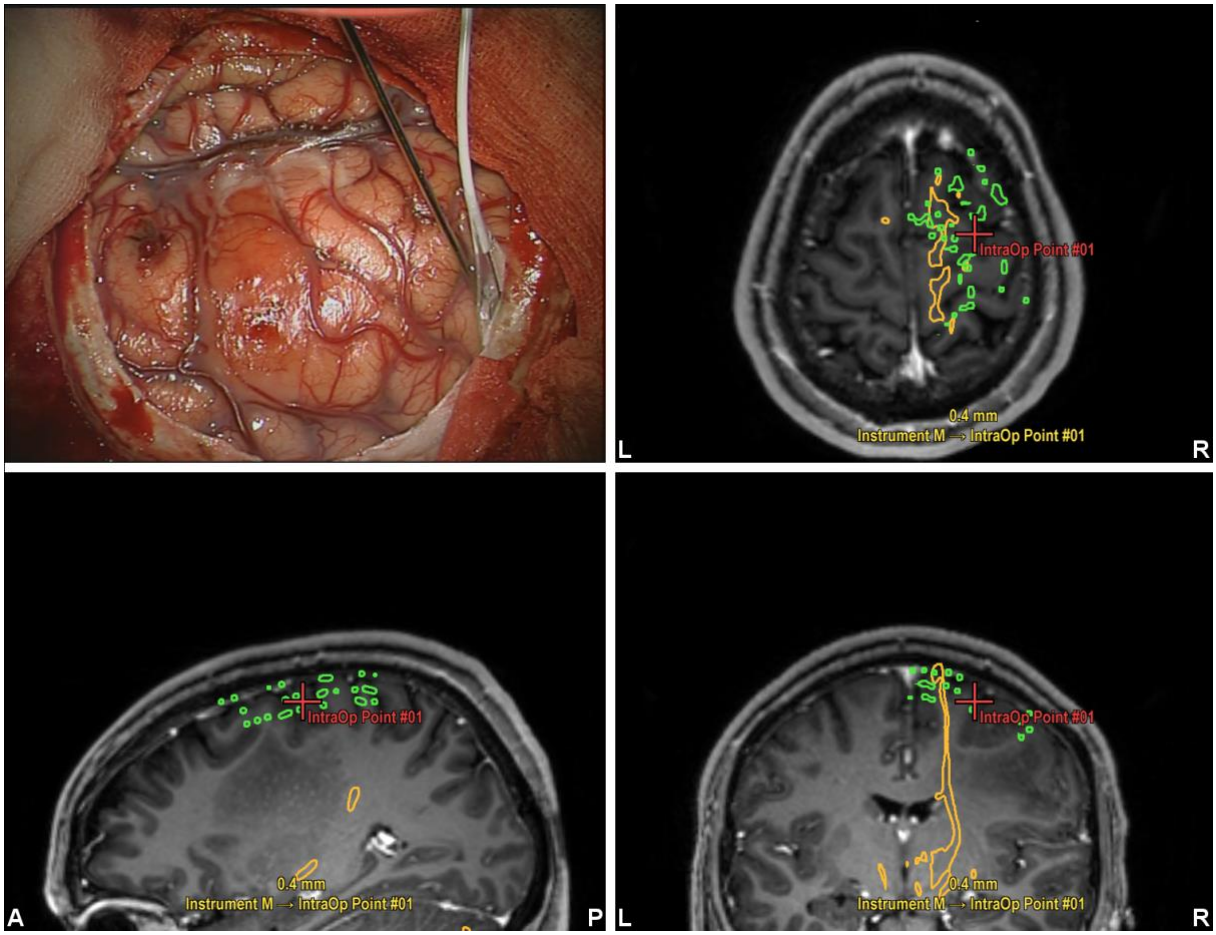

Fig. 5: Figure showing DES motor mapping; left upper image shows resection site during brain tumor surgery, other images show the location of the DES point stimulated by a strip electrode (red cross, IntraOP Point #01) as visualized in the intraoperative neuronavigation system; preoperative motor mapping results (shown in green) and corticospinal fibers (shown in yellow) were implemented in the neuronavigation data set as well

2.4. Preoperative motor mapping

2.4.1. Functional magnetic resonance imaging

Functional magnetic resonance imaging (fMRI) is commonly used for non-invasive location of cortical functions (for an example, see Fig. 6). It combines a structural image achieved from magnetic resonance imaging (MRI) with the possibility for functional testing. The technique of fMRI is based on alterations in blood oxygenation levels to measure changes of the cerebral blood flow between resting state and during visual, auditory, or sensorimotor motor tasks (Ogawa et al., 1992). It is assumed that cerebral blood flow correlates with neural activity; therefore, if an area shows higher blood flows during a task, this area is likely to be a part of pathways involved in the task (Logothetis et al., 2001; Ogawa et al., 1992).

The technique of fMRI is widely used for locating the motor cortex in patients with brain pathologies, such as tumors (Carpentier et al., 2001; Cramer et al., 1997). It can be used for preoperative motor mapping with good results that are consistent with those of the DES (Bizzi et al., 2008; Lehericy et al., 2000; Mueller et al., 1996; Roessler et al., 2005). Yet, while DES-based motor mapping measures the direct response to activation of a specific cortical area, fMRI measures merely surrogate parameters of neural activity, such as decreased blood oxygenation levels due to oxygen consumption. The actual relation between these signal alterations and neural activation and the spatial and temporal accuracy of the technique remain controversial, and noise signals and pathologies of the brain might lower the quality of fMRI results (Kong et al., 2016; Ugurbil et al., 2003). Hence reevaluation of the technique and comparison to newly developed methods for preoperative motor mapping is of importance.

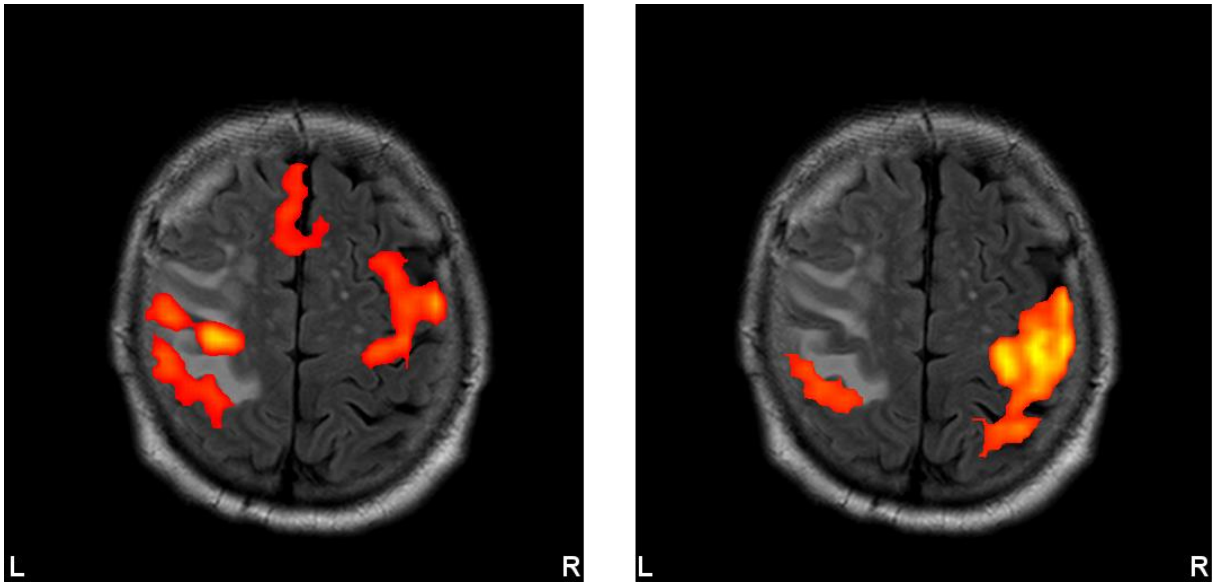

Fig. 6: Figure showing an fMRI scan of a patient with left-sided brain tumor; left image shows activation during movement of the right hand, right image shows activation during movement of the left hand; colored areas indicate areas active during movement; for both hands, highest activation was located in the PrG of the contralateral hemisphere

2.4.2. Positron emission tomography

Another method for depicting functional areas in patient's brain is the positron emission tomography (PET). This technique can measure neuronal activation using different radioactive tracer, such as water labeled with radioactive oxygen for detecting regional cerebral blood flow changes, or radioactively labeled fluorodeoxyglucose for detecting the glucose metabolism (Fox et al., 1987; Schreckenberger et al., 2001). Both techniques enable a precise location of the motor cortex, if investigated during a motor task, even in patients with lesions of the brain (Fox et al., 1987; Schreckenberger et al., 2001). Moreover, studies that aimed to compare PET, fMRI, and DES showed a good congruence of these 3 techniques (Krings et al., 2002; Reinges et al., 2004; Schreckenberger et al., 2001). In general, fMRI shows a higher spatial and temporal resolution than PET, with the benefit of being non-invasive, while it is more prone to artefacts from patient's movements or heartbeat; PET, on the other hand, is less susceptible to artefacts, yet needs injections of a radioactive tracer (Kong et al., 2016; Krings et al., 2002; Reinges et al., 2004). Nowadays, fMRI is wider available and therefore, currently of larger clinical impact (Krings et al., 2002). Some of the older studies investigating the motor cortex in patients with brain lesions used PET, though (Seitz et al., 1995; Weiller et al., 1993; Wunderlich et al., 1998).

2.4.3. Magnetoencephalography

Electroencephalography (EEG) and magnetoencephalography (MEG) are 2 further methods for non-invasive motor cortex location (for an example of an MEG scan, see Fig. 7) (Nagarajan et al., 2008; Pfurtscheller, 2001; Tarapore et al., 2012; Weiss & Mueller, 2003). Both methods have the same origin; they measure brainwaves along the patient's scalp, which are oscillations of electric/magnetic fields. A comparison of the brainwaves during resting state and during specific tasks (visual, auditory, or sensorimotor tasks) allows detection of areas involved in execution of cortical functions. The key to locate the motor cortex in EEG/MEG recordings is the event-related desynchronization, a desynchronization of brainwaves a short time before the onset and during the onset of movement, which is present mainly in the beta band frequency (Pfurtscheller, 2001; Pfurtscheller & Lopes da Silva, 1999). Different analytical approaches are available to locate the source of this desynchronization, which is considered the area of neural activity (Nagarajan et al., 2008). Preoperative motor mapping can be performed using MEG with comparable results to those of DES-based motor mapping (Nagarajan et al., 2008; Tarapore et al., 2012). Some studies indicate superiority of MEG when compared to fMRI (Keil et al., 2009). Due to its high acquisition and maintenance costs, MEG is available to only a limited number of centers.

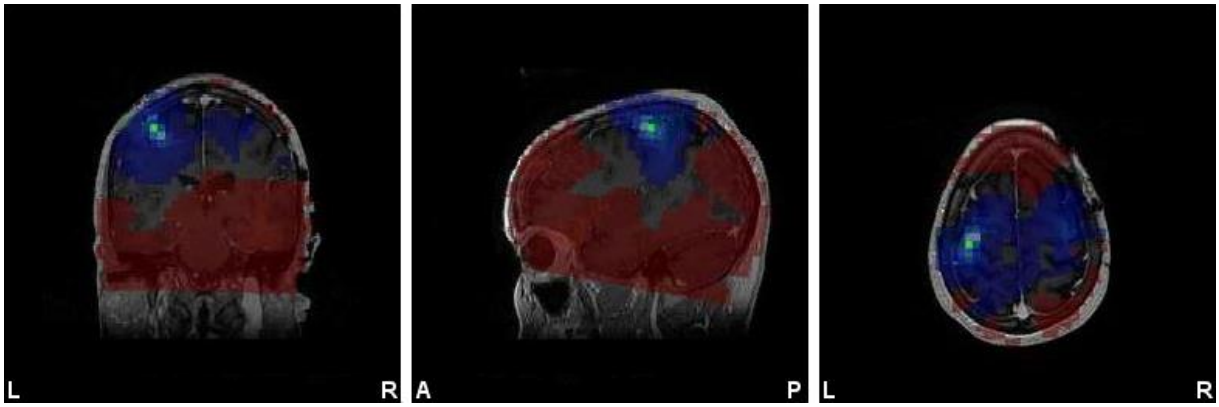

Fig. 7: Figure showing an MEG scan during right index finger motor task of a patient with a brain tumor; blue areas indicate higher activation during movement, red areas indicate no activation; green spot indicates the local maximum located in the left PrG; this picture was provided with the kind permission of Dr. Phiroz Tarapore, Department of Neurological Surgery, Biomagnetic Imaging Laboratory, University of California San Francisco

2.4.4. Principles of transcranial magnetic stimulation

The first report about transcranial magnetic stimulation (TMS) is from Barker et al. from the year 1985 (Barker et al., 1985). Before this date, non-invasive stimulation of cortical structures was performed using short, high-voltage electrical currents applied directly through electrodes on scalp as transcranial electrical stimulation (TES) (Merton et al., 1982). For their newly developed technique, Barker et al. utilized the principle of magnetic fields inducing an electrical field. Since then, TMS has undergone much development, yet the principles remain the same. A magnetic coil, placed over the scalp, is used to induce a short-lasting magnetic field. This field generates an electric field, which, with the adequate intensity and orientation, can excite cortical neurons and activate (or inhibit) corresponding cortical areas (Barker et al., 1985; Hallett, 2000; Ilmoniemi et al., 1999; Ravazzani et al., 1996; Rossini et al., 1994; Rossini et al., 2015; Ruohonen & Karhu, 2010). In terms of motor mapping, stimulation over the motor cortex evokes response movements in contralateral body parts which can be visualized as MEPs in the continuous EMG. They can be used to distinguish between motor positive (stimulation elicits MEPs) and motor negative (no MEPs) areas and to create a map of the motor cortex (for an example of a navigated TMS [nTMS]-based motor map, see Fig. 8). Other than the electrical current induced during TES, the magnetic field induced during TMS is not influenced by the tissue it passes, such as the skull or cerebral fluid. As the electric field is induced at deeper levels, lower intensities are sufficient (Barker et al., 1985; Ruohonen & Karhu, 2010).

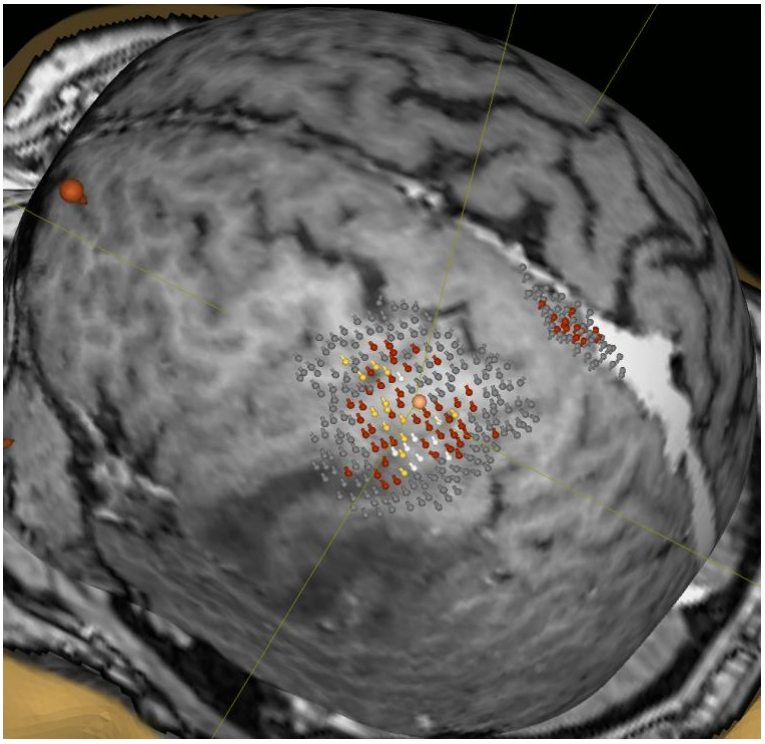

Fig. 8: Results of nTMS-based motor mapping of the upper and lower extremity in a patient with a left hemispheric tumor; grey dots indicate motor negative, colored dots indicate motor positive stimulation sites; the color scheme represents the MEP amplitude: red (50µV – 500µV), yellow (500µV – 1,000µV), white (> 1,000µV); upper extremity MEPs were located in the middle PrG around the handknob area (orange target at the center of the yellow crosshair), lower extremity MEPs were located in the superior part of the PrG

Motor mapping via TMS is generally well tolerated, as the induced electric field is of low intensity and therefore, the stimulation of structures on the scalp such as muscles and peripheral nerves is limited (Tarapore et al., 2016a; Tarapore et al., 2016b). Other than in earlier reports (Fauth et al., 1992), the risk of inducing seizures is minimal when using modern technology and adequate mapping protocols, resulting in TMS being considered a safe technique (Rossi et al., 2009; Tarapore et al., 2016a; Tarapore et al., 2016b).

To maximize the effects of TMS, it is important to consider the patient's skull morphology, as it influences the distribution of the electric field and hence the effect on the targeted structure (Ruohonen & Karhu, 2010). Moreover, the direction of the electric field in relation to the targeted structures is of importance (Rossini et al., 2015; Ruohonen & Karhu, 2010). To increase the precision of TMS, nTMS, which integrates the patient's structural MRI for navigating the coil, was developed. When stimulating the scalp, the coil's position is being visualized in the MRI scan allowing to target specific structures, such as the PrG, and to monitor the orientation of the induced electric field (Fig. 9) (Ruohonen & Karhu, 2010).

