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Using navigated transcranial magnetic stimulation to map the supplementary motor area

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Dissertation

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Für Margot und Richard Brunner.

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1. Abbreviations

AHS	Alien Hand syndrome
BG	Basal ganglia
BOLD	Blood-oxygen level dependent
CRB	Cerebellum
cTBS	Continuous theta burst stimulation
DCS	Direct cortical stimulation
DTI	Diffusion tensor imaging
DTI FT	Diffusion tensor imaging fibertracking
EEG	Electroencephalography
EMG	Electromyography
FET	Flexion-extension test
fMRI	Functional magnetic resonance imaging
FNT	Finger-nose test
FTT	Finger tapping test
JHFT	Jebsen-Taylor hand function test
LHO	Lifting heavy objects
LLO	Lifting light objects
LSO	Lifting small objects
M1	Primary motor cortex
MEG	Magnetoencephalography
MRI	Magnetic resonance imaging
NHPT	Nine-Hole peg test
nTMS	Navigated transcranial magnetic stimulation
PDT	Pronator drift test
PET	Positron-emission tomography

rs-fMRI	Resting-state functional magnetic resonance imaging
rTMS	Repetitive transcranial magnetic stimulation
SC	Stacking checkers
SDIT	Standard deviation of inter-tap-intervals
SF	Simulated feeding
SMA	Supplementary motor area
SMN	Spinal motoneurons
SPT	Simulated page turning
TCT	Test completion time
TMS	Transcranial magnetic stimulation
VCP	Visible coordination problem

2. Introduction

2.1. Brain Mapping: A Historic Perspective

The idea that specific cognitive and behavioral functions can be assigned to different parts of the human brain can be traced back at least as far as ancient Rome (Finger, 1994). Galen (130 – 200 A.D.) argues in his writings that structural differences between the cerebrum and cerebellum indicate a difference in their governing of bodily functions, notably sensory and motor purposes (Finger, 1994). Over centuries, this fundamental thought was refined and developed further. An early account of a model approaching the modern understanding of distributed function can be found in the works of Emanuel Swedenborg (1688 - 1772 A.D.), who was among the first to hypothesize a form of somatotopic arrangement in the motor cortex (Akert & Hammond, 1962).

Later, studies of clinical cases such as done by Paul Broca (1824 – 1880 A.D.) gave evidence for the concept of hemispheric dominance and resulted in the identification of the now famous Broca's Area involved in motor aspects of language generation (Finger, 1994; Riese, 1947). More advanced experiments, such as by direct electrical stimulation of animal cortices, were successful in demonstrating localized function, e. g. somatotopy of the primary motor cortex (Ferrier, 1875, 1886). These approaches show an early similarity to contemporary neurosurgical methods. Additionally, findings from anatomy, most prominently Brodmann's map of cortical cytology, made significant contributions to the understanding of differences in functional specification across the human brain (Finger, 1994).

In modern and contemporary neuroscience, the widespread utilization of advanced technological tools such as electroencephalography (EEG), magnetoencephalography (MEG), direct cortical stimulation (DCS), transcranial magnetic stimulation (TMS), magnetic resonance imaging (MRI) and functional MRI (fMRI) have revolutionized the generation of useful data regarding the localization of brain function. The scientific discipline dealing with this subject matter is now known under the term of brain mapping (Savoy, 2001).

2.2. The Cerebral Motor System

In order to better ground our elaborations regarding the SMA, this section aims to give a brief overview over the wider cerebral motor system.

Generally speaking, motor behaviour can be separated into two broad categories: reflexive movement and volitional movement (Schwartz, 2016). Due to the nature of this thesis, we will only examine volitional movement more closely.

While the concept of “intentions” and their potential mapping onto neurophysiological states is controversial (Uithol, Burnston, & Haselager, 2014), contemporary hypotheses often still refer to intentions as the basis for movement impulses (Desmurget & Sirigu, 2009). More specifically, reference is often made to frontal and parietal areas for the generation of these impulses, which were demonstrated to cause intentions when stimulated (Desmurget & Sirigu, 2009). In order to fulfill a specific goal, the brain is then forced to design a movement program engaging the right types of muscles in precisely the right amount and timing to fulfill the requirements.

Areas prominently involved in the computation of movement programs are the basal ganglia (BG) (Brittain & Brown, 2014; Park, Coddington, & Dudman, 2020) and the cerebellum (CRB) (Tanaka et al., 2021; Therrien & Bastian, 2019), which are connected to both the primary motor cortex (M1) (Hoover & Strick, 1999) and also to one another (Bostan & Strick, 2010). Their functional role within the motor system appears to be complex, since the involvement of these areas has been implicated in a number of aspects of motor control, such as e. g. movement timing, movement sensing or motor learning (Bostan & Strick, 2010; Tanaka et al., 2021). Additionally, lesions or diseases of these systems impact the subject’s capability for movement generation, albeit in different ways; a lack of dopaminergic projections into the BG loop for example contributes to the clinical symptoms of Parkinson’s Disease (Eisinger, Cernera, Gittis, Gunduz, & Okun, 2019), while damage to the cerebellum often causes motor problems such as e.g. ataxia (Tanaka et al., 2021). Therefore, while a clear mechanistic model is currently not constructable, it is relatively safe to assume that any prospective motor program would be influenced and refined by processes within the BG and CRB.

In a simplified model, the final stage of the cerebral motor system consists of motoneurons located mostly within layer V of M1 (Rivara, Sherwood, Bouras, & Hof, 2003). These neurons project directly toward the spinal motoneurons (SMN) in the anterior horn on the spinal level, thereby propagating their activation to the peripheral nerve, which eventually causes movement in the effector muscle (Lemon, 2008). Notably however, the SMA similarly contains neurons projecting toward the SMN, which have been estimated at about 10% of all corticospinal tract fibers (Nachev, Kennard, & Husain, 2008).

The circuitry involving the SMA and the rest of the motor system, most importantly M1, is complex and not explored exhaustively. Fig. 1 yields a simplified overview over the current understanding of structural SMA connectivity as pertaining to other prominent parts of the motor system.