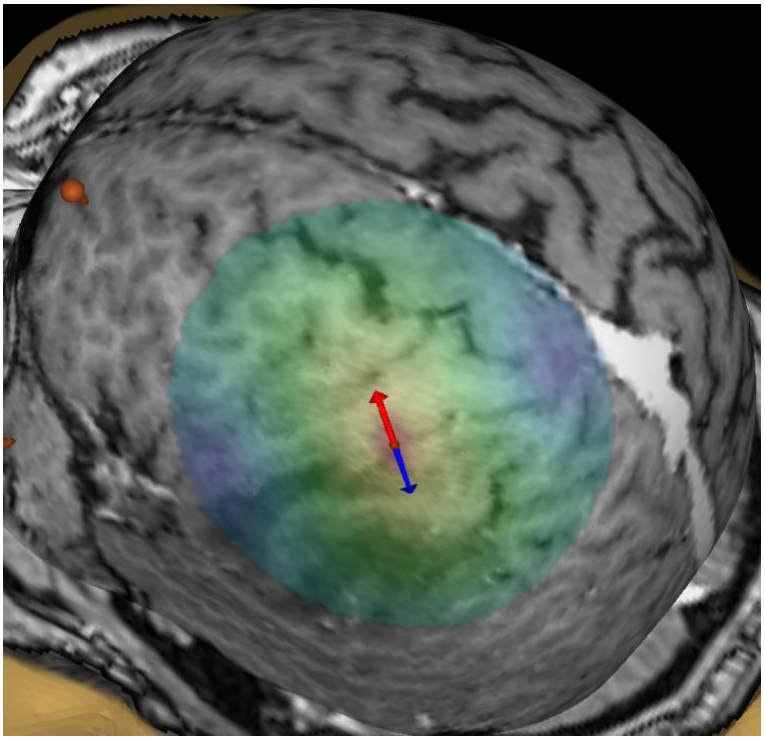

Fig. 9: Stimulation of the posterior border of the PrG in the same patient with a left hemispheric tumor; the electric field is strongest in the red dot at the cortex level; the arrow shows the direction of the induced electric field; the colored area (yellow to blue) shows the decreasing strength of the electric field

2.4.5. Clinical use of nTMS

In the neurosurgical setting, nTMS is an FDA approved technique for locating the motor and language cortex during preoperative planning. nTMS-based motor mapping is considered a highly reliable method, which is used as a matter of routine in many neurosurgical centers around the world (Krieg et al., 2012b; Picht et al., 2009; Tarapore et al., 2012). It gives results with a good spatial resolution and very good correspondence to DES (Krieg et al., 2012b; Picht et al., 2011; Tarapore et al., 2012; Weiss et al., 2013). There is evidence that it has the potential to improve outcomes in patients who undergo resection of motor-eloquent gliomas (Frey et al., 2014; Krieg et al., 2014; Krieg et al., 2015).

2.5. Objectives of the present study

For this study, we aimed to depict the tumor-induced functional reorganization of the motor cortex. Hence, we investigated the ipsilesional, nTMS-based motor maps in a cohort of 100 patients with brain tumors located in or adjacent to motor eloquent areas. We questioned

whether different tumor locations based on anatomical structures (tumor in the temporal lobe, the frontal lobe, in the PrG, in the PoG, or in the parietal lobe) cause different patterns of reorganization of the motor cortex.

In the following, we show that nTMS is able to not only locate the motor cortex spatially; it is able to detect mono- and polysynaptic projections within the motor cortex as well. Furthermore, we investigate patterns of reorganization of the motor cortex based on clinical factors, such as the above mentioned lesion location, yet also other factors, such as hemisphere dominance, or patient's motor deficit. Finally, we integrate our findings with evidence for cortical plasticity reported by other groups and validate the clinical impact of our data.

3. MATERIALS AND METHODS

3.1. Ethics approval

This study was designed and performed in accordance with ethical standards of Klinikum rechts der Isar of Technische Universität München and the Declaration of Helsinki from the year 1964. Our protocols were approved by our university's ethics committee, registration number: 2793/10. All our patients were informed about the risks and benefits of all procedures; of MRI scan by an experienced radiologist and of the nTMS investigation by the study investigator and the neurosurgeon in charge. The patients had the opportunity to discuss all their concerns before giving written consent to the exams. All patients agreed to the anonymized use of their personal data and information for purposes of this study.

3.2. Study design

This is an explorative, non-randomized, non-blinded study for prospective investigation of the motor cortex location in a cohort of 100 patients with brain tumors by use of nTMS.

3.3. Patient characteristics

A cohort of 100 patients with intra-axial brain tumors was enrolled in the study. All patients were scheduled for operative resection of their brain tumor at the Department for Neurosurgery, Klinikum rechts der Isar, between 2011 and 2013. Inclusion criteria consisted of age above 18 years, written informed consent, and brain tumor in or adjacent to motor eloquent areas. Motor eloquence was determined according to the preoperative MRI scan and consisted of tumors within or adjacent to the PrG, tumors within or adjacent to the corticospinal tract (CST), and patients with motor deficit and therefore impairment of motor pathways by tumor. Exclusion criteria were age under 18 years, general nTMS exclusion criteria, such as cochlear implant, pacemaker, deep brain stimulation electrodes, inability to elicit MEPs by nTMS, and decline of consent.

We performed our analysis among all 100 patients and among subgroups of patients, according to their anatomical tumor location. We distinguished between tumors in the frontal lobe, which includes the PMC, tumors in the PrG as the origin of the CST, tumors in the PoG, tumors in the remaining parietal lobe, and in the temporal lobe. We differentiated the PoG from the remaining parietal lobe because of its many projections to the PrG for sensory control of movement (Borich et al., 2015; Darian-Smith et al., 1993; Donoghue & Parham, 1983). Due to

the distance of temporal tumors from the PrG and the CST, we considered the subgroup of patients with temporal tumors as control group in this analysis.

To ensure comparability of our subgroups, further 7 factors were inspected in addition to tumor location: patient's age, gender, tumor entity, tumor site, hemisphere dominance, motor deficit, and previous surgeries (Tab. 1). Patient's age, gender (male vs. female), tumor entity (WHO° II, WHO° III, WHO° IV tumor, metastases, or other tumor entities), and previous brain tumor surgeries were extracted from our department's electronic medical record system. Patient's handedness (right-handed vs. left-handed) was determined according to a standardized questionnaire, but failed in 5 patients due to language difficulties. Tumor site (right vs. left) and tumor location (temporal, frontal, PrG, PoG, and parietal) were determined by the study investigator from the preoperative MRI scan. Patient's handedness and the tumor site allowed the assessment of the tumor hemisphere dominance (motor dominant hemisphere [DH] vs. non-dominant hemisphere [NDH]). Motor function was evaluated by the study investigator at the beginning of the actual nTMS-based motor mapping according to a standardized protocol; the strength of specific muscle groups in the upper and lower extremity was tested and a score was assessed according to the British Medical Research Council (BMRC) scale. BMRC score of 5/5 reflected no motor deficit; BMRC score < 5/5 in at least one muscle group reflected motor deficit.

		Temporal	Frontal	PrG	PoG	Parietal	Overall
Group size		5	24	35	17	19	100
Mean Age ± SD (years)		44.60 ± 13.47	51.54 ± 13.07	59.89 ± 14.79	45.24 ± 14.14	57.05 ± 13.01	54.09 ± 14.78
Gender	**M**	20%	63%	54%	65%	58%	57%
	F	80%	38%	46%	35%	42%	43%
Tumor Entity	**II**	20%	29%	11%	12%	-	14%
	III	-	13%	6%	12%	16%	10%
	IV	60%	29%	29%	35%	63%	38%
	MET	-	13%	37%	35%	16%	25%
	OTH	20%	17%	17%	6%	5%	13%
Tumor Site	**R**	60%	58%	46%	76%	74%	60%
	L	40%	42%	54%	24%	26%	40%
Hemisphere Dominance	**DH**	40%	50%	49%	35%	26%	42%
	NDH	60%	50%	49%	53%	63%	53%
Motor Deficit	**yes**	20%	17%	37%	35%	21%	28%
	no	80%	83%	63%	65%	79%	72%
Previous Brain Tumor Surgeries	**0**	60%	71%	83%	76%	84%	78%
	≥ 1	40%	29%	17%	24%	16%	22%

Tab. 1: Patient characteristics in subgroups of patients according to tumor location (tumor in the temporal lobe, frontal lobe, in the PrG, PoG, and the parietal lobe), and overall; data on hemisphere dominance were not available for 5 patients, therefore results don't sum up to 100%; abbreviations: SD = standard deviation, M = male, F = female, II = WHO II° tumor, III = WHO III° tumor, IV = WHO IV° tumor, MET = metastasis, OTH = other tumor entity, R = right, L = left, DH = motor dominant hemisphere, NDH = non-dominant hemisphere, 0 = no previous brain surgeries, ≥ 1 = at least 1 previous brain surgery

3.4. MRI

All patients obtained a preoperative MRI scan, which contained multiple sequences for clinical purposes and a T1-weighted 3-dimensional (3D) gradient echo sequence (TR 9 ms, TE 4 ms, 1 mm² isovoxel covering the whole head, 6 min 58 sec acquisition time) with and without intravenous administration of gadopentetate dimeglumine (Magnograf, Jenapharm GmH & Co. KG, Jena, Germany) for the uses with intraoperative and preoperative nTMS navigation. The MRI scanner was a 3 Tesla scanner in combination with an 8-channel phased array head coil (Achieva 3T, Philips Medical Systems, The Netherlands).

3.5. nTMS-based motor mapping

3.5.1. Neuronavigation of the nTMS system

Each patient underwent an nTMS-based motor mapping several days before a scheduled tumor resection surgery (Fig. 10). The 3D gradient echo sequence from the patient's preoperative MRI scan was transferred to the nTMS system (eXimia 3.2 and eXimia 4.3, Nexstim Plc, Helsinki, Finland) as DICOM export file to allow for navigated motor mapping.

Fig. 10: Experimental setup of the nTMS-based motor mapping from the investigator's perspective; the camera in the front records the patient's real time head position (represented by the head model); during mapping, the patient is sitting in a comfortable chair in order to allow for muscle relaxation; muscle activity is monitored via EMG (right screen); the investigator uses the magnetic coil to stimulate areas of the patient's brain (left screen)

The nTMS neuronavigation system consists of a stereotactic infrared camera (Polaris Spectra, Waterloo, Ontario, Canada), glasses with tracking units which mark the patient's head position, and a pointer with tracking units which allows detailed targeting (Figs. 11 & 12). The stimulation coil also contains tracking units (Fig. 11). The camera registers the position of the tracking units and in doing so, the system correlates the positions of patient's head and the pointer or stimulation coil to the structural MRI sequence in any moment during the examination. This is continuously visualized in the user interface, so that the investigator is able to target specific structures of the brain for stimulation (Fig. 11)

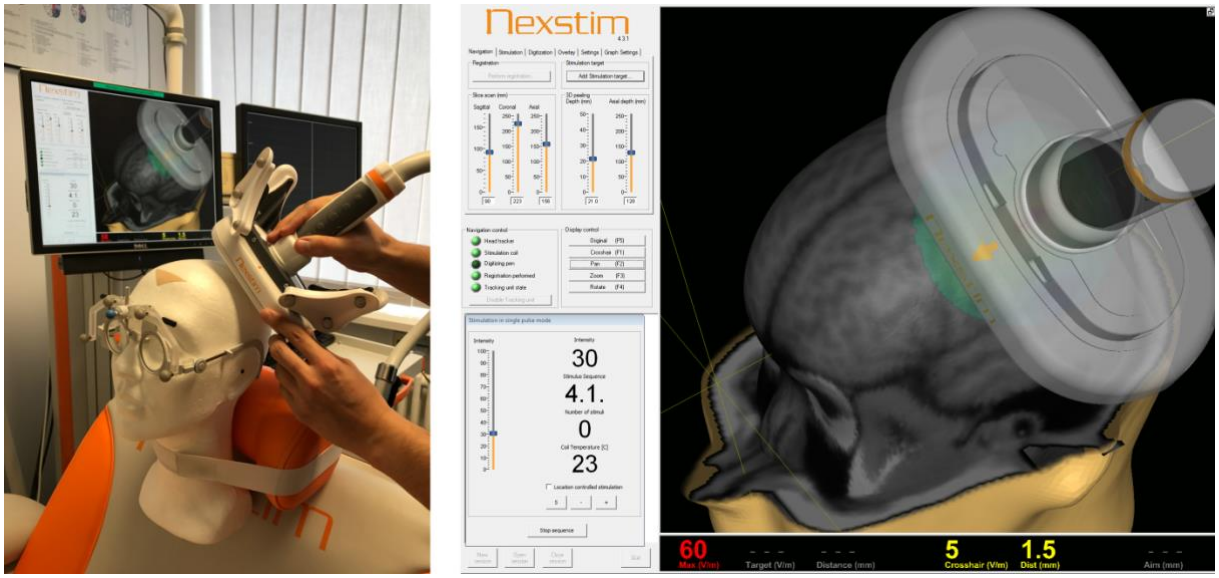

Fig. 11: Neuronavigation of the nTMS system; the head model represents the patient's head position; the patient is wearing glasses with tracking units, coil tracking units are also visible (left image); a camera (not in the picture) registers the position of the patient's head and the coil and superimposes the relative position of the coil on the 3D MRI sequence (right image)

To correlate positions of these structures, the 3D MRI sequence is co-registered with the patient's head position, marked by the glasses. To do so, the investigator locates specified anatomical landmarks, the nasion, left auricular and right auricular point, and 9 points along the patient's scalp, in the patient's MRI sequence and registers these points with equivalent points along the patient's head (Fig. 12).

Fig. 12: Co-registration procedure of the nTMS system; the patient is wearing glasses with tracking units and the investigator is holding a pointer with tracking units (left image); a camera (not in the picture) monitors the positions of the patient's head and the pointer; the investigator registers the location of 12 anatomical landmarks from the structural MRI scan (right image) to equivalent points along the patient's head (left image); landmarks consist of the nasion, left and right auricular points (3 red crosshairs in the upper section, right image) and 9 preset points along the scalp (brown circles, lower section, right image).

3.5.2. Determination of resting motor threshold

The first part of the actual motor mapping is the determination of the resting motor threshold (rMT), which is defined as the lowest stimulation intensity that elicits MEPs over 50 mV in amplitude in a relaxed abductor pollicis brevis muscle (APB) in at least 5 out of 10 stimulation trials (Rossini et al., 1994; Rossini et al., 2015). The nTMS system uses a biphasic figure-of-8 magnetic coil with a diameter of 50 mm for stimulation. It was shown that a figure-of-8 magnetic coil, the combination of 2 coils, induces a much more focal electric field than a single coil (Hallett, 2000). The muscle activity is being continuously monitored by EMG using pregelled surface electrodes (Neuroline 720, Ambu, Ballerup, Denmark). To determine the rMT, the investigator first needs to locate the motor hotspot, the site where stimulation elicits MEPs with highest amplitudes (Fig. 13). The motor hotspot of the hand is located in the handknob; an area in the middle of the PrG (Fig. 8, Fig. 13) (Campero et al., 2011).

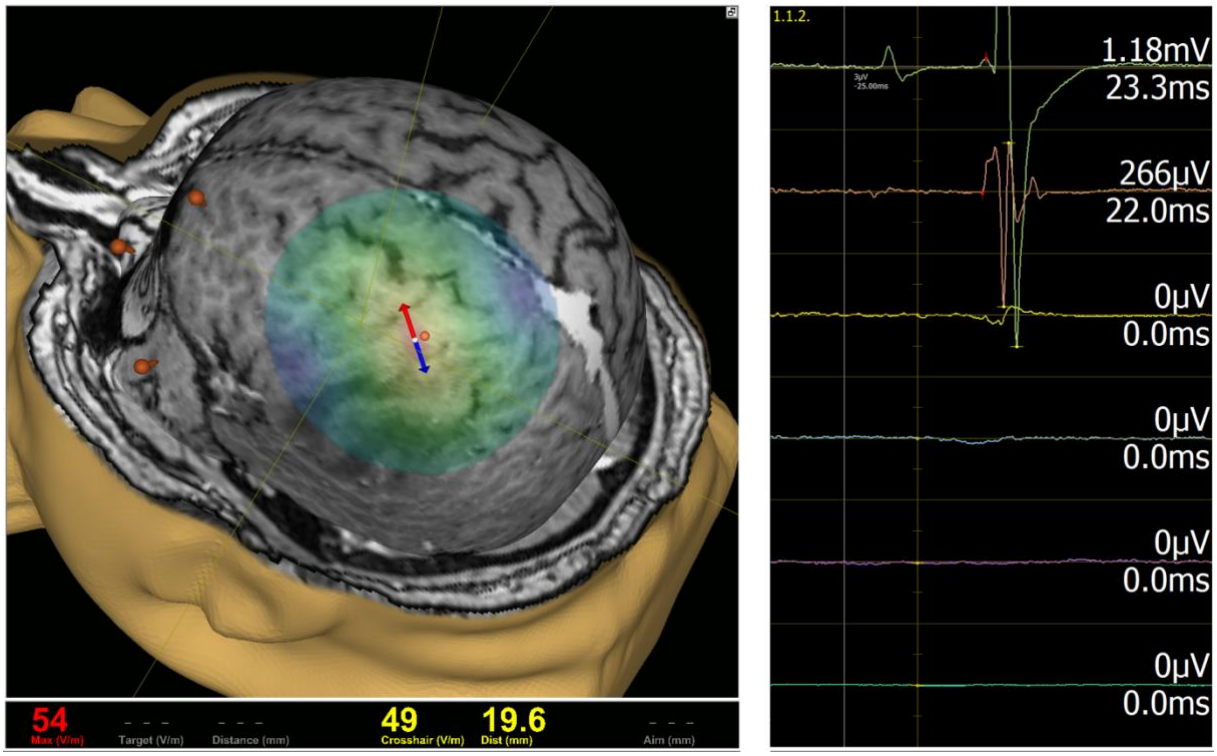

Fig. 13: Stimulation of the hotspot; left image: white dot represents the stimulation site with highest MEP amplitudes in a relaxed APB, the "hotspot", the orange dot next to the white dot represents the approximate location of the handknob; right image: a detail of the EMG recording, the green line shows the activity of the APB, the values show the MEP amplitude and latency

Lowest thresholds and strongest MEP responses can be achieved by orienting the electric field parallel to the corticospinal neurons (Day et al., 1989; Rossini et al., 1994; Rossini et al., 2015). In regards of anatomical landmarks, this means that the electric field should be oriented tangential to the scalp, in the posterior-anterior direction, perpendicular to the orientation to the central sulcus (Hallett, 2000; Rossini et al., 2015; Ruohonen & Karhu, 2010). With regards to individual factors, we determined the adequate orientation by stimulating the motor hotspot with the original, perpendicular, orientation as well as with orientations up to +45° and -45° to the original orientation (Fig. 14). The angle at which stimulation elicited MEPs with highest amplitudes was used for determining the rMT in the next step. Correspondingly to literature, the perpendicular orientation elicited the strongest responses in all cases. Motor mapping was then performed with an orientation of the electric field perpendicular to the gyrus (Rossini et al., 2015; Ruohonen & Karhu, 2010).