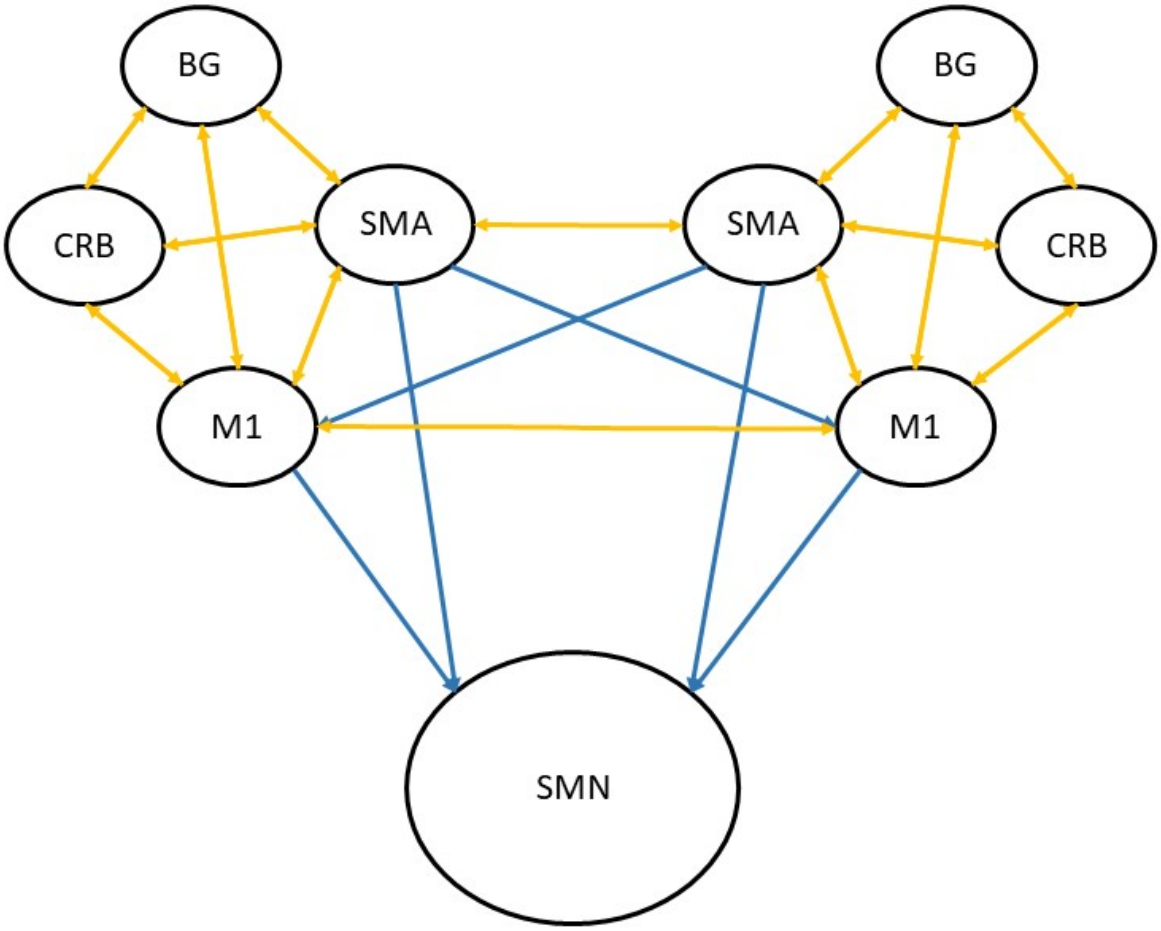

Figure 1 - Simplified structural connectivity of the cerebral motor system. Yellow arrows indicate bidirectional connectivity, blue arrows indicate unidirectional connectivity. Abbreviations: basal ganglia (BG); cerebellum (CRB); primary motor cortex (M1); supplementary motor area (SMA); spinal motoneurons (SMN).

The next section will examine one component of this motor system more closely, namely the SMA.

2.3. The SMA

The following sections will review a number of both initial and modern investigations into the function of the SMA, as well as the clinical relevance of lesions to this area.

2.3.1. Discovery and Initial Investigations

The SMA is a cortical region composed of two subregions found in the dorsal frontal lobe (Fig. 2) of both humans and, analogously, certain species of monkeys (Goldberg, 1985; Penfield & Welch, 1951). It was first described by Penfield & Welch in 1951, following a series of experiments in which the authors utilized DCS to stimulate the premotor cortex (Penfield & Welch, 1951). Penfield observed that behavior elicited from stimulation of this area, while for the most part relating to motor function, differed from reactions to stimulation of the Rolandic motor area. Specifically, Penfield noted that the SMA seemed to exercise control over both sides of the body, in contrast to the Rolandic motor area which governs only contralateral muscles (Penfield & Welch, 1951). He further characterized three different types of motor responses, namely the assumption of postures, complex maneuvers and quick movements without coordination (Penfield & Welch, 1951).

The role of the SMA in movement specifically rose to further prominence in the context of experiments published by Grey Walter, as well as Kornhuber and Deecke. Both groups used EEG methods to describe reproducible electric fields generated on the scalp prior to movement (Kornhuber & Deecke, 1965; Walter et al., 1964). Kornhuber and Deecke specifically located the peak of this “Readiness Potential” over the precentral region. They further observed that the potential was modulated by intention (Kornhuber & Deecke, 1965). The origin of this field was later determined to be the SMA (Shibasaki & Hallett, 2006).

The SMA’s Readiness Potential was further studied in the notorious Libet Experiment (Libet, 1999). Here, subjects were instructed to perform a voluntary wrist movement at a time point of their own choosing while having their EEG recorded. The experiment was designed to capture the moment in which the subject’s intention to move occurred in their consciousness, in order to then compare the timing to both the actual movement as well as to the recorded EEG patterns. The study famously found that the Readiness Potential preceded the subjects’ conscious intention to move by more than 300 ms (Libet, 1999). Based on this finding, the

subjects' capacity for sovereign choice in performing a seemingly voluntary, free action was questioned. While still controversial, this experiment and the lines of research having emerged from it have led to and continue to influence the wider discussion regarding the role of neurophysiology and neuroscience in the debate around the concept of free will (Sjöberg, 2021).