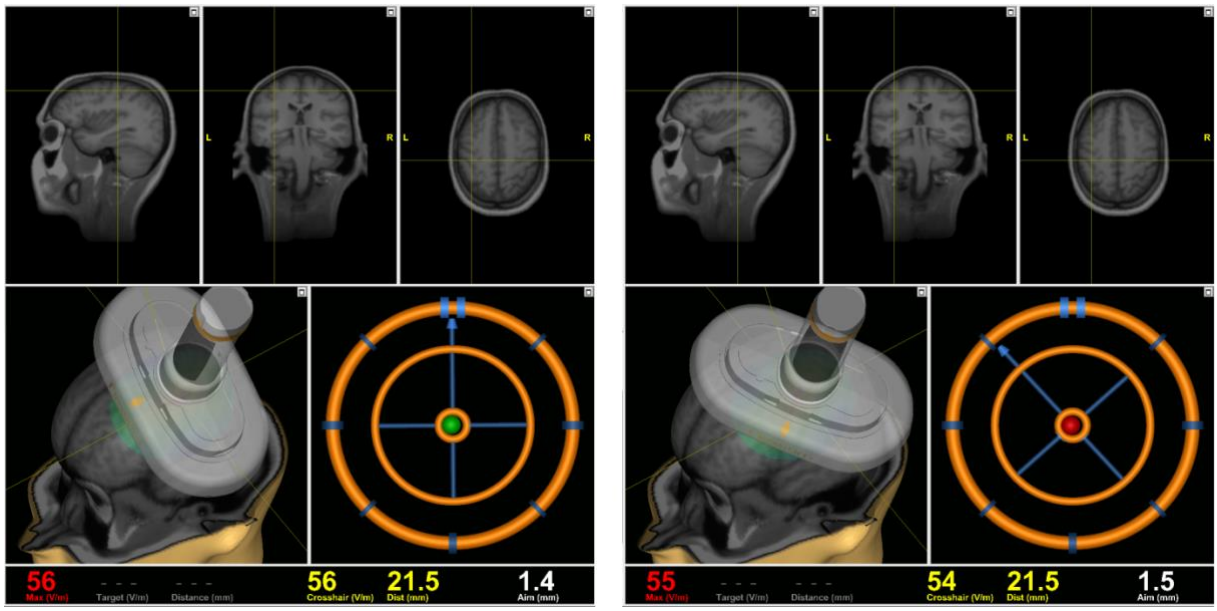

Fig. 14: Determination of the best electric field orientation; the investigator orients the coil perpendicular to the mapped gyrus, the PrG (left image), and -45° to the perpendicular orientation (right image), visible in the targeting tool in the right lower part of both images; the orientation of the coil and the electric field is visible by the orange arrow on front of the magnetic coil

The final rMT determination was performed over the hotspot with the determined coil orientation using the built in automated algorithm. The nTMS system automatically increases the stimulation intensity, if stimulation doesn't elicit MEPs over 50µV in amplitude, and decreases the intensity, if the MEP amplitude is higher, until the lowest stimulation intensity which still elicits MEPs over 50µV in a relaxed APB muscle is found, giving the patient's individual rMT. This is performed over maximum of 30 stimulation trials. For this step, it is crucial that the investigator visually checks the relaxation of the APB in the EMG; otherwise the automated determination might give a falsely low rMT (Fig. 15).

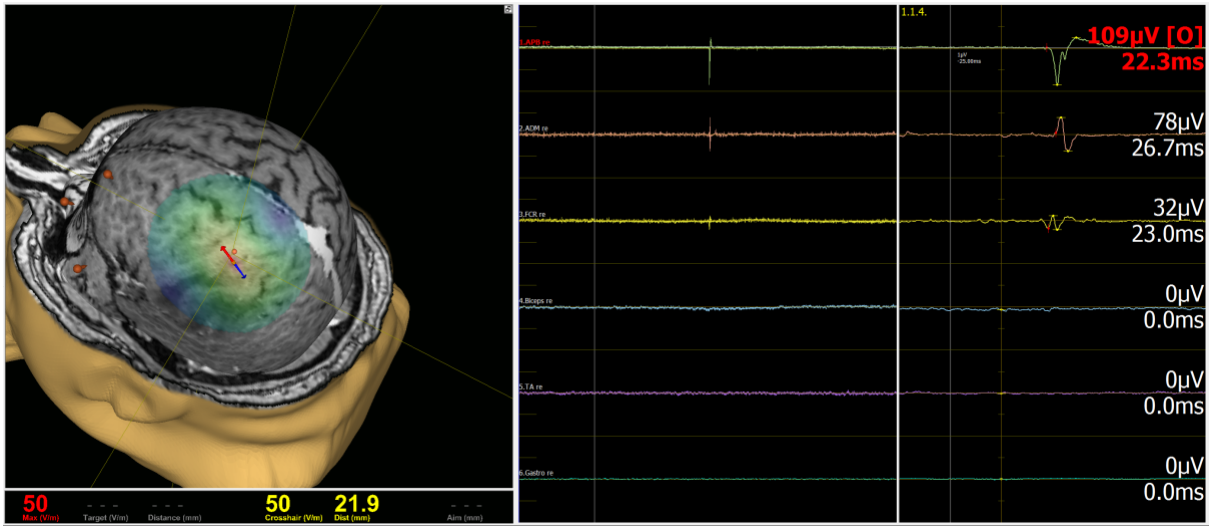

Fig. 15: Determination of the rMT; left image: the investigator stimulates the hotspot, the built in algorithm adapts the stimulation intensity until rMT is determined; middle and right image: EMG recordings, the green line depicts the APB activity; middle section shows muscle activity before and after stimulation, the flat green line indicates a good relaxation of the APB; right section gives a detailed view of the respective MEP (APB highlighted red)

3.5.3. Localizing the motor cortex

The actual mapping of the ipsilesional motor cortex was performed using stimulation intensities of 110% rMT for upper and a minimum of 130% rMT for lower extremity. Pregelled electrodes were placed over APB, abductor digiti minimi muscle (ADM), flexor carpi radialis muscle (FCR), biceps muscle (BCS), tibialis anterior muscle (TA), and gastrocnemius muscle (GCN) contralateral to the mapped hemisphere. The investigator started stimulating upper extremity motor areas around the hotspot and expanded the stimulation in all directions, until no motor positive stimulation sites were found. A motor positive stimulation site was defined as the site at which stimulation elicited MEPs over 50 µA in amplitude in at least 1 relaxed muscle. The electric field was oriented perpendicularly to the closest sulcus. The aim was to obtain a map of the motor cortex with a maximum distance of 1 cm between single stimulation sites, and a border consisting of 2 rows of motor negative stimulation sites (Fig. 8). Mapping of the lower extremity was started in the superior part of the PrG and expanded to adjacent gyri. If no response was found, the stimulation intensity was increased, as lower extremity motor cortex is located in deeper structures and higher stimulation intensities are needed for activation (Rossini et al., 2015). After each mapping session, the automated amplitude and latency determination of the nTMS systems was manually checked and MEPs were adjusted or removed, if falsified, visible for example by not sufficient relaxation of the muscle before

stimulation, or by MEP latency values that did not correspond to reference values from literature (Rossini et al., 1994; Rossini et al., 2015).

3.6. Statistical analysis

Our statistical analysis was conducted in 2 parts. First, we visualized the spatial location of the nTMS-based motor maps in subgroups of patients according to their tumor location. Second, we used MEP latencies to distinguish between short, monosynaptic and long, polysynaptic projections. We investigated whether clinical factors, such as tumor location, hemisphere dominance, and motor deficit, cause different patterns of distribution of polysynaptic latencies among the mapped gyri (PrG, superior frontal gyrus [SFG], middle frontal gyrus [MFG], PoG).

3.6.1. Spatial location

We visualized the spatial location of motor cortex using the SPM8 software (Functional Imaging Laboratory, Wellcome Trust Center for Neuroimaging, Institute of Neurology, UCL, London, UK) which runs under MATLAB (The MathWorks, Inc., Natick, MA, USA). To enable an interindividual comparison of motor maps, all maps were spatially normalized to fit Montreal Neurological Institute (MNI) standard space. To do so, we exported each patient's 3D gradient echo sequence and motor map, separately for upper and lower extremity mapping, into Analyze or NifTi file format using MRIcro software (Brett et al., 2001) (McCausland Center for Brain Imaging, Columbia SC, USA). To allow for exclusion of tumorous tissue from the normalization process, a mask of the tumor was created in the same step by masking the tumorous volume in each axial slice and exporting the tumor mask in the same file formats.
In the next step, we performed the normalization. Here, SPM8 was used to automatically determine a spatial transformation matrix which would convert a patient's individual MRI sequence into MNI standard space, disregarding voxels from the tumor mask. The same spatial transformation matrix was used for normalizing the patient's motor maps. The normalized MRI sequences were visually checked for congruence with the MNI standard space template included with the SPM8 software, considering anatomical landmarks such as the cranium and ventricles. To enhance the congruence, if necessary, the normalization process was repeated after adjusting the position of the 3D MRI sequence or, if the previous procedures were unsuccessful, without masking the tumor.
We present our results as heat maps of upper and lower extremity motor maps among our subgroups (tumor in the temporal lobe, frontal lobe, the PrG, PoG, and the parietal lobe). The white areas indicate highest overlap of motor function in the specific subgroup, the black areas

indicate lowest overlap. This allows us to depict varying locations of motor cortex according to different tumor locations. The heat maps were superimposed on the MNI standard space template and are displayed in 3D view created by MRIcron (McCausland Center for Brain Imaging, Columbia SC, USA).

3.6.2. MEP latency analysis

The nTMS-derived MEP latencies were analyzed considering their location in a specific gyrus (PrG, SFG, MFG, and PoG). The Shapiro-Wilk test showed a non-normal distribution of MEP latencies, which was confirmed by visualization of the distribution of MEP latency frequency histograms. First, we performed an overall analysis considering MEP latencies of all 100 patients. Kruskal-Wallis test for nonparametric distributions and Dunn's test for multiple comparisons of ranks as post-hoc test were conducted to test differences in mean MEP latencies between the mapped gyri (PrG, SFG, MFG, PoG) for each of the stimulated muscles (APB, ADM, FCR, BCS, TA, GCN). Second, we hypothesize that long latencies, i.e. MEP latencies longer than 1 standard deviation (SD) above mean MEP latency, are transmitted vie more than 1 synapse and therefore represent polysynaptic projections. We conducted a chi-square test to test for different distributions of polysynaptic projections among the mapped gyri (PrG, SFG, MFG, PoG) in subgroups of patients according to tumor location (tumor in the temporal lobe, frontal lobe, the PrG, PoG, and the parietal lobe), hemisphere dominance (DH vs. NDH), and a patient's motor deficit (no motor deficit vs. motor deficit). The distributions were tested separately for each muscle as well as combined for all muscles. All results are presented as mean ± SD. The level of significance was set at $p < 0.05$ for each statistical test. Statistical testing and visualization of results as graphs was performed using GraphPad Prism software (GraphPad Prism 6.05, La Jolla, CA, USA) and SPSS (IBM SPSS Statistics for Windows, Armonk, NY, USA).

4. RESULTS

Parts of this work were published previously and are presented here with kind permission of the Journal of Neurosurgery (Bulubas et al., 2016).

4.1. General considerations

nTMS mapping was performed successfully in all patients without any adverse events. The average rMT intensity was 33.83 ± 9.26% of maximum stimulator output. 4,712 motor positive stimulation sites were determined all together, with 47.12 ± 27.72 stimulation sites per mapping. The stimulation elicited 8,794 MEPs in 6 muscles (APB, ADM, FCR, BCS, TA, GCN) by stimulating 6 gyri (PrG, SFG, MFG, PoG, superior parietal lobule, inferior parietal lobule). 87.94 ± 64.45 MEPs were elicited per mapping on average. Stimulation of 1 stimulation site elicited MEPs in 1.87 muscles on average.

Our main objective was to perform an analysis in subgroups considering the patient's anatomic tumor location (tumor in the temporal lobe, frontal lobe, the PrG, PoG, and the parietal lobe). There were no statistically significant differences between these subgroups considering their gender, tumor entity, tumor site, hemisphere dominance, motor deficit, or previous surgeries. However the groups differed considering the patients' mean age (p = 0.003; Fig. 16). The post-hoc test showed statistical significance only for the comparison of patients with tumors in the PrG and the PoG (p = 0.005).

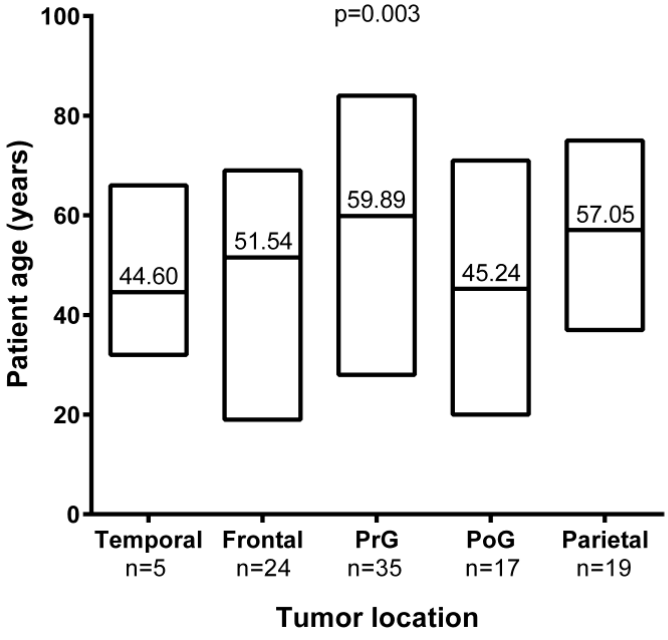

Fig. 16: Box plot displaying the age distribution in subgroups of patients according to their tumor location; the line represents mean age; patients with tumors in the PrG were older than patients with tumors in the PoG

4.2. Spatial location of nTMS-based motor maps

Heat map of upper extremity motor maps showed that the highest overlap of motor areas, represented by white/ yellow areas, was located in the handknob in the PrG, as expected (Fig.17 & 18). Yet individual motor areas spread widely around the handknob and adjacent areas, represented by red/black areas. Especially the dorsal parts of the MFG and the SFG seemed to contain motor representations in many patients, visible by white/yellow areas (Fig.17 & 18).

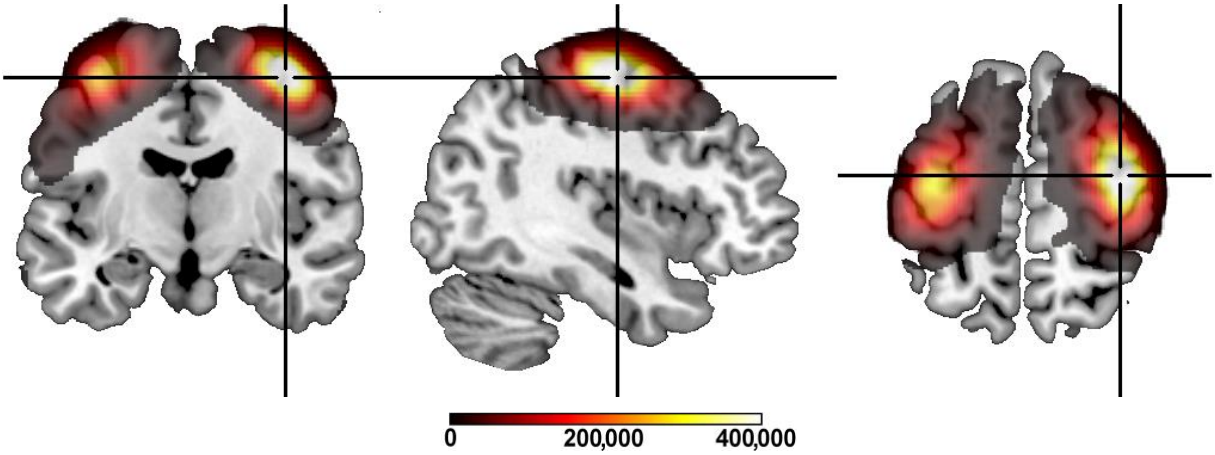

Fig. 17: Figure showing the heat map of upper extremity maps of all 100 patients in plane view; maps are superimposed on a standard brain template; white areas show highest overlap, black areas show lowest overlap of motor maps; left: coronal plane, middle: sagittal plane, right: axial plane

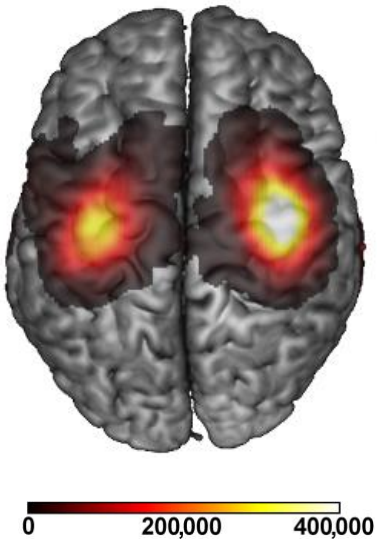

Fig. 18: Figure showing the heat map of upper extremity maps of all 100 patients in 3D view; maps are superimposed on a standard brain template; white areas show highest overlap, black areas show lowest overlap of motor maps

Considering each patient's tumor location, we observed that motor maps in patients with temporal tumors and tumors in the PrG were located rather closely together, resulting in compact heat maps. Patients with frontal and parietal tumors showed a rather wide spread of motor maps, with a spread along the anterior-posterior direction in the frontal subgroup and a spread into anterior and lateral areas in the parietal subgroup (Fig. 19).

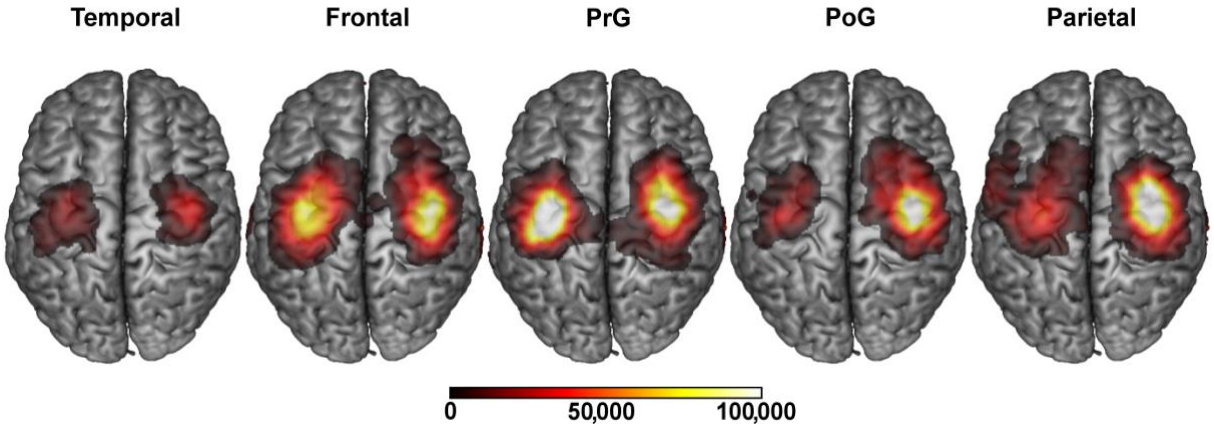

Fig. 19: Figure showing heat maps of upper extremity maps in subgroups of patients according to their tumor location in 3D view; maps are superimposed on a standard brain template; white areas show highest overlap, black areas show lowest overlap of motor maps

Lower extremity motor maps were available in 33 patients. Heat map of lower extremity motor maps showed highest overlap of motor function in the superior part of the PrG, as expected, with motor maps spreading mainly in the anterior-posterior direction, into the SFG and PoG (Fig. 20).

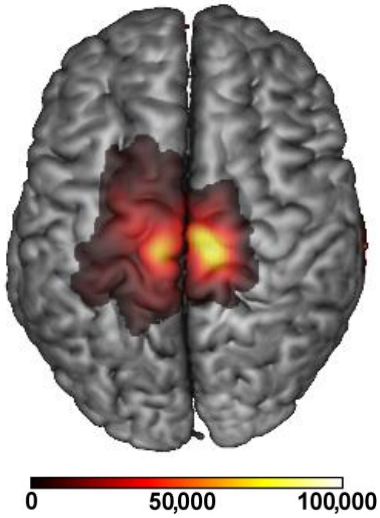

Fig. 20: Figure showing the heat map of lower extremity maps of 33 patients in 3D view; maps are superimposed on a standard brain template; white/yellow areas show highest overlap, black areas show lowest overlap of motor maps.

Considering distribution of lower extremity maps among tumor location subgroups, more than 2 maps were available only for patients with tumors in the frontal lobe (n = 11), in the PrG (n = 12), and in the PoG (n = 6). According to upper extremity motor maps, patients with tumors in the PrG and the PoG showed maps located closely to each other, while patients with frontal tumors showed a wide spread of lower extremity motor maps (Fig. 21).

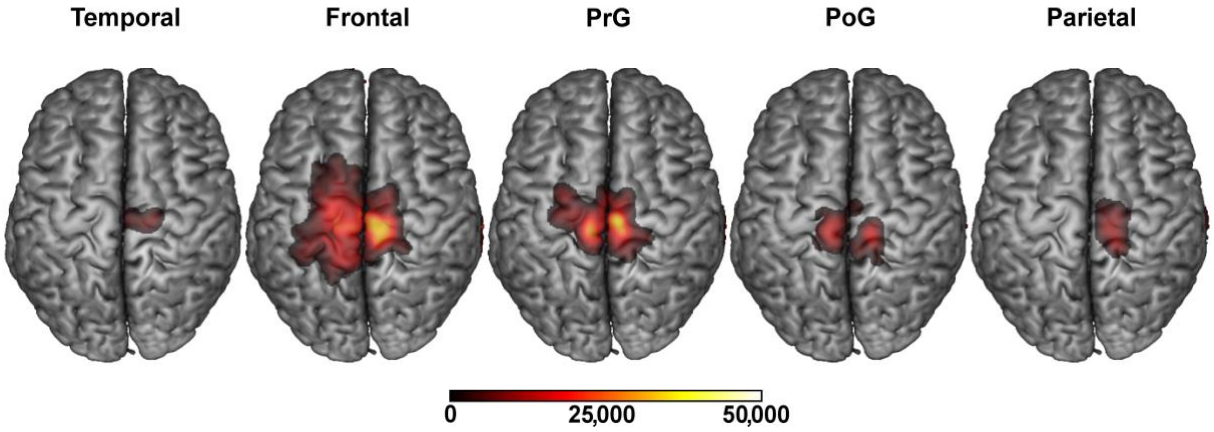

Fig. 21: Figure showing heat maps of lower extremity maps in subgroups of patients according to their tumor location in 3D view; maps are superimposed on a standard brain template; white areas show highest overlap, black areas show lowest overlap of motor maps.

4.3. MEP latency analysis

4.3.1. Distributions of MEPs and MEP latencies

As only 20 MEPs of the total 8,794 MEPs were elicited by stimulating the parietal lobes, they were excluded from the MEP latency analysis. This limits the analysis to 8,774 MEPs, elicited by stimulating 4 gyri (PrG, SFG, MFG, PoG). The majority of MEPs was found when stimulating the PrG (Fig. 22) and most of them were elicited in one of the hand muscles (APB, ADM; Fig. 23)

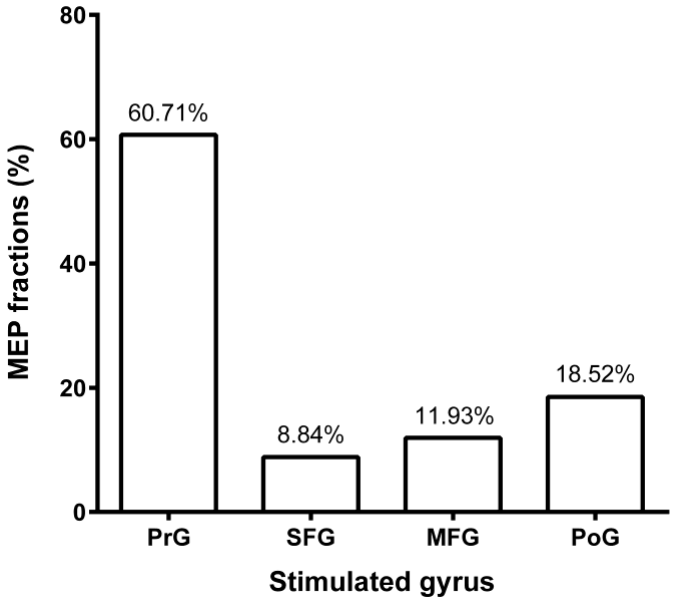

Fig. 22: Bar chart displaying MEP fractions in percent of overall MEPs elicited by stimulating one of the 4 mapped gyri (PrG, SFG, MFG, PoG).

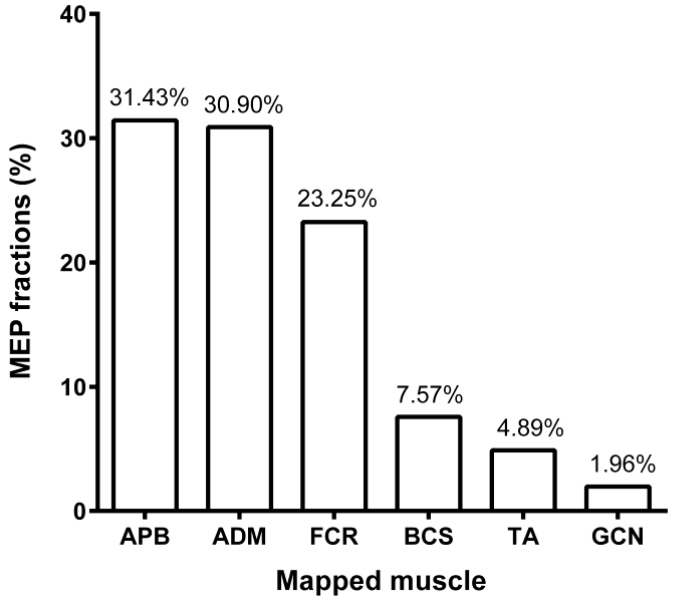

Fig. 23: Bar chart displaying MEP fractions in percent of overall MEPs elicited in one of the 6 mapped muscles (APB, ADM, FCR, BCS, TA, GCN).

The mean MEP latencies were longest for lower extremity muscles (TA, GCN), followed by hand muscles (APB, ADM) and more proximal upper extremity muscles (FCR, BCS; Fig. 24, Tab. 2).

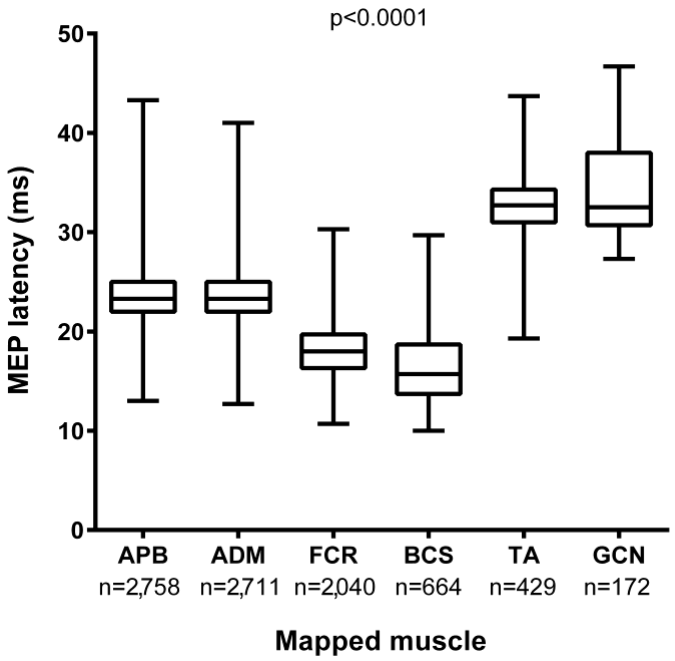

Fig. 24: Box-and-whiskers plot displaying median MEP latencies, 25 & 75 percentiles, and the range of MEP latencies (ms) elicited in one of the 6 mapped muscles (APB, ADM, FCR, BCS, TA, GCN.)

	APB	ADM	FCR	BCS	TA	GCN
Mean MEP latency ± SD (ms)	23.54 ± 2.54	23.72 ± 2.89	18.21 ± 2.54	16.17 ± 3.19	32.67 ± 3.01	34.15 ± 4.17
Median MEP latency (ms)	23.30	23.30	18.00	15.50	33.00	32.50

Tab. 2: Mean MEP latencies ± SD and median MEP latencies of single muscles.

Considering the distribution of mean MEP latencies among the stimulated gyri, most of the upper extremity muscles showed lowest mean MEP latencies in the PrG (ADM, FCR, BCS) and longest mean MEP latencies in frontal gyri (SFG & MFG: ADM, FCR, BCS, TA; Fig. 25, Tab. 3). APB mean MEP latencies were longer in the MFG or PoG, and shorter in the SFG, compared to the PrG (Fig. 25, Tab. 3). These distributions were statistically significant only for ADM and FCR (Tab. 3).

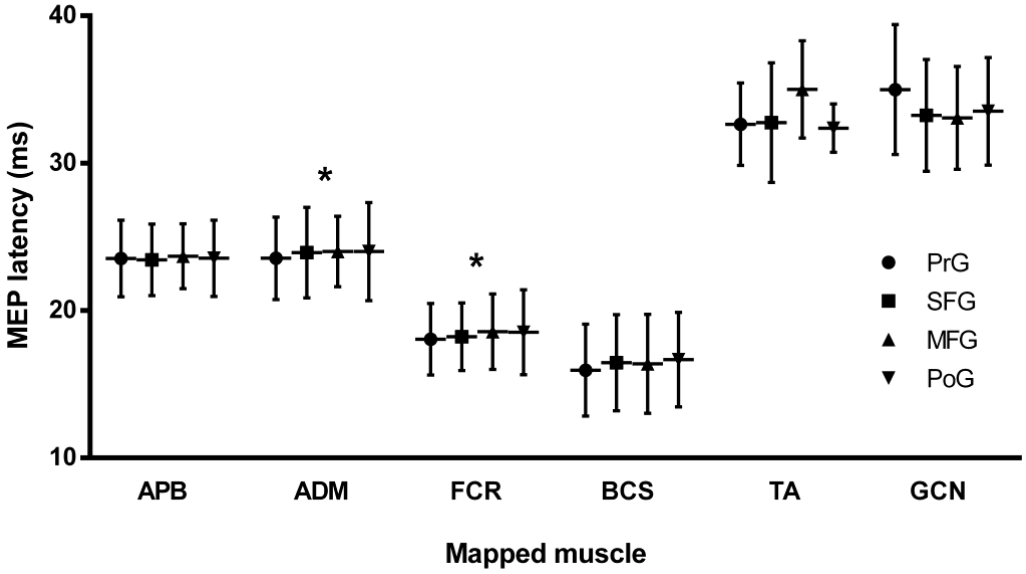

Fig. 25: Plot displaying mean MEP latencies ± SD (ms) separately for every gyrus and every muscle (p<0.05).*

	PrG	SFG	MFG	PoG	p
APB	23.53 ± 2.60	23.43 ± 2.43	23.69 ± 2.20	23.54 ± 2.59	0.34
ADM	23.54 ± 2.79	23.93 ± 3.07	24.00 ± 2.39	24.00 ± 3.33	0.0013
FCR	18.05 ± 2.43	18.22 ± 2.29	18.56 ± 2.56	18.53 ± 2.88	0.025
BCS	15.95 ± 3.12	16.46 ± 3.26	16.38 ± 3.35	16.67 ± 3.20	0.17
TA	32.64 ± 2.80	32.75 ± 4.06	35.00 ± 3.30	32.37 ± 1.64	0.23
GCN	34.99 ± 4.41	33.24 ± 3.79	33.07 ± 3.49	33.52 ± 3.65	0.12

Tab. 3: Mean MEP latencies ± SD (ms), shown separately for every muscle and every gyrus; we report significance levels for differences in mean MEP latencies between the mapped gyri for each muscle separately.

4.3.2. Distributions of polysynaptic projections, determined by long MEP latencies

In many patients, the frequency distributions of the individual MEP latencies showed a non-normal distribution, with many long latency MEPs. This effect was visible also when considering MEP latencies of all patients together (Fig. 26).

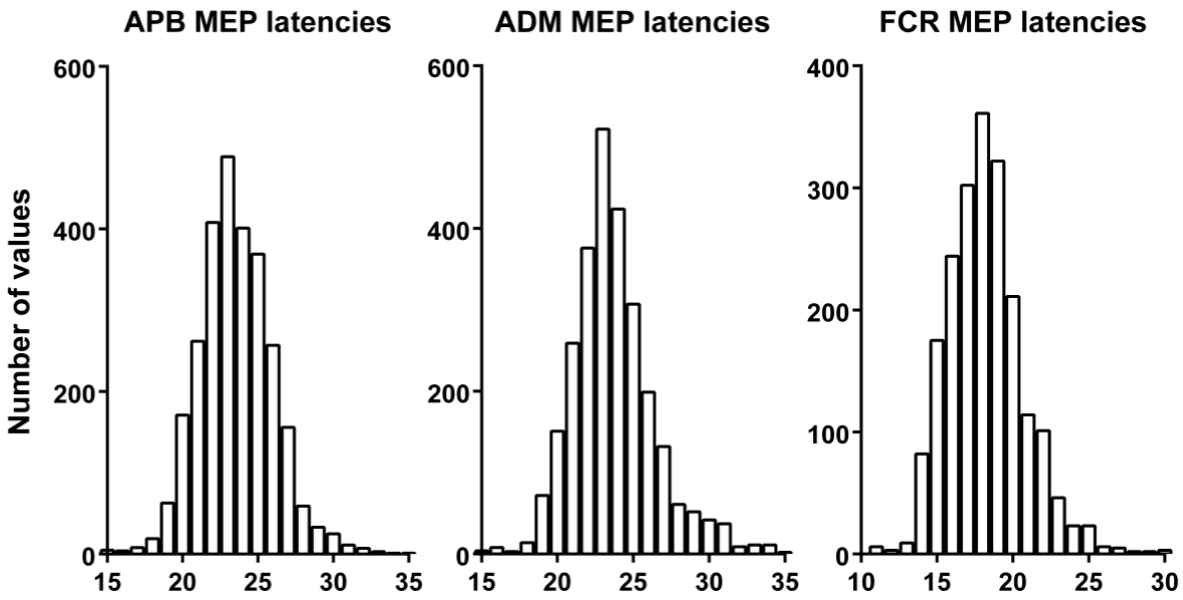

Fig. 26: Histograms showing frequency distributions of all MEP latencies of APB, ADM, and FCR, the muscles with highest MEP counts; many long latency MEPs were found, best visible in frequency distributions of ADM.

We hypothesized that these long latency MEPs, which we later defined as latencies longer than 1 SD above mean MEP latency, reflect polysynaptic projections, i.e. pathways that go through more than 1 synapse. We used the percentage of MEPs longer than 1 SD above mean MEP latency to calculate the percentage of polysynaptic projections. We originally calculated these polysynaptic projections separately for every muscle and every gyrus. For example, considering APB MEPs in the PrG, we found a mean MEP latency of 23.53 ± 2.60 ms. We determined a total of 1,694 APB MEPs in the PrG and a total of 251 polysynaptic MEPs, i.e. MEPs with latencies longer than 26.13 ms. This means that 15% of APB MEPs in the PrG represented polysynaptic projections (Tab. 4). This is the basic principle for our further analysis. We used it to investigate the influence of clinical factors such as tumor location, hemisphere dominance, and motor deficit. We determined the numbers of polysynaptic MEPs separately for every muscle and every gyrus. To increase the sample size, we present the results primarily combined for all muscles, distinguishing only the gyri (and the specific clinical factor; Figs. 27 - 29). We believe this facilitates the understanding of overall effects. In a second step, we report the original distributions of polysynaptic projections separately for every muscle and every gyrus, with limitation to APB, ADM, and FCR MEP latencies due to small MEP counts in the other groups (Tabs. 5, 7, 9). Considering different counts of polysynaptic projections, it is of interest to know whether these projections have an effect on mean MEP latencies, too. Therefore we additionally report the mean MEP latencies of single muscles between subgroups according to specific conditions (Tabs. 6, 8, 10).

	PrG	SFG	MFG	PoG
APB	15%	14%	15%	15%
ADM	15%	14%	12%	15%
FCR	14%	19%	17%	14%
BCS	18%	12%	23%	18%
TA	13%	12%	25%	19%
GCN	20%	15%	17%	23%

Tab. 4: Distribution of polysynaptic projections, shown separately for every muscle and every gyrus.