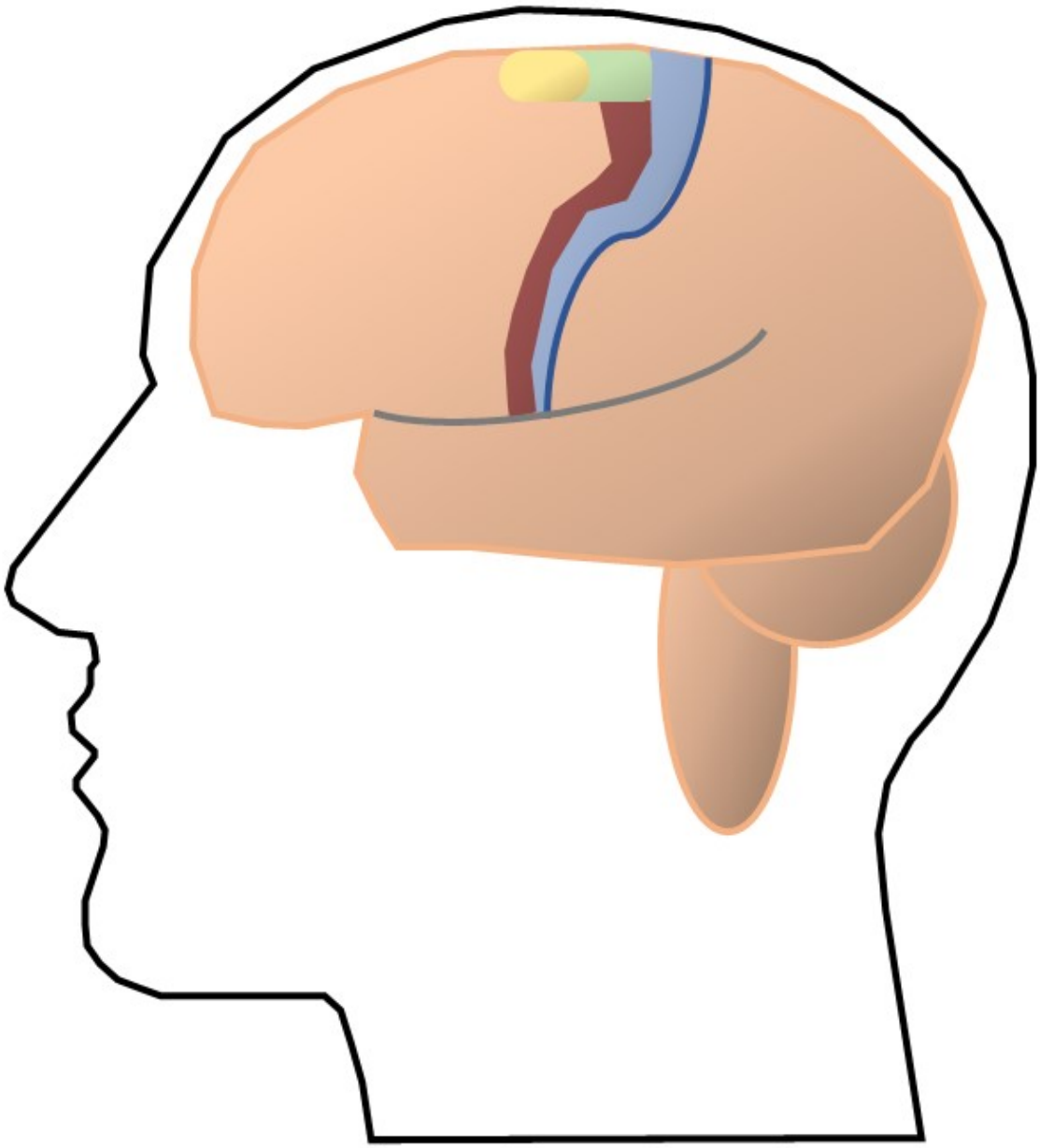

Figure 2 - Schematic display of supplementary motor area location. The two subregions of the supplementary motor area (SMA), SMA proper and pre-SMA are marked distinctly. Dark blue line: Sulcus Centralis; Grey Line: Sylvian Fissure; Light blue area: Primary motor cortex; Dark red area: Premotor cortex; Green area: SMA proper; Yellow area: Pre-SMA.

2.3.2. Modern Understandings of SMA Function

In contemporary research, the multitude of modern neuroscientific tools has enabled a wide array of research into the functions of the SMA. Some of the functions the SMA has been implicated in include: Estimation of time intervals (Casini & Vidal, 2011), mental geometric processing (Cona et al., 2017), working memory (Cañas et al., 2018), subjective experience of physical effort (Zénon et al., 2015), grip force scaling (White et al., 2013) and linguistic processing (Tremblay & Gracco, 2009). Nonetheless, the majority of SMA related research still appears centered on the more well-known role of the SMA in motor function per se (Alonso et al., 2012; Boccardi et al., 2002; Lang et al., 1991; Lee et al., 1999; Luppino et al., 1993; Serrien et al., 2002).

Herein, research has evolved toward a more differentiated understanding of the classical SMA, in which the region can be stratified into a more anteriorly located pre-SMA and a more posterior SMA-proper (Nachev et al., 2008) (Fig. 2). These subsections are different not only in anatomical connectivity (Liu et al., 2002; Luppino et al., 1993), but also appear to differ in terms of their functional role (Nachev et al., 2008).

For example, pre-SMA cells have demonstrated higher learning-related activity in motor learning compared to the SMA proper (Nakamura et al., 1998). Analogously, in a 1999 study, Nakamura et al. demonstrated that the inactivation of macaque pre-SMA via muscimol-injection interfered with the learning of new movement sequences, whereas already learned sequences were not affected. While a similar trend was observed after the inhibition of SMA proper, the latter did not reach statistical significance (Nakamura et al., 1999). Furthermore, pre-SMA specifically has shown relevance in the ability to switch between the execution of different tasks (Rushworth et al., 2002).

In contrast, the SMA proper appears to possess as somatotopic arrangement that when stimulated leads to the execution of complex movements (Fried et al., 1991). Thus, a tentative distinction between a more cognitively oriented function of the pre-SMA and a more directly motor-related SMA proper might be considered. One should however be careful in this distinction, since any such presumed functional borders are if present, then likely highly fluid in nature (Nachev et al., 2008).

2.3.3. Clinical Relevance of the SMA

In a more clinical context, the SMA is a structure of interest for intracranial tumor surgery due to its functional relevance. Estimates postulate that about 27 % of low grade gliomas and about 11 % of glioblastomas involve the SMA (Duffau & Capelle, 2004). Resections of, or indeed any

damage to this area may result in the occurrence of the so called SMA syndrome. Said syndrome typically consists of hemiparesis contralateral to the lesion often accompanied by mutism (Alonso et al., 2012; Rostomily et al., 1991; Zentner et al., 1996). Clinical rehabilitation is usually observed over a time course of multiple weeks, yet often residual deficits remain with the patient (Nachev et al., 2008).

The exact nature of the recovery mechanism is still debated. Some studies suggest that the contralateral SMA may play a role in taking over the function of the lesioned side (Acioly et al., 2015; Krainik et al., 2004). Supporting this hypothesis is evidence for strong transcallosal communication between the respective SMAs of both hemispheres (Liu et al., 2002). A diffusion tensor imaging study by Oda et al. has demonstrated a higher number of fibers connecting contralateral SMA and ipsilateral primary motor cortex to be correlated to shorter recovery time after SMA syndrome occurrence in brain tumor patients post resection (Oda et al., 2018). Additionally, a 2017 study by Vassal et al. demonstrated that in 6 cases of low-grade glioma that underwent partial resection of the SMA, interhemispheric functional connectivity between sensorimotor cortex ipsilateral to the tumor and contralateral SMA assessed by resting-state fMRI (rs-fMRI) increased with motor function rehabilitation (Vassal et al., 2017). Taken together, these findings could indicate that interhemispheric connectivity aids in the transfer and corresponding recovery of SMA function.

In spite of the transient nature of the SMA syndrome, hemiparesis nonetheless presents a significant disability and can facilitate the occurrence of significant, high-mortality medical complications such as e.g. pneumonia or emboly (Rolston et al., 2014; Wang et al., 2013). Therefore, minimizing the risk of SMA syndrome is a relevant concern for patients undergoing surgery involving the SMA.

A rarer complication of damage to the SMA can be found in the occurrence of the Alien Hand syndrome (AHS). This condition is characterized by the subjective loss of control over the actions of a given limb and resulting performance of involuntary actions (Assal et al., 2007; Scepkowski & Cronin-Golomb, 2003). It is hypothesized that the SMA may in healthy volunteers contribute to the inhibition of undesired motor programs, and that consequently damage to the SMA or its projections may lead to the realization of these otherwise suppressed programs (Scepkowski & Cronin-Golomb, 2003). In a case report of bilateral SMA damage, Boccardi et al. describe the clinical phenotype as “(…) patients being left at the mercy of environmental stimuli, unable to inhibit inappropriate actions.” (Boccardi et al., 2002). The presence of an AHS would naturally cause significant disability for any afflicted patients, which in turn should motivate procedures seeking to minimize the risk of its occurrence such as e. g. preoperative mappings.

2.4. nTMS: A Modality for Noninvasive Brain Stimulation

nTMS is a technology used in the noninvasive stimulation of brain tissue (Krieg, 2017; Rotenberg et al., 2014). This section will deal with the underlying principles, the basic setup and current clinical uses of nTMS protocols.