4.3.3. Polysynaptic projections subject to tumor location

We compared the distribution of polysynaptic projections among the gyri (PrG, SFG, MFG, PoG) between the temporal tumor subgroup, which we considered our control group, as the tumors were located within the largest distance of the motor cortex itself, and the other tumor location subgroups (in the frontal lobe, the PrG, PoG, and the parietal lobe). In the temporal subgroup, most polysynaptic projections were found in the frontal gyri (SFG & MFG; Fig. 27). Patients with frontal tumors showed a rather equal distribution of polysynaptic projections, with rather little polysynaptic projections in the frontal gyri compared to the temporal and other groups (p [frontal] = 0.013). Distribution of polysynaptic projections in patients with tumors in the PrG didn't differ from those of the temporal subgroup (p [PrG] = 0.23*)*. It seemed as if, of all gyri, there were less polysynaptic projections in the SFG (Fig. 27). In patients with tumors in the PoG, most polysynaptic projections were found in the PrG, and least in the PoG itself (p [PoG] = 0.013*)*. Patients with parietal tumors showed little polysynaptic projection in the PrG and MFG, and relatively many in the PoG (p [parietal] = 0.0002; Fig. 27).

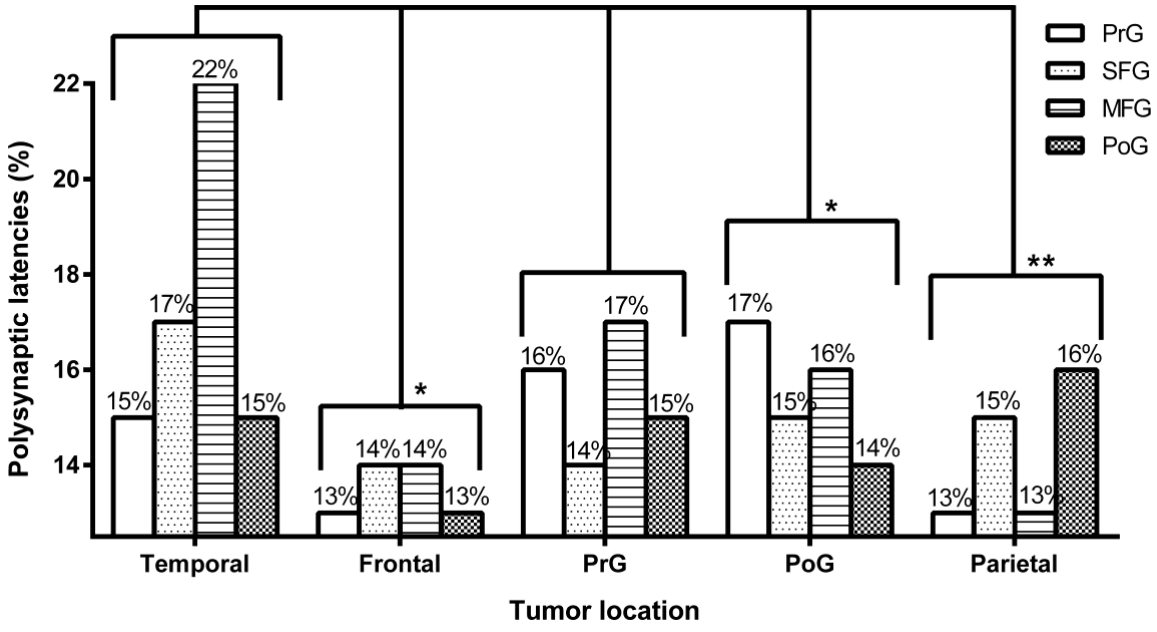

Fig. 27: Bar chart showing the distributions of polysynaptic projections among the mapped gyri between the temporal and the other tumor location subgroups (p<0.05; ** p<0.001); polysynaptic projections of all muscles were combined here*

In the single muscle analysis, most of the distributions reflected the distributions mentioned above. Statistically significant levels were achieved only for APB (Tab. 5). Here, the distribution of the temporal subgroup differed from the distribution with all muscles combined, showing clearly less polysynaptic projections in frontal gyri (SFG & MFG; Fig. 27, Tab. 5). The distributions of other subgroups reflected the above described distributions, with frontal group showing a rather homogenous distribution with most polysynaptic projections in frontal gyri; the PrG group showing little projections in the SFG (except FCR), the PoG group showing most projections in the PrG (except FCR), and the parietal group showing little projections in the PrG (except FCR; Tab. 5). Still these results should be considered with caution.

		PrG	SFG	MFG	PoG	p
APB	**Temporal**	22%	10%	13%	13%	-
	Frontal	13%	17%	14%	12%	0.0056
	PrG	14%	11%	18%	15%	0.014
	PoG	18%	15%	14%	14%	0.015
	Parietal	13%	16%	16%	17%	0.0001
ADM	**Temporal**	13%	27%	20%	9%	-
	Frontal	12%	12%	11%	14%	0.26
	PrG	17%	14%	15%	16%	0.50
	PoG	18%	7%	14%	12%	0.75
	Parietal	13%	24%	10%	15%	0.10
FCR	**Temporal**	10%	13%	40%	22%	-
	Frontal	13%	15%	15%	9%	0.45
	PrG	17%	24%	18%	15%	0.96
	PoG	13%	23%	20%	14%	0.35
	Parietal	12%	6%	13%	14%	0.85

Tab. 5: Distributions of polysynaptic projections among the mapped gyri between tumor location subgroups, separately for every muscle; we report significance levels for differences in distributions between the temporal and the other subgroups

Just like in the previous paragraph, even if we observed more polysynaptic projections for a specific muscle gyrus combination, we didn't necessarily observe increased mean MEP latencies here (Tab. 6).

		PrG	SFG	MFG	PoG
APB	**Temporal**	22.57 ± 2.05	24.20 ± 2.02	23.79 ± 1.95	21.35 ± 1.00
	Frontal	23.45 ± 2.50	24.11 ± 2.22	24.60 ± 1.95	23.52 ± 3.33
	PrG	23.74 ± 3.04	22.94 ± 2.51	22.97 ± 2.30	24.26 ± 2.53
	PoG	23.34 ± 2.14	22.80 ± 1.92	22.81 ± 1.85	23.23 ± 2.10
	Parietal	23.93 ± 2.41	24.91 ± 2.65	24.08 ± 2.08	23.43 ± 2.21
ADM	**Temporal**	22.51 ± 1.70	22.75 ± 1.74	21.81 ± 3.06	24.82 ± 2.51
	Frontal	23.76 ± 2.92	25.14 ± 3.45	24.69 ± 2.68	24.73 ± 3.95
	PrG	23.66 ± 3.02	23.23 ± 2.80	23.20 ± 1.81	24.46 ± 3.00
	PoG	22.88 ± 1.79	22.62 ± 1.43	23.30 ± 1.37	21.74 ± 2.03
	Parietal	23.89 ± 2.89	25.58 ± 3.58	24.15 ± 2.07	23.86 ± 3.12
FCR	**Temporal**	18.47 ± 1.25	19.81 ± 1.52	18.74 ± 0.95	19.54 ± 2.45
	Frontal	18.33 ± 2.71	18.72 ± 2.60	18.94 ± 2.49	18.66 ± 2.46
	PrG	17.99 ± 2.76	19.06 ± 2.41	18.36 ± 2.77	19.12 ± 3.46
	PoG	17.29 ± 1.85	17.38 ± 1.62	18.47 ± 2.30	17.07 ± 2.11
	Parietal	18.11 ± 2.08	17.60 ± 2.38	18.28 ± 2.59	18.41 ± 2.36

Tab. 6: Mean MEP latencies ± SD (ms) of tumor location subgroups separately for every muscle and every gyrus

4.3.4. Polysynaptic projections subject to hemisphere dominance

We found in general more polysynaptic projections in the DH, compared to the NDH (Fig. 28). Moreover, most of the polysynaptic projections in the DH were located in the frontal gyri ($p < 0.0001$; Fig. 28).

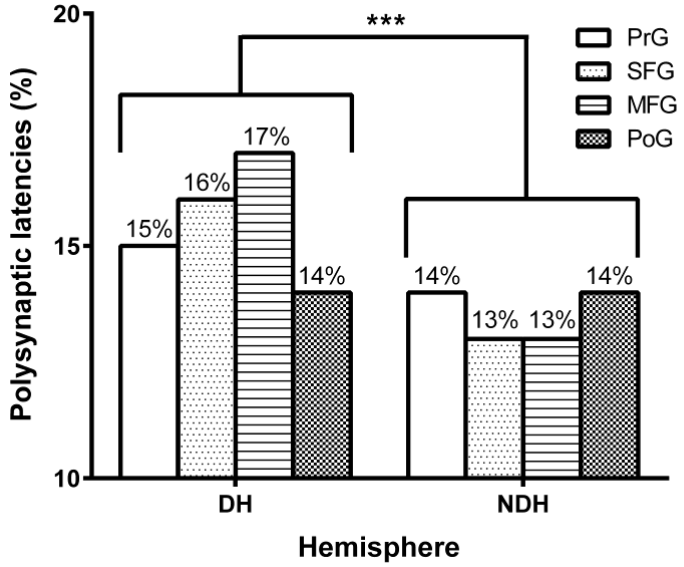

*Fig. 28: Bar chart showing the distribution of polysynaptic projections among the mapped gyri between the DH and NDH (*** p<0.0001), polysynaptic projections of all muscles were combined here*

Same patterns were observed considering polysynaptic projections of every muscle and every gyrus separately, with most polysynaptic projections in frontal gyri of the DH, either in the MFG (APB), in the SFG (ADM), or both the SFG and MFG (FCR); they were statistically significant only for ADM (Tab. 7).

		PrG	SFG	MFG	PoG	p
APB	**DH**	15%	15%	18%	12%	0.10
	NDH	13%	11%	15%	13%	
ADM	**DH**	13%	18%	13%	17%	0.011
	NDH	16%	11%	12%	15%	
FCR	**DH**	14%	20%	19%	13%	0.077
	NDH	13%	20%	12%	15%	

Tab. 7: Distribution of polysynaptic projections among the mapped gyri between the DH and NDH, separately for every muscle; significance levels reflect differences in distributions between the DH and NDH

Although more polysynaptic projections were observed for specific muscle gyrus combinations, we didn't observe an increase of mean MEP latency here (Tab. 8).

		PrG	SFG	MFG	PoG
APB	**DH**	23.73 ± 2.52	23.36 ± 2.37	23.62 ± 2.37	23.89 ± 2.77
	NDH	23.33 ± 2.69	23.54 ± 2.54	23.79 ± 1.96	23.31 ± 2.44
ADM	**DH**	23.93 ± 3.11	24.51 ± 3.44	24.22 ± 2.73	25.13 ± 3.69
	NDH	23.23 ± 2.43	22.82 ± 1.86	23.81 ± 2.03	23.17 ± 2.75
FCR	**DH**	18.23 ± 2.52	18.81 ± 2.36	18.90 ± 2.92	19.51 ± 3.36
	NDH	17.87 ± 2.36	17.30 ± 1.84	18.36 ± 2.31	17.98 ± 2.52

Tab. 8: Mean MEP latencies ± SD (ms) in the DH and NDH separately for every muscle and every gyrus

4.3.5. Polysynaptic projections subject to motor deficit

Patients with motor deficits showed similar patterns of distribution of polysynaptic projections compared to patients without motor deficit, with most polysynaptic projections in the frontal gyri. Patients with motor deficits showed slightly less polysynaptic projections in the PrG ($p = 0.028$; Fig. 29).

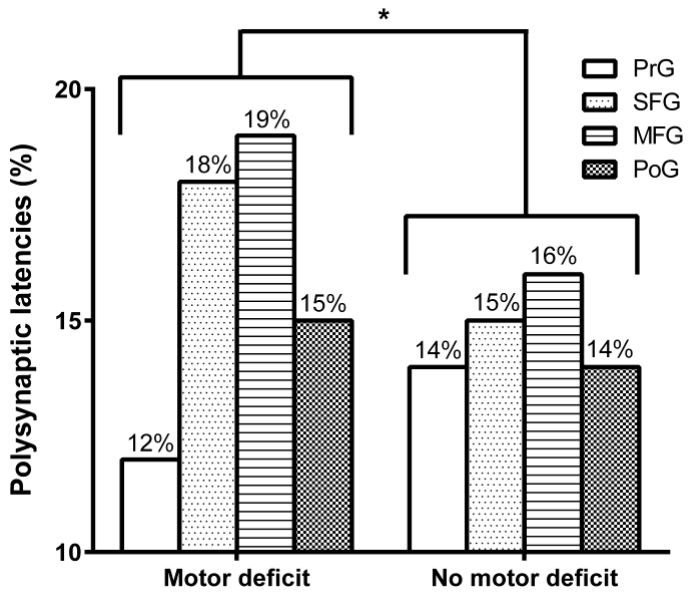

Fig. 29: Bar chart showing the distribution of polysynaptic projections among the mapped gyri between patients with and without motor deficit (p<0.05), polysynaptic projections of all muscles was combined here.*

Considering single muscles, other than in the combined analysis above, we observed varying patterns among patients with and without motor deficits (Tab. 9). APB, ADM, and FCR do not show statistically significant differences, these derive from BCS ($p = 0.017$) and TA ($p = 0.028$) MEP latencies (Tab. 9). Due to the small numbers of BCS and TA MEPs (Fig. 23), yet a wide range of distributions (Fig. 24), we do not consider these muscles reliable enough to conclude on real effects of patient's motor deficit on the distribution of polysynaptic projections.

	Motor Deficit	PrG	SFG	MFG	PoG	p
APB	**yes**	10%	5%	12%	8%	0.19
	no	14%	16%	18%	13%	
ADM	**yes**	13%	13%	19%	18%	0.53
	no	14%	17%	15%	14%	
FCR	**yes**	13%	23%	23%	14%	0.90
	no	14%	16%	14%	14%	

Tab. 9: Distribution of polysynaptic projections among the mapped gyri between patients with and without motor deficits, separately for every muscle; significance levels reflect differences in distributions between patients with and without motor deficits.

Accordingly to the above mentioned results, we didn't observe any effect of increased numbers of polysynaptic projection on mean MEP latency (Tab. 10).

	Motor Deficit	PrG	SFG	MFG	PoG
APB	**yes**	23.76 ± 2.74	23.86 ± 2.90	23.27 ± 2.19	24.33 ± 2.99
	no	23.45 ± 2.56	23.37 ± 2.35	23.77 ± 2.20	23.31 ± 2.41
ADM	**yes**	23.47 ± 3.26	24.11 ± 3.91	25.69 ± 3.58	24.73 ± 4.29
	no	23.57 ± 2.62	23.89 ± 2.80	23.69 ± 1.95	23.78 ± 2.95
FCR	**yes**	17.78 ± 2.56	17.13 ± 1.76	18.29 ± 2.03	18.66 ± 3.04
	no	18.13 ± 2.37	18.50 ± 2.33	18.60 ± 2.63	18.49 ± 2.82

Tab. 10: Mean MEP latency ± SD (ms) in patients with and without motor deficit separately for every muscle and every gyrus.

5. DISCUSSION

We were able to perform an nTMS-based motor mapping successfully and without any adverse event in all our patients. This allowed us to show again that nTMS is a feasible and tolerable method for locating the motor cortex, even in patients with brain tumors (Krieg et al., 2012b; Picht et al., 2011; Tarapore et al., 2016a).

5.1. General considerations and terminology

In literature, many terms for addressing areas of the motor cortex are in use - Brodmann areas, M1, PMC, extrapyramidal cortex, non-primary motor area, or supplementary motor cortex. Furthermore, it is possible to distinguish areas that evoke movement directly through corticospinal pathways, such as the CST, or areas that evoke movement through interconnections, or cortico-cortical projections, to other motor cortex areas (Dum & Strick, 2002). In terms of DES and nTMS, researchers often speak of excitable areas. It is important not to confuse the term “primary” and “non-primary” with terms describing the excitability of the motor cortex. DES as well as TMS elicit motor responses when stimulating areas in the PrG, as well as areas outside the PrG, in the SFG, MFG, and PoG (Foerster, 1936; Kombos et al., 1999; Penfield & Boldrey, 1937; Pitkanen et al., 2015; Teitti et al., 2008).

Usually the term M1 is used to describe the origin of CST, the posterior part of the PrG, or Brodmann area 4, whereas non-primary is understood as an area without direct projections to the spinal cord. Extrapyramidal motor cortex, PMC, supplementary motor cortex, or Brodmann area 6 are often used synonymously in literature to describe the non-M1; with special confusion considering the term supplementary motor cortex, due to the differentiation of the SMA, which is just one part of the PMC. Studies on primates showed that as well the areas in the PrG, as areas in the PMC contain cortico-cortical and corticospinal projections (Dum & Strick, 2002). Due to this confusing nomenclature, we preferably use the gyral anatomy to refer to the location of stimulation sites in this manuscript.

We were able to elicit MEPs not only by stimulating the PrG, but also in the SFG, MFG, and PoG. It seems as the locations of our stimulation sites in the SFG and MFG correspond to the location of the PMC, as originally defined by Dum and Strick (Dum & Strick, 2002). Although they investigated motor areas in brains of primates, many studies tried to locate these areas in human brains, too. The consensus on the location of the PMC in human brains is not as strong as in primates, yet generally, PMd and PMv are believed to be located on the lateral surface of the brain, in the posterior part of the frontal lobe, just anteriorly to the PrG, while the SMA lays rather on the medial surface of the hemisphere, in the most superior part just

anteriorly to the PrG (Fig. 30) (Fink et al., 1997; Fridman et al., 2004; Mayka et al., 2006; Tanji, 1994).

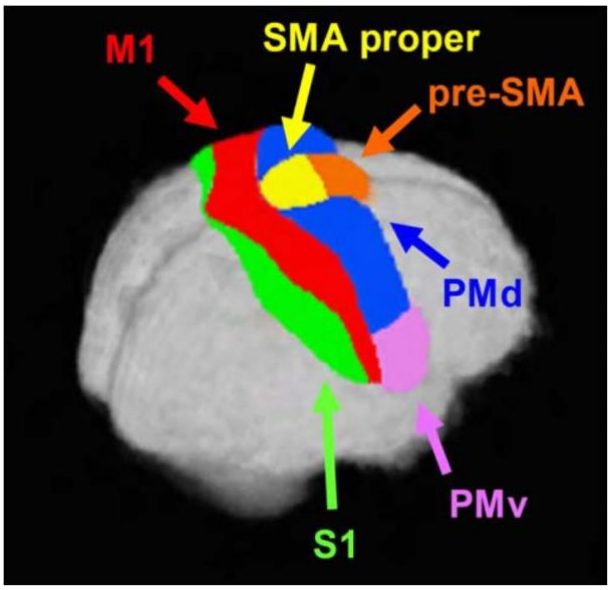

Fig. 30: Human motor area template based upon data generated from probability distributions of the PMC along with previously established anatomical criteria (data derived from 126 articles), reprinted from Mayka, M. A., Corcos, D. M., Leurgans, S. E., & Vaillancourt, D. E. (2006). Three-dimensional locations and boundaries of motor and premotor cortices as defined by functional brain imaging: a meta-analysis. Neuroimage, 31(4), 1453-1474, with permission from Elsevier

Considering the location of our nTMS-based stimulation sites, it is imaginable that our nTMS investigation of SFG elicited motor responses by activating the SMA and hence, the location of the medial SMA might project on the lateral surface during nTMS mapping in our case (Bulubas et al., 2016). While we did not restrict our measurements to the posterior parts of frontal gyri, which represent the PMd and PMv (Fig. 30) (Mayka et al., 2006), most of the stimulation sites were indeed elicited from here. Taken together, we believe that we were able to elicit motor responses stimulating the PMC, namely the SMA in the most superior part of the SFG, and PMd (and eventually PMv) in the remaining posterior SFG and the posterior MFG (Bulubas et al., 2016).

To distinguish between areas with direct projections to the spinal cord, and areas indirectly exciting motor neurons in the spinal cord through other connections, we use the terms monosynaptic and polysynaptic projections. As polysynaptic projections have longer pathways, we assumed that we could detect them as those MEPs having longer latencies, as described in the methods section (Bulubas et al., 2016). As explained in the previous paragraph, it might be confusing to use the terms primary, non-primary, or premotor to express whether an area has corticospinal projection or excites motor neurons indirectly, especially

because of the increasing evidence that all motor cortex areas consist of a combination of both, corticospinal and cortico-cortical projections (Dum & Strick, 2002; Teitti et al., 2008). Therefore we investigate the polysynaptic projections separately for every location where we were able to elicit MEPs, in the PrG, SFG, MFG, and PoG.

5.2. Locating motor areas outside the PrG

Already in the middle 20th century, neurosurgeons investigating brains with pathologies were able to elicit motor responses when stimulating areas outside the PrG (Fig. 2) (Foerster, 1936; Fulton, 1935; Penfield & Boldrey, 1937). These findings were confirmed in later intraoperative studies, for example in a study comparing monopolar and bipolar cortical stimulation for intraoperative location of the motor cortex; bipolar stimulation detected 37.85% of motor positive stimulation sites anterior and 7.85% posterior of the PrG (Fig. 31) (Kombos et al., 1999).

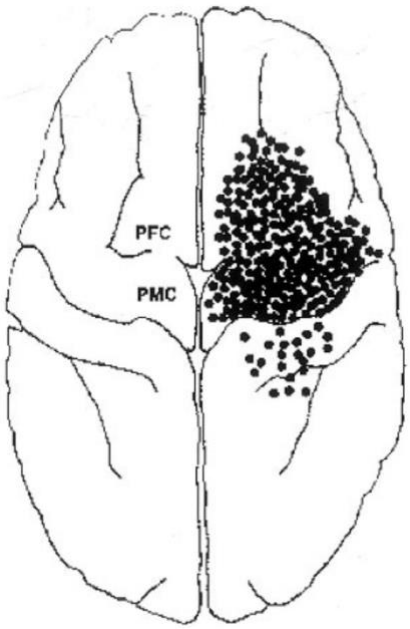

Fig. 31: Distribution of motor areas identified by bipolar cortex stimulation via DES; reprinted from Kombos, T., Suess, O., Kern, B. C., Funk, T., Hoell, T., Kopetsch, O., & Brock, M. (1999). Comparison between monopolar and bipolar electrical stimulation of the motor cortex. Acta Neurochir (Wien), 141(12), 1295-1301, with permission of Springer

Detection of premotor areas in primates inspired many studies to locate these areas in human brains. In healthy subject, imaging studies using PET and fMRI were able to locate corresponding areas in the frontal lobe that were active during execution of movement (Fig. 30) (Boecker et al., 1994; Fink et al., 1997; Rao et al., 1993). Some stimulation studies were

also able to show that it is possible to evoke movement when stimulating areas of the frontal cortex in healthy subjects. In a series of studies from Finland, the authors were able to elicit MEPs in distal hand muscles when stimulating a part of the SFG, corresponding to the PMd, pointing to their short MEP latencies and, hence, their monosynaptic character (Teitti et al., 2008; Vaalto et al., 2011). Another study used TMS to induce movement when stimulating area 44 (corresponds to PMv), also pointing to their monosynaptic character (Uozumi et al., 2004). Stimulation studies are more common in patients with brain pathologies. A study using subdural electrodes in patients with epilepsy gives numbers comparable to the intraoperative stimulation study mentioned above – 65.9% of motor positive stimulation sites were located within 10 mm anteriorly of the Rolandic fissure (corresponding roughly to the location of the PrG), 24.4% were located more anteriorly, and 7.3% were located posterior to the Rolandic fissure (Uematsu et al., 1992). In patients with epilepsy due to a brain lesion, only 28.1% of motor positive stimulation sites were located less than 10 mm anterior of the Rolandic line, while 62.5% were found more anteriorly, indicating a shift of motor function into more anterior areas in these patients (Uematsu et al., 1992). Yet it should be considered that distributions of motor responses in the group of patients with brain lesions were reported only in relation to the Rolandic line, which is less precise as its determined using landmarks of the skull, compared to the Rolandic fissure, which is determined in the MRI scan; therefore the actual extent of the shift might be smaller (Uematsu et al., 1992).

These 2 studies underline our findings. We found 60.71% of MEPs stimulating the PrG, 20.77% stimulating the frontal gyri, and 18.52% stimulating the PoG (Fig. 22). These numbers correspond to numbers reported in the previous paragraphs, with more MEPs in the PoG (Fig. 31) (Kombos et al., 1999; Uematsu et al., 1992). Several theories might explain the higher counts of MEPs in the PoG in our study – direct corticospinal projections in the PoG might be more prone to TMS stimulation rather than intraoperative stimulation, TMS might be more likely to activate cortico-cortical projections to the PrG, or our cohort of patients might simply have more motor representations in the PoG than cohorts from other studies, for example because we included more patients with tumors in the parietal lobe (Kombos et al., 1999). However, it should be considered that we report MEP counts, not counts of motor positive stimulation sites. We found more MEPs than actual stimulation sites due to the overlapping muscle representations in motor areas (Farrell et al., 2007; Sanes et al., 1995). We didn't analyze the counts of motor positive stimulation sites according to their location in a gyrus, although their location and distribution is visualized in the form of heat maps (Figs. 17 – 21). Accordingly, these maps show that upper extremity motor areas were located mainly in the PrG, and to a lesser extent also in areas of the frontal lobe anterior to the PrG, as well as in the PoG (Fig. 18 & 19). These findings indicate that, alongside the PrG, the PMC in the posterior parts of the

SFG and MFG, and the PoG, play a role for reorganization of motor function in patients with motor eloquent brain tumors.

5.3. The role of the motor cortex in the frontal lobe

A large part of motor representations in our cohort of patients was located in the frontal gyri (Fig. 18 & 19). In terms of motor cortex, the frontal lobe includes the PMC: the SMA, PMd, and PMv, which – at least in primates – contain direct corticospinal projections to contralateral muscles and non-direct projections to other cortical areas, such as the PrG (Fig. 4, 30) (Dum & Strick, 1991, 2002). The importance of these motor areas in lesioned brains has been discussed extensively in literature.

Studies on primates showed the major importance of PMv and PMd for maintaining motor function, when the PrG is impaired. They used a GABA agonist to inactivate motor representations in the PrG and in the PMC in primates, whose PrG hand muscle representations has been previously lesioned, to simulate a lesion of the PrG. They observed that, 3-4 months after initial lesioning of the PrG, the primates' motor functions recovered. In their reorganized brain, inactivation of the ipsilesional PrG didn't have any effect on motor function, yet inhibition of the PMC worsened the recovered motor function, indicating that motor function moved from the lesioned PrG to the ipsilateral PMC (Liu & Rouiller, 1999). Interestingly, a corresponding study performed in infant monkeys showed that, if the PrG is lesioned at an early age, motor function is more likely to reorganize recruiting regions adjacent (medially) to the lesion, instead of the more distant PMC, as observed in older primates (Rouiller et al., 1998).

In human studies, corresponding hypotheses were raised. It is believed that reorganization of motor pathways due to impairment of the regular motor cortex or motor tracts is enabled through different patterns (for reviews, consider Duffau, 2006; Kong et al., 2016; Nudo, 2013; Rossini & Pauri, 2000; Sanes & Donoghue, 2000). In general, 3 different mechanisms for functional reorganization of the motor cortex were described, as explained in the following.

First, areas adjacent to the lesion, or tumor, can be recruited, often corresponding to an enlargement of the respective motor area (Cicinelli et al., 1997; Traversa et al., 1997; Weiller et al., 1993). This theory is supported by the idea that there is not just one single cortical area representing a muscle or muscle group, instead there are several, overlapping representations targeting the same muscles in the PrG (Farrell et al., 2007; Sanes et al., 1995). These areas are interconnected through horizontal fibers, which might be the mechanism explaining how other areas can be recruited, if one of them is impaired by a lesion (Jacobs & Donoghue, 1991).

An fMRI study proposed that in patients with brain tumors, in comparison to, for example, patients with congenital cortical malformations, the functional reorganization takes place

mainly through recruitment of adjacent areas, while in the other group, alternative mechanisms are of major importance (Carpentier et al., 2001).

Second, more distant areas in the ipsilateral hemisphere can be recruited, such as the PMC. The role of the SMA for motor function is well known since mid-20th century. Resection of the SMA leads to transient motor (and speech) deficits, which is commonly known as the SMA syndrome (Krainik et al., 2001; Laplane et al., 1977; Zentner et al., 1996). In terms of recovery from stroke, studies point to the importance of the ipsilesional PMC. A TMS study in patients recovering from stroke with motor impairment was able to show that TMS inhibition of the ipsilesional PMd leads to a disruption of motor function, while inhibition of the contralesional PMd doesn't show any effect, pointing to the role of the ipsilesional PMd for motor recovery (Fridman et al., 2004). Imaging studies in patients recovering from stroke with motor impairment as well as in patients with brain tumors showed increased activation of both, the ipsi- and the contralesional PMC during movement of the affected hand (Cramer et al., 1997; Meyer et al., 2003; Seitz et al., 1998; Weiller et al., 1993).

This leads to the third mechanism - motor areas of the contralesional motor pathways can be recruited. Studies using TMS showed that stimulation of the contralesional PMd disrupts movement of affected hand in patients with recovered motor function after stroke with motor impairment but, in patients with higher motor impairment, it rather facilitates the activation of the ipsilesional motor cortex (Bestmann et al., 2010; Johansen-Berg et al., 2002; Werhahn et al., 2003). This indicates that areas of the contralesional motor cortex modulate the activity of the ipsilesional motor cortex through inhibiting transcallosal projection, by increasing or decreasing the inhibitory effects. More than that, studies were able to find correlation of the degree of reorganization, be it the enlargement of motor areas or the activation of contralesional motor areas, with less recovery and, hence, higher motor function impairment (Bestmann et al., 2010; Cicinelli et al., 1997; Johansen-Berg et al., 2002; Meyer et al., 2003; Traversa et al., 1997; Turton et al., 1996). Some pointed out that, with progressing motor function recovery, motor function might shift from the contralesional motor cortex back to the ipsilesional motor cortex (Werhahn et al., 2003).

Which compensatory mechanisms will be ultimately recruited is highly individual. All these patterns were found in similar cohorts of patients recovering from stroke (Weiller et al., 1993). A study investigating 6 patients with brain tumors in the hand motor cortex showed the individual reorganization patterns between these patients, consisting of areas within the PrG, ipsilateral areas anterior and posterior to the PrG, as well as, in 1 patient, contralateral motor cortex areas, which was in particular nicely visible by comparison to the motor cortex of the non-affected hemisphere (Fig. 32 c) (Seitz et al., 1995).

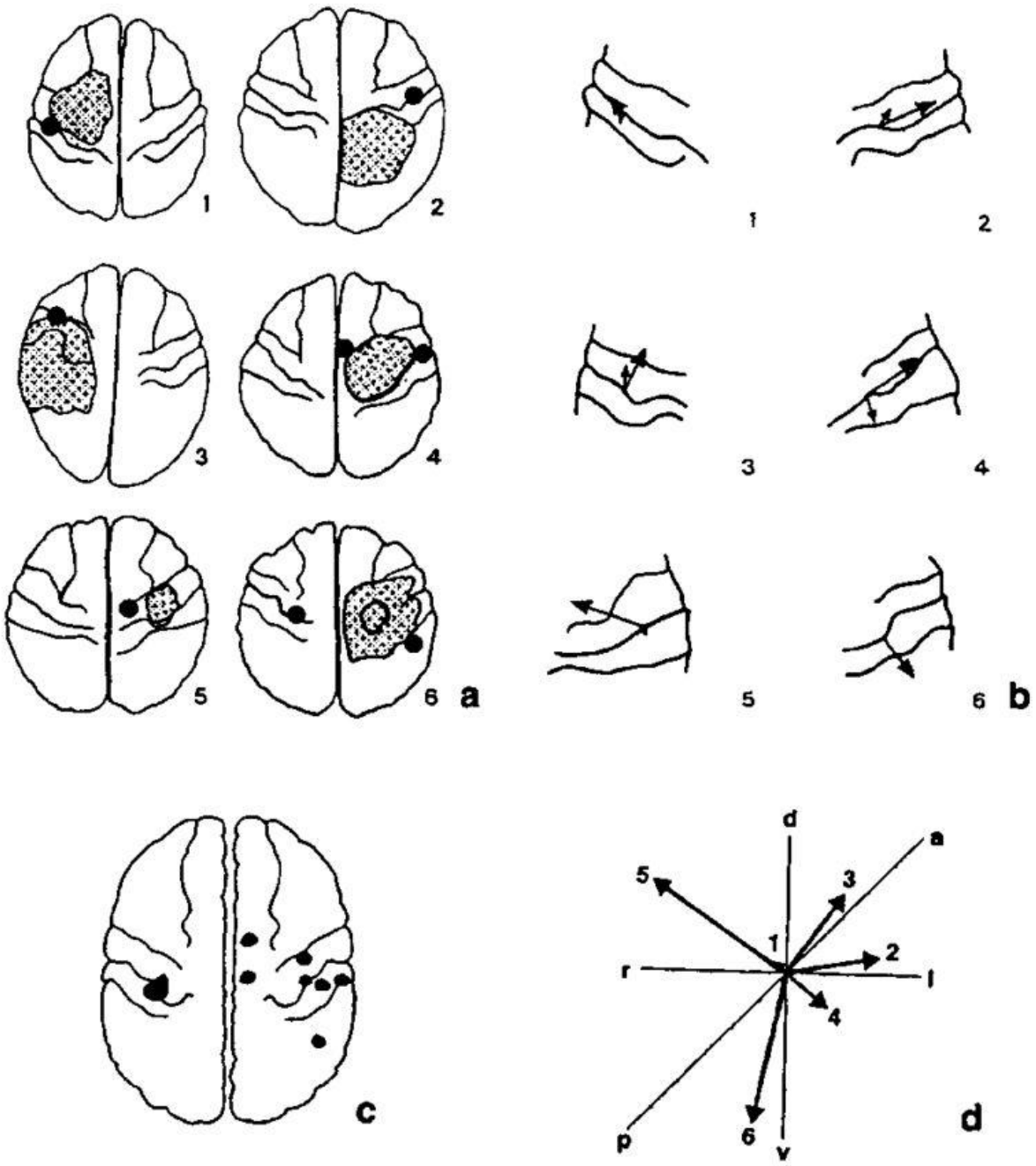

Fig. 32: Patterns of reorganization of the motor cortex in 6 patients with brain tumors within the hand motor cortex; motor cortex was located by PET; a) motor cortex location (black dots) in relation to the tumor (shaded area); b) displacement of central sulcus (small arrows) was different than displacement motor cortex (large arrows); c) in the healthy hemisphere (left), the motor cortex was located in a small area in the middle central sulcus; in the affected hemisphere (right), its location varied in and around the PrG; d) displacement of the motor cortex in the affected compared to the healthy hemisphere (a=anterior, p=posterior, d=dorsal, v=ventral, r=right, l=left); reprinted from Seitz, R. J., Huang, Y., Knorr, U., Tellmann, L., Herzog, H., & Freund, H. J. (1995). Large-scale plasticity of the human motor cortex. Neuroreport, 6(5), 742-744, http://journals.lww.com/neuroreport/Pages/default.aspx, with permission from Wolters Kluwer

In summary, several reorganization mechanisms including areas in the ipsi- and contralateral hemisphere take place when the original motor pathways are impaired. Many of these findings were derived from patients suffering from stroke. Although our cohort has some similarities with cohorts from the previous stroke studies, such as a lesion in some part of the motor tract, resulting in impaired motor function, there are some differences, too. The essential difference is that most of our patients developed a lesion during a significantly longer period of time and – probably due to this temporal factor - only a minority of our patients actually developed motor deficits, indicating that the functional reorganization in our cohort was more present or of higher quality (Desmurget et al., 2007; Duffau, 2006). Yet it is commonly accepted to transfer reorganization patterns observed in patients with strokes, or in human and animal models, to those of patients with brain tumors (Duffau, 2006).

Our study is in accordance with the previously mentioned studies that report different reorganization patterns in lesioned brains. We found different distributions of motor areas among our patients, indicating shifts along the anterior-posterior and the medial-lateral direction in some individuals, as well as different sizes of motor areas between individuals, giving evidence for the existence of reorganization mechanisms in the lesioned hemisphere (Fig. 17 - 21). We observed that, in the vast majority of our patients, the motor areas were indeed located in the PrG; in and adjacent to the handknob area for upper extremity muscles, and around the superior part of the SFG for lower extremity muscles (Fig. 17 – 21). As most of the motor cortex was actually located in or adjacent to its original location, this might be seen as evidence that recruitment of adjacent areas was a reorganization mechanism displayed in our cohort. Due to the small sample size of lower extremity muscles (Figs. 20 & 21), we limit the discussion to the upper extremity motor maps.

Many patients showed a high overlap of upper extremity muscles in the posterior parts of the SFG and MFG, indicating that these areas contain detectable projections to upper extremity muscles in a large number of our patients with brain tumors (Figs. 18 & 19). As explained earlier, the nTMS-based stimulation sites we found in the SFG and MFG might correspond to the PMC, namely SMA and PMd (Fig. 30) (Fink et al., 1997; Mayka et al., 2006). This shows that we also observed the second reorganization mechanism described earlier, the recruitment of more distant areas the ipsilateral hemisphere. Our finding underlines the importance of these 2 mechanisms for functional reorganization of motor function in patients with motor eloquent brain lesions.