2.4.1. Underlying Technology

Fundamentally, nTMS works via the principle of electromagnetic induction. A brief, strong electrical current is sent through the stimulation coil, which in turn creates a rapid change in the magnetic field near the coil (Pascual-Leone, 1999; Rotenberg et al., 2014). This magnetic field change can in turn interact upon charged particles and, for cerebral tissue specifically, can lead to the depolarization and subsequent firing of neurons (Romero et al., 2019). While studies investigating the direct cellular effects of TMS are rare, one such study performed by Romero et al. published in 2019 demonstrated that single pulse TMS affects neurons in a roughly 2 mm^3 volume, and results for the most part in a short term (< 50 ms post-stimulus) spike of firing rate for the exposed neuron (Romero et al., 2019).

Herein, coil shape is an important variable influencing the spatial aspect of the induced magnetic field. As an example, figure-of-eight coils are used for higher focality or H-coils for deeper penetration of the induced stimulation compared to simple round coils (Rotenberg et al., 2014). The temporal aspect of the pulse on the other hand can be manipulated via the pattern of current flow within the coil (e.g. monophasic vs. biphasic pulses) (Krieg, 2017; Rotenberg et al., 2014).

Aside from the stimulator output and the means of optimizing it, the other foundational component of nTMS is the neuronavigational system. One of the most prevalent ways to achieve neuronavigation is frameless stereotactic navigation, which is applied for a variety of purposes in neurosurgery (Grunert et al., 2003; Roessler et al., 1997). Imaging data, usually in the form of MRI data, is used to create a virtual analogue of the patient's head (Herwig et al., 2001). By marking corresponding anatomical landmarks both on the virtual and physical head, the system develops an internal model of position and orientation of the patient in relation to a reference (e.g. a headband fixed to the patient's forehead) (Herwig et al., 2001). During the application of nTMS, the position of this reference (and thereby the patient head) and any tools such as e. g. pointers or stimulation coils is continuously tracked via an infrared camera system. This results in a constantly updated reference on the position of the coil in relation to the individual cortical anatomy (Fig. 3). From this, the maximum of the evoked electric field can be inferred via line-navigation or e-field-navigation. In line-navigation, the maximum e-field is

presumed to be located on a line perpendicular to the coil plane, while in e-field navigation additional factors such as individual anatomy are taken into account when calculating the invoked e-field (Sollmann et al., 2016).

The use of navigation enables a variety of key options in researching cortical function, such as repeatable targeting of a specific location for longitudinal stimulation, high-resolution raster-mappings of e. g. motor function or mapping of altered cortical anatomy where landmark-based orientation may not be applicable anymore. The latter aspect is of special interest in the context of presurgical mapping of brain functions. On one hand, tumors often alter standard neuroanatomy in a significant way by exerting pressure on or infiltrating the surrounding tissue, which may cause e. g. displacement of specific gyri in relation to standard anatomical landmarks. On the other hand, experiments have demonstrated that for corticospinal motor neurons, functionally relevant representations are not always fixed to the precentral gyrus but may also exist in premotor areas (Moser et al., 2017; Teitti et al., 2008). In order to properly target and afterwards correlate function to these areas, navigated stimulation is necessary. Furthermore, processes such as diffusion-tensor imaging (DTI) based fibertracking (DTI FT) seeded via the individual mapping data are possible by exporting the identified cortical sites as DICOM files (Negwer et al., 2017; Sollmann, Zhang, et al., 2018).

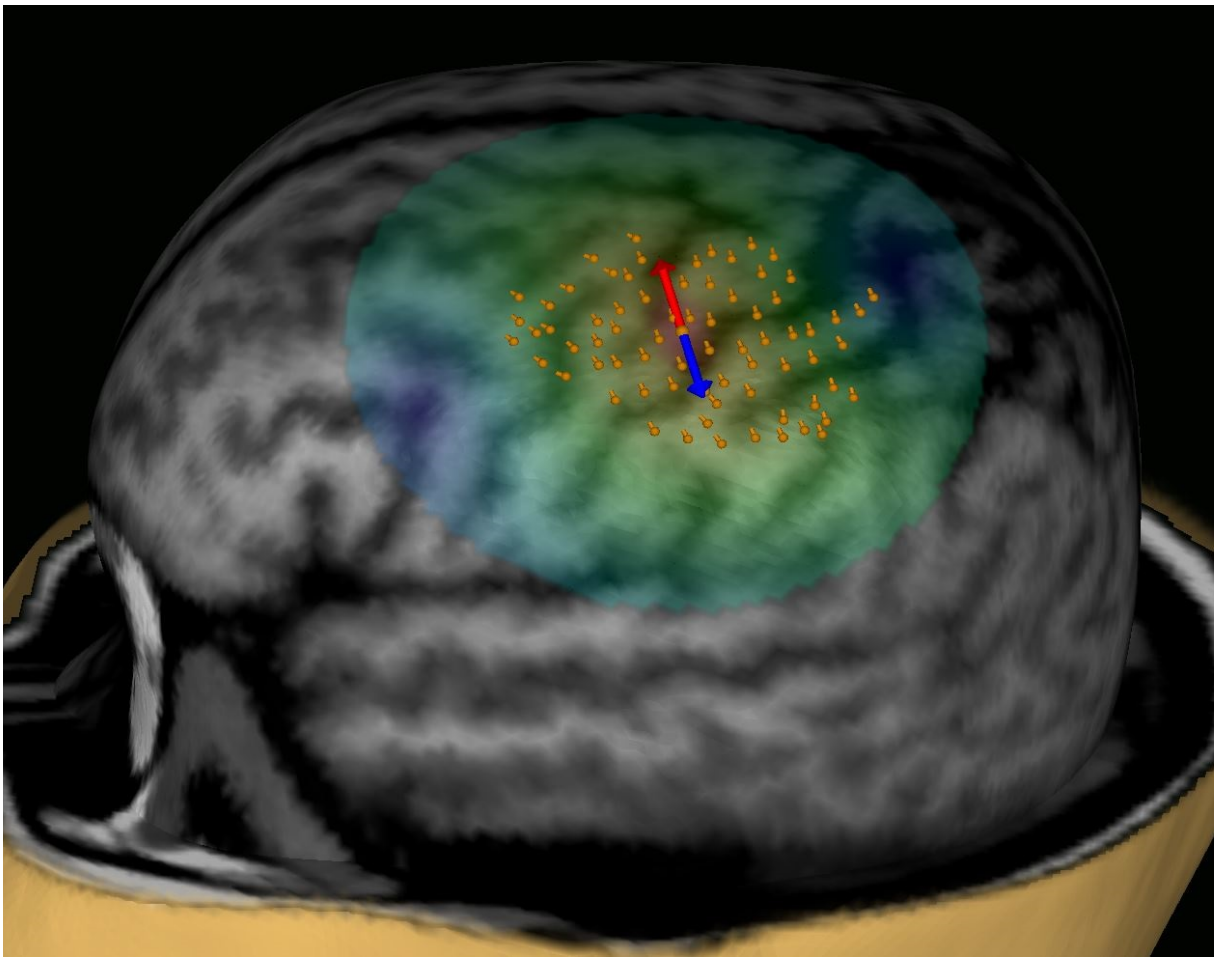

Figure 3 – Neuronavigation in navigated transcranial magnetic stimulation. Figure 3 displays a typical visualization of neuronavigation in navigated transcranial magnetic stimulation (nTMS). Relevant structures such as e. g. the precentral gyrus can easily be identified. Red/blue arrow: induced current direction; Coloured area: Total induced e-field; Orange pins: Individual stimuli.

2.4.2. System Setup

A basic nTMS setup consists of multiple distinct elements (see methods). The two essential components are a stimulation device containing an electromagnetic coil and a neuronavigation system. Depending on the individual use case, various additions such as electromyography (EMG) or behavioral task modules (e.g. display screen for visual tasks) can be added to the setup (Krieg, 2017).

As of today, most available systems rely heavily on being used by experienced personnel. While assist devices may be employed to reduce the weight of the coil or fix it in a given position, most mapping protocols (see below) require both the capacity for momentary fixation as well as quick redeployment of the coil, which can best be achieved by freely handling the coil manually. Basic neuroanatomical knowledge is required for optimal stimulation target placement and selection of primary stimulation sites (e. g. in motor mapping).

The stimulation trigger is usually present in the form of a foot pedal, thereby leaving both hands free for coil handling. Additional information is provided in the methods section.

2.4.3. Functional Mappings

Due to its ease of application, relative lack of side-effects and its patient-oriented benefits, nTMS has found increasing acceptance in the neurosurgical community over the course of the last decade, over which nTMS has been used to map a wide variety of cognitive function. The following section will give a brief overview over currently applied mappings and is also meant to illustrate the rationale for our experiments regarding the mapping of the SMA.

2.4.3.1. Motor Mappings

One of the most prominent uses of diagnostic nTMS in brain surgery is the mapping of functional primary motor cortex to the individual brain anatomy. In brief, the examination is performed as follows (Krieg et al., 2017):

After co-registration to the previously gathered structural MRI data as detailed above, the patient is instructed to sit/lie in a relaxed position. EMG recording electrodes are affixed to the respective muscles of interest, which may be chosen either according to a given in-house standard or according to the specific location of the lesion of interest. Common target muscles include abductor pollicis brevis, adductor digiti minimi, flexor carpi radialis, biceps brachii, quadriceps femoris, tibialis anterior and gastrocnemius. In recording the EMG, it is imperative to avoid sources of EMG noise such as electronic devices. While it has been shown that motor

mappings are performable even when high amounts of ambient electromagnetic noise are present (Schramm, Haddad, et al., 2020), all non-essential sources of noise should be removed for optimal data. In the context of nTMS motor mappings, a motor evoked potential (MEP) is considered valid if it shows both plausible latency (which depends on the respective muscle of interest) and possess an amplitude of at least 50 µV.

After EMG noise has been sufficiently reduced, a rough mapping is performed by applying stimuli with a supra-MEP-threshold intensity to the hand-knob and surrounding cortical areas. The goal of this rough mapping is the identification of a motor-hotspot, i. e. the cortical point at which an MEP for a specific muscle is most easily evoked with a maximal amplitude (Krieg et al., 2017). After identification of this hot-spot, the optimal angulation of e-field to local gyrus in an iterative process of testing out different angulations and narrowing down to the optimal one, which the literature states is usually 90° in relation to the local gyrus (Krieg et al., 2017; Raffin et al., 2015).

Once the optimal angulation has been verified, the resting motor threshold (rMT) can be obtained. The rMT is defined as the minimum stimulation intensity necessary for obtaining valid MEPs in 50 % of all trials. By stimulating the motor hotspot multiple times with varying frequencies and taking into account the respective resulting MEPs, the rMT can be estimated within specified confidence intervals. The rMT is considered a measure of cortical excitability and correlates with a number of other measures, e.g. microstructural properties of white matter, sex and antiepileptic medication (Klöppel et al., 2008; Sollmann, Tanigawa, et al., 2017).

Subsequent to the rMT determination, the actual motor representation mapping takes place. Herein, a stimulation intensity of 105% of rMT is used to serially stimulate the cortical area around the motor-hotspot until locations are found where no valid MEPs can be evoked anymore (Krieg et al., 2017). The result is an individualized map of cortical motor representations (Fig. 4). This map can then be exported and used for further planning, such as e. g. by DTI FT with the motor representations used as seed Regions of Interest (ROI) for delineation of the corticospinal tract (Sollmann, Wildschuetz, et al., 2017). Fig. 5 demonstrates an example of DTI FT visualization for both motor seeds and language seeds (see below).

The use of nTMS for preoperative motor mappings has nowadays been practiced for almost a decade, and investigations into its clinical use have found a number of related benefits regarding patient outcome. By informing the surgeon of the current state of the patient's motor system, especially in relation to any present intracranial lesions, the surgical planning and resection procedure can be optimized and tailored to the individual case. This has been demonstrated to result in e. g. higher confidence of the surgeon during resection, higher percentage of gross-total resections, smaller craniotomy size and importantly, fewer long-term

surgery-related neurological deficits, thereby providing benefit to patient care (Krieg et al., 2014; Krieg et al., 2012).

Other usages of nTMS motor mappings can be found in circumstances where examinations of the motor system are desirable, but not obtainable through regular neurologic examinations. One example for this can be found in the intensive care environment, where sedation often prohibits patient cooperation. nTMS motor mappings have been employed in this context to gain diagnostic insights into the patient's motor system when these were otherwise unobtainable (Schramm, Haddad, et al., 2020).