As we didn’t investigate the healthy hemisphere in our cohort, we can’t discuss the role of the contralesional hemisphere here.

5.4. The role of the motor cortex in the parietal lobe

Studies investigating the location of motor function in healthy and lesioned brains often concentrate on motor areas in the frontal lobe, the PMC, as described in the previous chapter. This is understandable, as the discovery of premotor areas in the frontal lobe of primates was conducted just recently and it revolutionized the understanding of the motor cortex (Dum & Strick, 1991, 2002). It is surprising how many studies found motor areas in the parietal lobe and didn't consider this finding considerably important as that of frontal motor areas.

Already the intraoperative stimulation studies mentioned earlier showed that a significant portion of motor areas was located posterior of the PrG (Foerster, 1936; Kombos et al., 1999; Nii et al., 1996; Penfield & Boldrey, 1937; Uematsu et al., 1992). PET and fMRI studies in patients recovering from stroke with motor impairment and in patients with brain tumors were also able to show increased activation in the PoG and the parietal cortex, indicating that these areas might be of importance for compensation of motor function (Cramer et al., 1997; Meyer et al., 2003; Weiller et al., 1993). Single reports repeatedly provided evidence that, if the PrG is impaired by a tumor, motor areas can shift posteriorly from the PrG into the PoG and the parietal cortex (Fig. 32) (Almairac et al., 2014; Hayashi et al., 2014; Seitz et al., 1995; Takahashi et al., 2012). A shift of motor areas from the PrG – at least partially – to the PoG was also observed in the majority of 50 young patients with epilepsy (Haseeb et al., 2007).

The role of the PoG for motor function isn't surprising. PoG contains cortico-cortical projections to the PrG, underlining its role for motor function (Borich et al., 2015; Darian-Smith et al., 1993; Donoghue & Parham, 1983; Jones et al., 1978; Matyas et al., 2010). For a long time, it was believed that the PoG provides sensory feedback and modulates the execution of motor function in the PrG. However, recent studies pointed to a more complex cooperation between these 2 structures and suggested that the PoG might harbor potential for recovery of motor function after stroke or for motor skill learning (Brodie et al., 2014; Gandolla et al., 2014; Meehan et al., 2011). It is still unclear if this part of motor function takes place solely through cortico-cortical projections into the PrG, through projections into the PMC, or through direct corticospinal projections, as the parietal lobe contains the structural potential for using all these pathways (Galea & Darian-Smith, 1994; Jones et al., 1978).

We found a significant number of excitable motor areas in the PoG in our cohort (Figs. 18 – 21). Taken together with the results from intraoperative stimulation studies mentioned earlier, it seems as the PoG might play a more important role for functional reorganization of the M1 than assumed. The mechanisms behind eliciting motor responses by stimulating the PoG still remain unclear – an explanation might be that we elicited MEPs by stimulating the regular, cortico-cortical projection to the PrG and, hence, induce movement by indirectly activating the PrG. Another explanation is that we actually detected reorganized projections in the PoG. Here, it would be of interest to differentiate whether this reorganization corresponds to the

unmasking of so far inhibited corticospinal projections, or rather takes place through recruitment of other cortical areas. Altogether, our findings should be considered with caution. There are also converse findings, for example a large study that investigates 165 patients with brain tumors who received DES motor mapping during surgery and who found motor responses in the PoG only rarely, indicating that the extent of motor representations in the PoG might be influenced by methodological differences (Desmurget & Sirigu, 2015; Tate et al., 2014).

As only 20 out of the 8794 MEPs were located posterior to the PoG in the remaining parietal lobe, we cannot provide evidence for the existence of motor areas in the parietal lobe other than in the PoG. Moreover, we didn't investigate the whole parietal cortex; we started the motor mapping in the PrG and extended the mapping parietally until we weren't able to elicit any more MEPs in two rows. If there was motor negative cortex between the PrG and PoG and other parts of the parietal cortex, we weren't been able to detect it. Other studies pointed to the importance of parietal-frontal motor networks (Fogassi & Luppino, 2005; Matelli & Luppino, 2001; Schucht et al., 2013).

5.5. nTMS-based motor maps and their correlation to tumor location

A study from 1998 suggested that, if a tumor is located in the superior part of the brain, it induces an inferior shift of the motor cortex, while, if it is located in the inferior part of the brain, it induces a superior shift of the motor cortex (Fig. 33) (Wunderlich et al., 1998). Although this finding was not confirmed in other studies, predicting what areas will overtake the motor function when specific regions of the brain are impaired would be a huge help for the neurosurgeon and the patient; it would enable predicting motor deficits as a result of tumor location and would facilitate decisions for or against resection of tumors in motor eloquent areas. Hence, we asked – how does a tumor location influence the motor cortex location; does a frontal tumor induce a parietal shift, and does a parietal tumor induce a frontal shift?

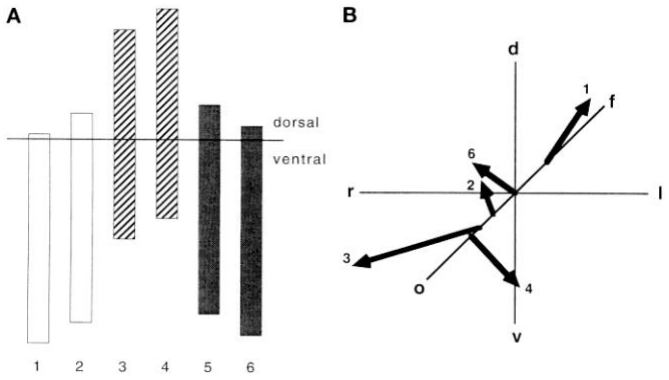

Fig. 33: A: Tumor volume and distribution in relation to the motor hand area (black line), each bar represents 100% of the tumor volume; B: 3D-vector displacements of the PET-based hand motor cortex in the affected hemisphere, origin corresponds to the healthy hand motor cortex; d=dorsal, f=frontal, l=left, o= occipital, r=right, v=ventral; reprinted from Wunderlich, G., Knorr, U., Herzog, H., Kiwit, J. C., Freund, H. J., & Seitz, R. J. (1998). Precentral glioma location determines the displacement of cortical hand representation. Neurosurgery, 42(1), 18-26; discussion 26-17, by permission of Oxford University Press

Several studies tried to find a pattern of reorganization of the motor cortex. A PET study investigating motor activation in patients with brain tumors found that the motor cortex shifted along the anterior-posterior or medial-lateral direction, causing shifts of 9 – 43 mm within the PrG or from the PrG into the premotor or parietal cortex (Fig. 32) (Seitz et al., 1995). They included patients with tumors affecting the PrG, yet didn't investigate the effects of the exact anatomical location of the tumor. Correspondingly, a stimulation study in patients recovering from stroke with motor impairment found mainly shifts along the medial-lateral direction, and stated that shifts along the anterior-posterior direction were observed mainly in cases with a longer period of time between examination and the stroke episode (Byrnes et al., 1999). Other stimulation studies in patients recovering from stroke with motor impairment indicated that motor maps decrease in size shortly after stroke, and increase in size as motor function improves (Liepert et al., 1998; Traversa et al., 1997). Moreover, they showed that the center of the motor cortex area shifted medially or laterally, as motor function improved (Liepert et al., 1998). An fMRI study observed similar patterns, with increased activation of motor areas and increasing posterior shift in patients with larger motor impairment (Bestmann et al., 2010).
However, these results are varying through studies and usually based on small cohorts. We are the first to systematically investigate whether we can predict the extent or direction of functional reorganization of the motor cortex from the tumor's anatomical location in a large cohort (Fig. 18 – 21). We observed that the spread of motor areas seemed smaller in patients with tumors in the PrG (n = 35) and in the temporal lobe (n = 5). While the spread of the temporal tumor subgroup might be explained simply by the small patient count, the small

spread of motor maps in the PrG tumor subgroup is surprising. Most of the literature addressing brain tumors provides data on spatial reorganization of the motor cortex that was based on lesions within the PrG (Seitz et al., 1995; Wunderlich et al., 1998). However, we observed the smallest spread in this subgroup.

There might be several explanations for this finding. First, it might indicate that, if the PrG is directly impaired by a tumor, functional reorganization takes place rather by recruitment of adjacent areas, than more distant areas, leading to a smaller spread. Second, the difference might be based on different plasticity potentials of different brain areas. While the cortex is capable to extensive functional compensation, functional reorganization of lesions in the white matter tracts is observed only rarely (Herbet et al., 2016; Ius et al., 2011). Moreover, different cortical areas show different plasticity potential, too. Areas in the frontal lobe, especially the prefrontal cortex and the SMA, are easily compensated for, in opposite to areas in the PrG and PoG (Herbet et al., 2016; Ius et al., 2011). Further investigation showed a distinction within the PrG, whereas the ventral part of the PrG could be compensated for, and the dorsal part couldn't (Herbet et al., 2016). These findings might explain the smaller motor cortices in the PrG tumor subgroup.

The aspect of different plastic potential of cortical and subcortical structures would be interesting to include in further investigations. As we included tumors affecting the motor cortex and the CST, there might be more subcortical tumors in the non-PrG groups, which would contradict the findings from above (Herbet et al., 2016; Ius et al., 2011). Interestingly, other studies reported converse findings, too, such as a study investigating patients with lesions in the PoG which found more functional reorganization in patients with subcortical lesions, compared to those with cortical lesions (Rossini et al., 1998).

Allover, we observed different reorganization patterns of the motor cortex between our subgroups. While our findings support theories about different mechanisms for functional reorganization within the ipsilesional hemisphere, we were not able to predict the location of the motor cortex from tumor location alone. This indicates that functional reorganization is a highly complex, multifactorial process that needs to be assessed by further studies, as it might have clinical impacts on patient outcomes.

5.6. MEP latencies – general consideration

The exact activation mechanisms behind TMS stimulation are based on several principles. Studies showed that TMS excites predominantly axons and activates functional circuits via cortico-cortical and corticospinal projections; the latter mechanism being currently considered the predominant mechanism for stimulating the motor cortex (Rossini et al., 2015). What structures are prone to stimulation depends largely on the orientation of the electric field in

relation to the targeted structure, with an orientation perpendicular to the central sulcus being the optimal orientation for activating the motor cortex (Hallett, 2000; Rossini et al., 2015). It also depends on the stimulation intensity, with 110% rMT being the widely used stimulation intensity for mapping of the motor cortex (Rossini et al., 2015). Deviation from the optimal stimulation parameters might affect MEP latencies by recruiting neurons through different pathways. Studies have observed that suprathreshold stimulation results in shorter MEP latencies and the higher the stimulation intensity, the shorter the MEP latency (Rossini et al., 2015; van der Kamp et al., 1996).

Moreover, MEP latencies were shown to correlate with height and, as men are often taller than women, with gender (Saisanen et al., 2008; van der Kamp et al., 1996). This shows that the time between stimulus and response depends on the distance from stimulated area and response area (Livingston et al., 2010). Logically MEP latency differs among stimulated muscles. Several studies reported normative values for different nTMS and TMS stimulation parameters, such as MEP latencies. For example, Rossini et al. (1994) reported normative values determined by TMS in 50 healthy subjects, namely for APB of 20.1 ± 1.8 ms, for BCS of 11.6 ± 1.2 ms, and for TA of 26.7 ± 2.3 ms. Another study using the same nTMS system as we did provided normative values from investigations in 65 healthy subjects; in particular for APB of 23.6 ± 1.4 ms for men, 22.1 ± 1.3 ms for women, and for TA of 33.3 ± 2.5 ms for men, and 30.2 ± 1.8 ms for women (Saisanen et al., 2008). Accordingly to the literature, we observed shortest MEP latencies in proximal extremity muscles (FCR, BCS), followed by distal upper extremity muscles (hand muscles; APB, ADM), and by lower extremity muscles (TA, GCN; Fig. 24, Tab. 2). On the other hand, our mean MEP latency values were longer than some of those reported in literature (Rossini et al., 1994; Rossini et al., 2015; Saisanen et al., 2008). This might be caused by the fact that 60% of our patients were men, or it might indicate that MEP latencies tend to be higher in the ipsilesional motor cortex of patients with brain tumors than in healthy brains. This might be explained by affection of the CST by tumor or the peritumorous edema, which might impair the signal conduction and cause slower conduction times. Corresponding observations with by trend higher MEP latencies in the ipsilesional hemisphere were made for patients recovering from stroke (Cakar et al., 2016; Cicinelli et al., 1997; Traversa et al., 1997; Turton et al., 1996).

A study comparing specifically nTMS mapping parameters in the ipsi- and contralesional hemisphere of patients with brain tumors reported MEP latency medians of 24 ms in the contralesional, and 23 ms in the ipsilesional hemisphere (Picht et al., 2012). We observed corresponding values, with a median of 23.30 ms for APB and ADM in our cohort (Tab. 2). As we observed a non-normal distribution of MEP latencies, it is beneficial to provide the median values, yet, to allow comparison with normative values reported in other literature, we provide

the mean and SD values of MEP latencies as well (Fig. 24, Tab. 2). An analysis with regards to the non-normal distribution of MEP latencies is provided in the following.

5.7. Use of MEP latencies for distinction of mono- and polysynaptic projections

We hypothesized that MEP latencies can be used to distinguish between monosynaptic and polysynaptic projections. In particular, we hypothesized that MEP latencies longer than 1 SD above mean MEP latency represent muscle activation through longer, polysynaptic pathways, possibly through the reorganized pathways (Bulubas et al., 2016). The idea that longer MEP latencies represent either slower conducting or polysynaptic, hence non-direct motor pathways, can be found throughout literature (for example Kallioniemi et al., 2015).

A series of studies using nTMS in healthy subjects was able to induce short-latency motor responses stimulating the posterior SFG, i.e. the PMC, and they hypothesized that these motor pathways were monosynaptic, direct corticospinal projections (Teitti et al., 2008; Vaalto et al., 2011). Hence they were able to find support for theories established in primates, stating that the PMC in the frontal lobe contains direct corticospinal projections (alongside to the cortico-cortical projections to the PrG) (Dum & Strick, 1991, 2002). Our findings also support the existence of direct, corticospinal projections in the PMC and the PoG. We were able to elicit motor responses by stimulating the frontal gyri and the PoG as well, and as the mean MEP latencies in all gyri were roughly in the same range, this indicates that large numbers of our motor responses were elicited via monosynaptic, direct corticospinal pathways (Fig. 25, Tab. 3).

Yet the monosynaptic pathways reflect only one part of the organization of the motor cortex. We observed that a high number of our patients showed rather prolonged MEP latencies in the frontal gyri, i.e. the PMC, and the PoG (Figs. 25, Tab. 3). A study using nTMS in healthy subjects provided support for our finding; they observed that MEP latencies tend to be shorter in the center of motor cortex, usually around the handknob area of the PrG, and longer at the border of the motor cortex (Kallioniemi et al., 2015). This would explain our findings, as in our cohort the centers of motor cortices were located usually in the PrG, around the handknob area, yet the borders spread into the SFG, MFG, and the PoG (Fig. 18 – 19, 25, Tab. 3). Additional support was provided by studies in patients recovering from stroke with motor impairment that showed corresponding results, with shortest MEP latencies in the center of the motor area, and longer MEP latencies in surrounding areas (Cicinelli et al., 1997).

Moreover, this study showed that this pattern was inversed shortly after stroke, and recovered weeks later, potentially linked to motor function recovery (Cicinelli et al., 1997). A study comparing patients who recovered from stroke with motor impairment to healthy subjects found longer MEP latencies in the PMd, compared to the PrG, in both hemispheres of healthy

patients, and in the contralesional hemisphere in stroke patients, yet shorter MEP latencies in the PMd in the ipsilesional hemisphere of patients (Fridman et al., 2004). This is interesting, as the finding in the ipsilesional hemisphere reflects the idea of inversed organization of short and long MEP latencies mentioned in the previous paragraph and shows that this reorganization can also persist for a longer period of time than just for the acute phase after stroke. On the other hand, the findings in healthy subjects and the contralesional hemisphere provide evidence controversial to those of Teitti et al. (2008). However, a study using DES to investigate MEP latencies in the PMv of patients with brain tumors found longer MEP latencies here than in the PrG (Fornia et al., 2018). In summary, there is more evidence that shows that MEP latencies tend to be longer in the PMC, especially in patients with brain lesions, which is in accordance with our findings (Figs. 25, Tab. 3).

Furthermore, we found that the MEP latencies among our patients were non-normally distributed with a high number of longer MEP latencies, i.e. presumably polysynaptic projections (Fig. 26). We wondered whether these polysynaptic projections correspond to the observed increased mean MEP latencies and whether, as we investigated only patients with motor eloquent brain tumors, whether the polysynaptic projections might be a hint to the reorganized motor pathways (Bulubas et al., 2016). We hypothesized that tumor location would be the strongest influencing factor that causes reorganization of the motor cortex and changes in MEP latencies; hence we investigated the distributions of polysynaptic projections among the mapped gyri (PrG, SFG, MFG, PoG) in subgroups of patients with different tumor locations (Fig. 27, Tabs. 5 & 6). Yet, other factors are likely to influence MEP latencies, too. For example, as the dominant hand is used more frequently than the non-dominant hand, these training-related effects of the human motor cortex are well described in literature (Karni et al., 1995; Sanes & Donoghue, 2000). Therefore we hypothesized that the location of stimulation sites in the DH (usually left) and NDH (usually right) might be an influencing factor as well (Fig. 28, Tabs. 7 & 8). Finally, the MEP latency partially reflects the central motor conduction time (CMCT), which can be used to display the integrity of the CST (Groppa et al., 2012; Rossini et al., 2015). Therefore, we hypothesized that the integrity of the CST, visible as motor deficits, might be another factor influencing the distribution of polysynaptic projections among the gyri (Fig. 29, Tabs. 9 & 10).

5.8. Effects of tumor location on distribution of motor areas

Considering distributions of polysynaptic projections among gyri of different tumor location subgroups, we used the temporal tumor group as control due to the largest distance of tumor to motor pathways. We found many polysynaptic projections in the frontal gyri of the temporal tumor subgroup, which was different than in the other subgroups (Fig. 27, Tab. 5). This might

reflect the normal state of the brain – mainly monosynaptic projections in the PrG, and many polysynaptic projections in the PMC, projecting either directly to the PrG or through other cortical areas to the spinal cord (Cicinelli et al., 1997; Fornia et al., 2018; Fridman et al., 2004; Kallioniemi et al., 2015).