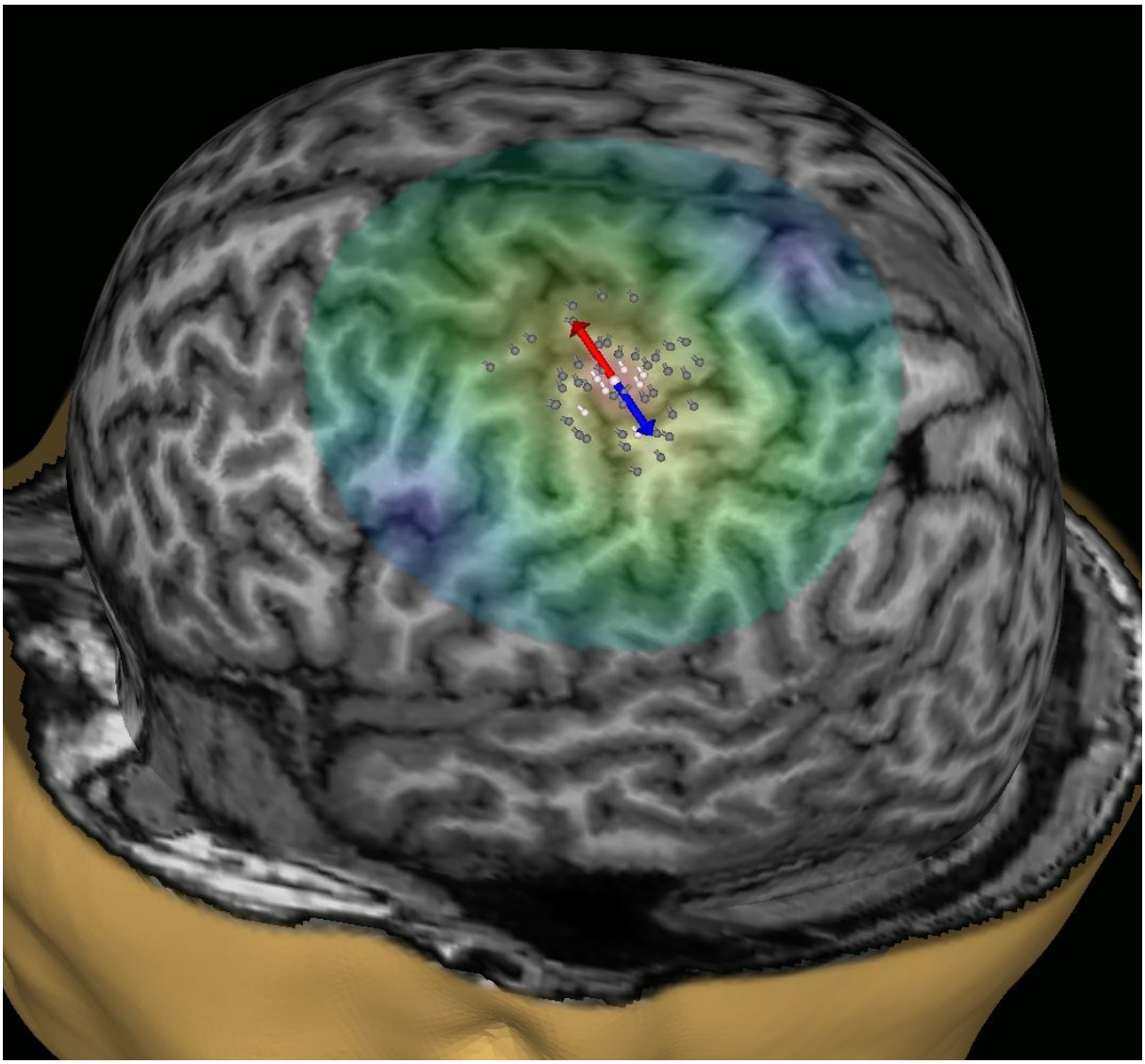

Figure 4 – Exemplary motor mapping. Figure 4 demonstrates the results of a navigated transcranial magnetic stimulation (nTMS) motor mapping of the abductor pollicis brevis muscle. Motor positive points are depicted in white, motor negative points in grey. Current direction is indicated by the arrow.

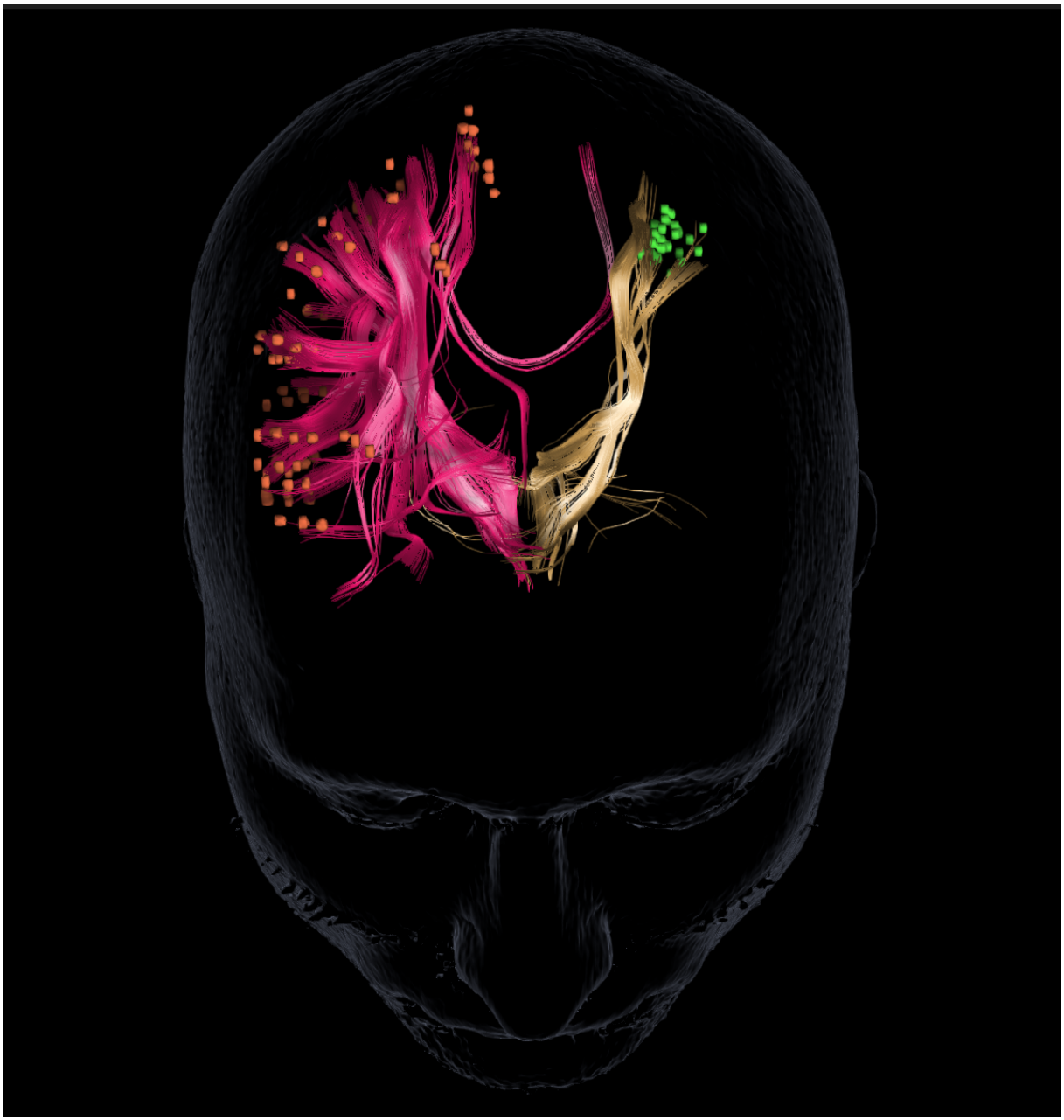

Figure 5 - Exemplary diffusion tensor imaging fibertracking. Figure 5 demonstrates the results of two navigated transcranial magnetic stimulation (nTMS) based fibertrackings (FT). The right hemisphere shows fiber reconstructions based on language-positive nTMS points as seeds (seeds in orange, fibers in pink), the left hemisphere demonstrates the results of motor-positive nTMS points as seeds (seeds in green, fibers in gold).

2.4.3.2. Language Mappings

Another application of diagnostic nTMS is the use of repetitive TMS (rTMS) to identify cortical areas involved in the generation of language. Previous studies utilizing DCS have discovered that the cortical areas involved in language generation are distributed with significant variability among different individuals (Ojemann et al., 1989). Since resection of a language-involved cortical area or fibertract may result in the postoperative occurrence of aphasia (Sollmann et al., 2019), there is value in diagnostic modalities that allow for the precise localization of function on cortical tissue.

The principle of localizing language function fundamentally differs from the nTMS paradigm employed in motor mappings: In motor mappings, the examiner aims to activate corticospinal neurons in order to localize them. In language mappings however, the function can not be visualized in this manner, since no nTMS paradigm exists to the authors knowledge that can consistently force the occurrence of language. Thus, one resorts to the opposite, the induction of a virtual lesion.

The term virtual lesion is loosely defined and refers to the acute disruption of normal cortical function via the application of rTMS protocols (Pascual-Leone, 1999). The neurophysiological basis for the virtual lesion is not extensively studied. In practice, a virtual lesion is observed when rTMS stimulation leads to disruptions of normal task-related behavior, e. g. when a patient is unable to speak during a naming task. Current models assume that the underlying basis for this effect is a momentary lowering of signal-to-noise ratio, which in turn decreases the brains capacity for regulated output (Silvanto & Cattaneo, 2017). Importantly, not every rTMS protocol works in the same manner for every task; authors have noted that some rTMS paradigms can actually lead to increases in cognitive function depending on the involved neuron populations, stimulation strength and brain state (Silvanto & Cattaneo, 2017).

In terms of clinical usage, one commonly applied protocol uses 10 Hz rTMS combined with a picture-naming task (Sollmann, Fuss-Ruppenthal, et al., 2018). Herein, the patient is presented with a number of pictures of common objects (e. g. a house, dog, etc.) and is instructed to name the object shown as quickly and accurately as possible. In a baseline paradigm, the patient is first presented with the entire picture set. Any pictures that elicit unusual responses or that the patient fails to name adequately is removed from the picture pool. In a second baseline run, this procedure is repeated to ensure that the eventual testing set of pictures elicits stable responses under normal conditions.

For the actual mapping, a grid of 42 stimulation targets per hemisphere is overlaid on the individual cortical anatomy according to a standardized template (Sollmann et al., 2013). The subject resumes the prior task and is then stimulated sequentially on every stimulation target

using a 10 Hz/5 stimuli protocol. The performance is recorded, and after the mapping has been conducted, language errors are evaluated on video and the corresponding stimulation target is marked as “language-positive”.

The respective language-positive points are then exported and can be used in preoperative planning similar to the procedure applied in motor mappings. Specifically, DTI FT can be used to visualize fibers emerging from the language ROIs. Investigations into the relation between visualized fibers and intracranial lesions have demonstrated that a risk-stratification regarding postoperative language deficits can be made based on the distance of language fibers to the resection (Sollmann et al., 2019). In this way, language mappings provide both the patient as well as the physicians with treatment-relevant information.

Outside the purely clinical use of nTMS language mappings, the disruption of cognitive processes may hold promise for the use in neuropsychological studies related to the generation of language. In one approach, the language-positive points of 40 patients with brain tumors were separated according to the nature of the language disruption (e. g. no-response, semantic error, performance error, etc.). The authors then demonstrated that the visualized networks differed based on the nature of the language error (Sollmann, Zhang, et al., 2018). In another line of experiments, the classic noun-generating picture naming task has been compared to a verb-generating task for the use in nTMS language-mappings and DTI FT (Ohlerth et al., 2021a; Ohlerth et al., 2021b). Notably, verb-generation differs from noun-generation on the basis of additional grammatical processes such as e. g. declension. Analysis of the mapping results revealed that verb-generation compared to noun-generation led to both higher language error rates during the mapping, as well as a higher number of visualized tracts, specifically those belonging to the ventral stream.

One potential implication of these experiments is that neural correlates of distinct cognitive aspects of language generation could potentially be visualized using nTMS and DTI FT, which might in turn be of interest to e. g. neurolinguists in endeavors related to the study of language generation.

2.4.3.3. Mappings of Other Cognitive Functions

While motor and language mappings are currently the most researched and applied cases of functional mappings using nTMS, other cognitive functions have likewise been explored. The corresponding protocols mostly rely, analogous to language mappings, on the induction of virtual lesions to infer local cortical function. Similar to language mappings, these studies served an important role in the hypothesis generation for the present thesis, demonstrating precedents for successful disruption of higher order cognitive functions via rTMS.

One example can be found in the mapping of arithmetic function to cortical anatomy using simple mathematical operations as a test (Maurer et al., 2016). Here, the authors have adapted the picture-naming paradigm as detailed above, but instead of displaying objects have opted to show brief calculation problems (e.g. “5 + 6”, “9 : 3”) to the subject. The subject was instructed to name the correct solution as quickly as possible, first without and subsequently during stimulation. In analyzing the responses during stimulation, the authors were able to demonstrate that stimulation of different cortical sites elicited different error rates for the subject’s responses, and conclude that rTMS could potentially be used to map the capacity for simple calculations (Maurer et al., 2016).

In another approach, an rTMS paradigm has been used to influence visuospatial computations in healthy subjects, with the intention to identify areas that when damaged might contribute to e.g. neglect (Giglhuber et al., 2017). In these studies, a line bisection landmark test and a greyscale test respectively were used to gauge visuospatial processing. In this task, the subjects were presented with a horizontal line bisected at varying points by a vertical line. The subjects were instructed to name which part of the horizontal line (left or right of the bisection) was larger, both in a baseline setting sans stimulation, and afterwards during stimulation of a number of cortical sites. An erroneous attribution of greater length to the in fact smaller section of the line would in this setting be interpreted as a form of rTMS-induced neglect. The authors report that stimulation led to primarily neglect symptoms ipsilateral to the stimulation, and that this approach may hold promise for future perioperative diagnostics (Giglhuber et al., 2017).

Further examples of cognitive functions that were shown to be disruptable via rTMS include e.g. face processing and visuospatial attention as measured by a grayscale task (Giglhuber et al., 2018; Maurer et al., 2017).

2.5. Mapping of the SMA

Due to the potential ramifications of lesions to the SMA as outlined previously and the demonstrable clinical benefits of mappings of other functions as detailed above, mappings of functional SMA could potentially be of use in the perioperative diagnostics for patients suffering from lesions in this area. A better visualization of functionally relevant structures could plausibly lead to better preoperative planning, more informed decisions on the resection process and consequently to a better outcome for the patient. The following section will briefly detail previous research on nTMS and mappings of the SMA and will then establish the hypotheses for the present thesis.

2.5.1. Previous TMS Studies of the SMA

TMS has been previously applied to the SMA for a number of purposes. One consistent pattern within the literature has been the use of SMA stimulation to probe its connectivity to M1. For example, one study demonstrated that TMS of the SMA enhanced short-interval intracortical facilitation, but not the contralateral silent period or short-interval intracortical inhibition in M1 (Shirota et al., 2012). Interestingly, another study employing a similar design found that when stimulation conditions were tightly controlled, prior SMA stimulation was on one hand able to heighten MEPs elicited from M1, but did on the other hand not influence silent period, short-interval intracortical inhibition or intracortical facilitation (Arai et al., 2012), thus demonstrating partially reversed results to Shirota et al.

In another study, the authors employed a neuromodulation paradigm by using rTMS to investigate the potential effects of modulated SMA activity on M1. In their investigations, the authors found that 5 Hz rTMS to the SMA proper caused significant heightening of MEPs invoked via TMS of M1 for a short period after neuromodulation. Other measures such as short-interval cortical inhibition, intracortical facilitation and silent period were not influenced by SMA stimulation (Matsunaga et al., 2005).