The frontal tumor subgroup showed less polysynaptic projections in general, and a rather equal distribution of polysynaptic projections among the gyri, with little polysynaptic projections in the frontal gyri, indicating that these projections do not represent the newly formed, compensatory motor pathways, yet rather that the polysynaptic projections are more vulnerable than the monosynaptic pathways (Fig. 27, Tab. 5) (Bulubas et al., 2016). This is partially seen in the PrG subgroup, too, which shows that especially projections in the SFG were prone to impairment by a tumor in the PrG, and in the PoG subgroup, which shows least polysynaptic projections in the PoG (Fig. 27, Tab. 5). If the tumor impairs polysynaptic projections first, one might wonder why there are not less polysynaptic projections in the PrG of the PrG subgroup. Yet this finding actually makes sense, as the PrG still is the location of the main motor function and hence is most robust and least capable of functional reorganization (Herbet et al., 2016; Ius et al., 2011).

In the parietal subgroup, surprisingly little polysynaptic projections were found in the PrG and MFG and many in the PoG. This pattern is difficult to explain, as one would not expect an impairment of frontal motor areas by parietal tumors. Yet there are motor areas in the parietal cortex which are part of a parietal-frontal motor network and which have cortico-cortical projection to the frontal PMC (Fogassi & Luppino, 2005; Matelli & Luppino, 2001; Schucht et al., 2013). Eventually the impairment of these areas leads to a reduced input from these areas to the PMC and causes a reduced output of the PMC itself, which we observed as decreased numbers of polysynaptic projections in the MFG (Fig. 27, Tab. 5).

When considering the mean MEP latencies in subgroups of patients according to their tumor location, instead of the numbers of polysynaptic projections, we didn't observe any relation of increased numbers of polysynaptic projections with the mean MEP latencies (Tab. 6).

5.9. Effects of hemisphere dominance on distribution of motor areas

TMS studies in healthy subjects as well as in patients recovering from stroke or with brain tumors usually don't report differences between the right and left, or the DH and NDH (Cakar et al., 2016; Livingston et al., 2010; Picht et al., 2012; Saisanen et al., 2008; van der Kamp et al., 1996). Yet single studies found converse findings. Although they do not report general differences between the DH and NDH, Saisanen et al. (2008) found longer MEP latencies in the APB of the DH, when using monophasic magnetic stimulation. Previously, we showed that the mean of the normally distributed MEP latencies is longer in patients with left-sided tumors,

corresponding to the DH (Sollmann et al., 2017). Further studies reported differences between the DH and NDH, concerning larger motor representations as well as lower rMT in the DH (Kallioniemi et al., 2015; Pitkanen et al., 2015; Triggs et al., 1994). To sum up, although the current consensus in literature is that there are no significant differences between the DH and the NDH that can be visualized using TMS, in specific patients groups and under specific conditions, differences might be present.

This idea seems even more reasonable, as differences in organization of the motor cortex were shown to depend on hemisphere dominance. On the one hand, the dominant hand is usually better trained than the non-dominant, and motor training influences the motor cortex representations (Karni et al., 1995; Sanes & Donoghue, 2000). Furthermore, studies showed that the SMA is prominently important in the - usually left - DH (Rogers et al., 2004). In the DH, the PrG and SMA have stronger interconnections than in the NDH, as shown by fMRI-based connectivity analysis during hand movement (Pool et al., 2014). These theories might help explain our findings. We observed more polysynaptic projections in patients with tumors located in the DH, with especially many polysynaptic projections in the frontal gyri compared to the NDH (Fig. 28, Tab. 7). These higher counts of polysynaptic projections might represent more cortico-cortical projections from the PMC to the PrG and, hence, might indicate the stronger interconnection of the SMA and the PrG in the DH, compared to NDH (Bulubas et al., 2016).

When considering the mean MEP latencies in the DH and NDH, instead of the numbers of polysynaptic projections, we didn't observe any relation of increased numbers of polysynaptic projections with the mean MEP latencies, corresponding to the results discussed in the previous paragraph (Tab. 8). Hence an increased number of polysynaptic latencies doesn't necessarily prolong the mean MEP latency, which might by caused by the non-normal distribution of our data.

5.10. Effects of motor deficit on distribution of motor areas

As we investigated the motor cortex in a cohort of patients with brain tumors, it is important to consider to which extent functional reorganization took place here. We were able to elicit motor responses in all of our patients, meaning that they had at least some remaining motor function in the affected extremity at the time of the motor mapping. On the other hand, a part of our patients suffered from motor deficits, which is important because paresis might prolong the MEP latency. MEP latency, although not limited to this factor, reflects the CMCT. The CMCT is increased in generalized neurological disorders such as multiple sclerosis and amyotrophic lateral sclerosis, yet also in patients with compressive cervical myelopathy or other compression of the CST, such as stroke (Cakar et al., 2016; Caramia et al., 1991; Chen et al.,

2008; Groppa et al., 2012; Rossini et al., 2015). Although spinal tumors cause cervical myelopathy and in many cases, also prolong the CMCT, to what extent this is true also for intracerebral lesion is less clear. In terms of stroke, the ipsilesional hemisphere often shows increased MEP latencies, or the CMCT, compared to the contralesional hemisphere (Cakar et al., 2016; Cicinelli et al., 1997; Traversa et al., 1997; Turton et al., 1996). Moreover, shorter MEP latencies and shorter CMCT were observed with improved motor function recovery (Cakar et al., 2016; Cicinelli et al., 1997; Turton et al., 1996). As mentioned earlier, although many findings from patients after stroke can be transferred to patients with brain tumors, especially the different temporal extent of the disease and smaller counts of motor deficits in our cohort might hinder a simple transfer of basic theories.

Conversely to the studies in patients recovering from stroke, a study investigating nTMS parameters in a cohort of over 50 patients with brain tumors did not find any effects of clinical factors, such as the patient's motor function, on MEP latency (Picht et al., 2012). Actually 50 patients (out of 55 patients for who MEP latency was obtained) showed almost the same MEP latencies in the ipsi- as in the contralesional hemisphere, hence the presence of a tumor didn't seem to prolong the MEP latency (Picht et al., 2012). This is basically in accordance with our findings. The distribution of overall polysynaptic latencies showed slightly more latencies in patients with motor deficits than in patients without motor deficits, yet this finding couldn't be confirmed in the single muscle analysis (Fig. 29, Tab. 9). The mean MEP latencies seemed to be increased more often in patients with motor deficits than without motor deficits, when considering these values for every muscle and every gyrus separately, yet, again, this does not seem to be a representative result, as the variety between the single muscles and gyri was very large (Tab. 10). Hence, although we observed a slight trend towards increased numbers of polysynaptic projections in patients with motor deficit in general, we do not consider this finding reliable enough to conclude what effects a patient's motor deficit has on the distribution of polysynaptic projections (Bulubas et al., 2016).

5.11. Clinical implications

Before and during operative tumor resection, it is of importance to locate areas of the brain that contribute to motor function. This was shown by 3 independent studies, which showed improved outcomes in patients, when nTMS-based motor mapping was performed before surgery (Frey et al., 2014; Krieg et al., 2014; Krieg et al., 2015). The observed effects consisted of expanded surgical indications and extents of resections, higher rates of gross total resections with lower rates of residual tumors, better survival outcomes, improved motor outcomes, less newly developed motor deficits, smaller craniotomies, and shorter inpatient stays (Frey et al., 2014; Krieg et al., 2014; Krieg et al., 2015).

Intraoperative motor mapping using DES and intraoperative neuromonitoring are considered the “gold standard” for distinction of motor eloquent and non-eloquent areas (Cedzich et al., 1996; De Witt Hamer et al., 2012; Duffau et al., 2005; Kombos et al., 2000; Szelenyi et al., 2010). The validity of this approach was shown when resecting tumors in the SMA, for example. Here, resection might induce motor deficits, yet only of transient character; the patients will recover within months (Krainik et al., 2001; Zentner et al., 1996). This phenomenon is well described in literature and referred to as the SMA syndrome (Bannur & Rajshekhar, 2000; Kim et al., 2013; Krainik et al., 2001; Laplane et al., 1977; Russell & Kelly, 2007; Zentner et al., 1996). This indicates that not all of the motor areas we found in the SFG might be motor eloquent.

However, conclusions should be made carefully; being able to resect tumor of the SMA without permanent deficits does not necessarily mean that resecting the SMA when the PrG itself is impaired by a tumor will not cause permanent deficits. When the SMA is impaired by a tumor, functional reorganization involving other areas of the PMC and the PrG takes place (Krainik et al., 2004). Eventually resection of the SMA without deficits was made possible by functional reorganization that has already taken place preoperatively. On the other hand, when the PrG is impaired by stroke or a tumor, areas of the PMC play a more important role (Cramer et al., 1997; Fridman et al., 2004; Meyer et al., 2003; Seitz et al., 1998; Weiller et al., 1993). A recent study investigated patients with nTMS-based motor areas in the PMC that were resected during tumor resection, and showed that 62% of these patients suffered from permanent postoperative motor deficits (Moser et al., 2017). It is conceivable that the PMC might overtake motor function and might become a motor eloquent structure. This underlies necessity of performing DES-based motor mapping, even in areas outside the PrG.

However, there are contradictory reports in the literature, too. A study showed that resecting areas of the PMd that previously evoked MEPs using chronic subdural electrodes induces only transient motor deficit, similar to those of the SMA syndrome (Mikuni et al., 2007). Another study reported a single case of a patient scheduled for operative resection of an epileptogenic focus, where nTMS, fMRI, and MEG localized the motor cortex in the PMC, rather the PrG (Makela et al., 2013). DES did not confirm this finding, and therefore the area in the PMC was resected without causing transient deficits (Makela et al., 2013). Altogether, an individual approach that includes multimodal motor mapping should be followed to ensure the best possible surgery outcomes for patients with motor eloquent brain tumors. Intraoperative motor mapping via DES should be considered even in patients with far frontal tumors, if nTMS locates motor function in these areas.

5.12. Limitations of the study

This study is an explorative study performed to investigate the location and the characteristics of the motor cortex in a cohort of 100 patients with motor eloquent brain tumors. The emphasis was to determine the influence of individual tumor location on the functional reorganization of the motor cortex.

Our cohort consisted of individuals; differences among our patients were observed considering patient characteristics, such as their age, gender, and height, and disease characteristics, such as individual tumor location, tumor size, and tumor pathology. Considering tumor pathology, we included patients with slow-growing low-grade gliomas (LGG) as well as aggressive high-grade gliomas and metastases. There are hints in literature that a slower developing lesion might give more room for functional reorganization leading to less functional deficits (Desmurget et al., 2007). The effect of time was shown during subsequent tumor resection surgeries, when (motor) function moved from areas adjacent to the tumor, to more distant areas, from first to second surgery, a couple of years apart (and enabled the main finding of this study, that a two-staged surgical approach might allow for complete tumor resection in patients with LGG) (Duffau et al., 2003; Duffau et al., 2002; Robles et al., 2008). Sometimes the tumor resection itself is regarded as an inducer of plasticity (Duffau et al., 2002). Moreover, different infiltrative characters of the tumor might lead to differences in the affection of the CST, visible for example by peritumorous edema, and the therapy that is appropriate. 22% of our patients presented with recurrent tumors, hence most of them previously underwent radiotherapy and chemotherapy. The vast majority of patients with brain tumors scheduled for operative resection at our department receive antiepileptic drugs, usually levetiracetam. While all these factors might influence the plasticity of the motor cortex, we do not expect these factors to influence our results, as they should be distributed equally among our subgroups. We were able to show an equal distribution of clinical factors that were available to us among our tumor location subgroups, with the exception of age (Tab. 1). Yet the readers should be aware of this limitation.

Considering the age, patients in the PrG subgroups were older than patients in the PoG subgroup (Fig. 16). This might play a role as it was shown that the plastic potential of the brain decreases in older patients (Muller-Dahlhaus et al., 2008; Pascual-Leone et al., 2011; Polimanti et al., 2016). Some studies were able to show age-dependency of some nTMS parameters, for example linearly increasing rMT with age from 20 years up to 60 years, showing a decreasing on older patients, while others didn't observe any effects (Picht et al., 2012; Saisanen et al., 2008). Yet the mean age in our subgroups ranged from mid-40's to late 50's, which is still rather close. Yet this should be kept in mind when considering our results.

Other factors that showed high variability were nTMS-mapping parameters such as the numbers of stimulation sites among patients (47.12 ± 27.72), numbers of MEPs among

patients (87.94 ± 64.45), and numbers of MEPs among gyri and muscles (Fig. 22 & 23). APB, ADM, and FCR were the muscles where eliciting MEPs was easiest, hence these were the muscles we considered most reliable for MEP latency analysis. It is important to consider that our analysis of distributions of polysynaptic latencies relies on defining polysynaptic projections as those MEPs with latencies longer than 1 SD above mean MEP latency. This is not a fact stated in literature, more of an approximation to find an approach that enables the distinction of polysynaptic and monosynaptic projections. Yet, as no evidence for exact duration of monosynaptic and polysynaptic latencies, other studies use the mean MEP latency and the SD as approximation, too (Kallioniemi et al., 2015).

Additionally, technical limitations of our study consist of the precision of the nTMS system we used. The developers report that the figure-of-8 biphasic magnetic coil stimulates an area of 0.68 cm^2, which is the area stimulated by 98% of the electric field intensity. Targeting this area is possible with an accuracy of 0.57 cm, that consists of errors caused by the optical tracking system, movement of the head, computation of the electric field using coil characteristics and individual head models, and the registration of the individual head position to the MRI scan (Ruohonen & Karhu, 2010). This lines up well with the variability of nTMS-based motor positive sites and those determined by DES, which was reported to be 0.57 ± 0.46 cm (in newly diagnosed tumors) (Krieg et al., 2013). Considering the size of the hand motor cortex in the PrG (apprx. 11.2 mm), this might seem rather large (Campero et al., 2011). However, the spatial resolution of DES is approximately 1 cm, too, and both methods allows a reliable location of the M1 within the PrG, so nTMS seems to be comparable to the “gold standard” DES (Kong et al., 2016). Moreover, the nTMS system used in this study is comparable to other systems currently in use (Ruohonen & Karhu, 2010). One of its advantages is the calculation of the electric field strength individually for each patient approximately at the cortical level, ensuring that the maximum electric field activates cortical areas (Ruohonen & Karhu, 2010; Sollmann et al., 2016).

Further technical limitations concern the steps performed to enable a spatial visualization of the motor cortices. To avoid mass effects of tumors causing displacement of anatomical structures, we performed a normalization of each patient’s MRI scan and motor map. The normalization procedure might be negatively affected by tumors, yet this can be minimized by masking the tumor and excluding the pathological tissue from determining the normalization parameters, as was done in this study (Brett et al., 2001). Yet, as the lesion size increases, there is less ‘healthy’ brain tissue to use for determining the normalization parameters, which results in poorer normalization results. Hence, if normalization with masking the lesion didn’t give satisfactory results, the normalization was repeated without masking the lesion.

Further mathematical limitations of our study consist of the fact that we did not perform correction for multiple testing, nor did we correct for possible confounding factors, such as the

age, as mentioned previously. Yet, this approach is acceptable for explorative studies. It is important to keep in mind that this was an explorative study aiming to investigate various effects, and the significance of our results has yet to be proven in further studies. Studies with larger, yet more uniform cohorts, including additional information, such as from the contralesional hemispheres, should be performed to validate our results.

6. CONCLUSION

This explorative study provides further information about functional reorganization of the ipsilesional motor cortex in patients with motor eloquent brain lesions. In this cohort, cortical areas other than the PrG, such as the PMC (SFG+MFG) and PoG, contain motor function, presumably due to functional reorganization of the motor cortex. Tumor location alone did not account for the large variability in spatial location of the motor cortex. Nor did it sufficiently explain different distribution of mono- and polysynaptic projections throughout these cortical areas. Taken together our findings indicate that functional reorganization is a complex process that depends on many clinical and individual factors and tumor location alone does not fully predict the functional reorganization of the motor cortex.

7. SUMMARY

Introduction: In patients with brain tumors, the motor cortex can be found in varying locations in and around the precentral gyrus [PrG] of the brain. In neurosurgery, this fact is important to consider as the aim of tumor resection is to resect the tumorous tissue without harming the motor pathways. Brain plasticity is believed to be the mechanism behind the functional reorganization of the motor cortex allowing for its relocation to different brain areas. In this explorative study, we investigated whether a varying tumor location (tumor in the temporal lobe, the frontal lobe, in the PrG, in the postcentral gyrus [PoG], or in the parietal lobe) causes different patterns of reorganization of the ipsilesional motor cortex in a cohort of 100 patients with motor eloquent brain tumors using navigated transcranial magnetic stimulation (nTMS).

Methods: During preoperative nTMS-based motor mapping, a coil is used to create a magnetic field that induces a short-lasting electric field which activates the underlying motor cortex. The muscle responses to this activation can be measured via electromyography as motor evoked potentials (MEPs). Hence, motor areas can be detected and located using the patient's magnetic resonance imaging scan, creating an individual motor map for each patient. We used 2 approaches to analyze these motor maps with regard to the tumor location: For a spatial approach, we visualized the normalized motor maps in subgroups of patients according to their tumor location. The second approach investigated the polysynaptic projections, i.e. motor pathways connected through more than 1 synapse. These were defined as originating from those stimulation sites where stimulation elicited MEPs with latency longer than 1 standard deviation above mean MEP latency. The differences in their distribution among the 4 mapped gyri (PrG, superior frontal gyrus [SFG], middle frontal gyrus [MFG], PoG) were analyzed between subgroups of patients according to tumor location, as well as hemispheric dominance and motor deficit.

Results: Motor cortex was located mainly in the PrG, yet showed a wide spread into adjacent gyri – the posterior SFG and MFG and the PoG. Motor areas in patients with tumors in the temporal lobe and in the PrG were located rather closely together compared to the other subgroups. Monosynaptic and polysynaptic projections were found in all gyri. The distribution of polysynaptic projections showed a dependency on tumor location, with many polysynaptic projections in the frontal lobe of patients with temporal tumors ($p<0.001$). Investigating other factors than tumor location showed a dependency on hemisphere dominance with more polysynaptic projections in the frontal gyri of the dominant hemisphere ($p<0.0001$).

Conclusion: Alongside the PrG, the premotor cortex (SFG+MFG) and the PoG contain motor function in patients with motor eloquent brain tumors. Tumor location alone does not predict the different patterns of reorganization of the ipsilesional motor cortex sufficiently, indicating that functional reorganization is a complex process.

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9. FIGURES

10. TABLES

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12. PUBLICATIONS

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