Aside from connectivity studies, and perhaps more relevant to the purpose of this thesis, are attempts of using TMS to disturb various aspects of SMA function. One study for example has employed off-line continuous theta burst stimulation (cTBS) to the SMA to influence perception of effort in a grip force task and found that after stimulation, the task was perceived as less effortful as assessed by a number of metrics (Zénon et al., 2015). SMA stimulation was again related to grip force in another study, where TMS over the left but not the right SMA increased the grip force of healthy participants (White et al., 2013). As a notable limitation, but studies only investigated a small amount of participants (12 and 8 respectively).

In an approach relatable to our study, the pre-SMA has been subjected to 10 Hz rTMS during a language task, resulting in increased reaction times of participants compared to sham stimulation (Tremblay & Gracco, 2009).

The clearest parallels to previous studies and the present experiments can likely be drawn to two studies investigating motor planning and bimanual movement control respectively. Serrien et al. observed that 5 Hz offline rTMS of the SMA lowered both the accuracy of rhythmic, bilimbic finger movements as well as the coupling of C3 and C4 EEG electrode recordings (corresponding to M1) compared to pre-stimulation in six subjects (Serrien et al., 2002). Since the TMS application was performed outside the actual task performance, the study did not create a virtual lesion, but rather observed changes attributable to neuromodulation. In another study, Makoshi et al. observed that depending on the exact timing of a single TMS pulse to the

SMA, healthy subjects' (n =10) ability to hold a sudden weight was impaired in different ways, which the authors interpret as reflecting a timing-dependent disruption of motor planning (Makoshi et al., 2011).

Importantly, despite some studies potentially demonstrating successful disruption of SMA function, none of the mentioned studies have attempted to derive a mapping procedure from their findings, but have rather studied the functionality of the SMA per se.

2.5.2. fMRI Mapping Approaches

Previous attempts at preoperative localization of the SMA have been performed with fMRI paradigms (Kokkonen et al., 2009; Qiu et al., 2014; Wongsripuemtet et al., 2018). fMRI uses recordings of fluctuations in the blood-oxygen level dependent (BOLD) signal to indirectly infer neuronal activity (Logothetis, 2008). While fMRI boasts impressive spatial resolution, it suffers from low temporal resolution, which some studies attempt to compensate for with simultaneous other modalities such as e.g. EEG (Huster et al., 2012).

One 2009 study by Kokkonen et al. was able to visualize a sensorimotor network using both task-based fMRI (finger-tapping task) and rs-fMRI. Notably however, the SMA could not be visualized in the resting-state paradigm in this study, and was not visualized in some of the patients even in the task-based paradigm (Kokkonen et al., 2009).

In another fMRI approach, Wongsripuemtet et al. used as seed-based analysis of rs-fMRI connectivity to visualize bilateral SMAs in a cohort of 66 brain tumor patients and 21 healthy controls. The authors report that visualization of SMA both in patients and controls was successful in more than 95 % of cases using a bilateral handknob seed (Wongsripuemtet et al., 2018).

The SMA has also been visualized as a by-product during investigations into preoperative mappings of the wider sensorimotor network. For example, Qiu et al. used a seed-based approach to analyze both rs-fMRI and task-based fMRI data in 17 patients undergoing tumor resection. In most cases, a seed placement into the hand-knob contralateral to the tumor site was able to visualize the entire sensorimotor network including the SMA (Qiu et al., 2014)..

While studies such as these certainly demonstrate that fMRI can in principle be used for SMA localization, one weakness of seed-based fMRI approaches is that they require a priori assumptions about functional distribution, which in turn may be inaccurate in patients suffering from intracranial lesions (Duffau, 2005; Yang et al., 2007). nTMS could circumvent this issue, since its mechanism for mapping does not rely heavily on a priori assumptions of functional

distribution and has high sensitivity comparable to DCS in e.g. language mapping (Picht et al., 2013).

Additionally, potential methodological concerns regarding the usage of fMRI for function localization in brain tumors exist. Some evidence suggests that tumor neovascularization may influence the BOLD-signal fluctuations underlying fMRI analyses (Hou et al., 2006). This might cause additional problems in localizing function in a lesioned brain. Here again, nTMS might offer an avenue that does not suffer from weaknesses related to altered tissue perfusion.

2.5.3. MEG Mapping Approaches

While fMRI is arguably the most common imaging modality for investigations into functional cortical architecture, MEG and positron-emission tomography (PET) are other modalities that have found applications in this field.

In MEG, brain activity is investigated by interrogating miniscule changes in the magnetic field on the head surface arising from electrical activity of cortical neurons. Herein, MEG not only demonstrates high spatial as well as temporal resolution, but is also directly measuring the effects of neuronal activity as opposed to the effects of neurovascular coupling for fMRI (Papanicolaou et al., 2005). Due to these technical strengths, MEG has seen usage in, among other fields, the perioperative mapping of function to cortical anatomy in the neurosurgical context (Papanicolaou et al., 2005).

While not tailored to the SMA exclusively, one 2019 study by Zimmermann et al. demonstrated that the SMA could be visualized among other motor areas using a movement task of the contralesional hand in a cohort of 13 patients suffering from gliomas, hemangiomas and arterio-venous malformations (Zimmermann et al., 2019).

One potential weakness of MEG in the registration of SMA activity has been hypothesized to be that SMA activation is often found bilaterally and in close proximity to one another, thereby causing signals that may cancel each other out and thus complicate detection via MEG (Lang et al., 1991). This might partially explain the relative scarcity of studies investigating MEG-based SMA detection. Another contributing factor could arguably be the low availability of MEG imaging in general.

2.5.4. Aims and Hypotheses

In light of the literature presented in the previous sections, the hypotheses for this thesis become clear:

1) Based on the previous reports of rTMS usage in mapping cognitive functions, we hypothesize that rTMS protocols can be used to elicit virtual lesions in the SMA.
2) We further hypothesize that these virtual lesions can be objectified using clinically established test of fine motor skills, as per the established main function of the SMA.
3) We further hypothesize that testing of different stimulation parameters will demonstrate a difference in observed effects, thereby yielding a preferential protocol for the induction of virtual lesions.

The main goal of the experiments conducted in this thesis is to generate a protocol that can be used for clinical application in nTMS-based SMA mappings.

3. Methods

3.1. Ethics

The experimental protocol underlying this thesis was approved by the Ethics Committee of Technical University Munich. The study complied with the guidelines set forth in the Declaration of Helsinki. Each participant underwent a detailed education regarding the study protocol, and all potential questions regarding the experimental procedure were answered. Written informed consent was obtained from all participants. Since video material of the experiments was collected, additional explicit consent was obtained regarding the publication of this material (Schramm et al., 2019).

3.2. Subjects

For this prospective pilot study, we recruited a total of 20 subjects. Participants were acquired via word-of-mouth. For participation in the entire experimental process, a sum of 100 € was given to each subject as compensation.

Inclusion criteria were defined as the following (Schramm et al., 2019):

1) Full legal age (18 years) or older
2) Informed consent on part of the participant
3) Right-handedness according to the Edinburgh Handedness Inventory (Oldfield, 1971).

Exclusion criteria were defined as the following (Schramm et al., 2019):

1) Contraindications for MRI, including but not limited to: Pacemakers, intracardial defibrillators, artificial heart valves, intracranial devices such as deep brain stimulation, intrauterine devices, non-removable piercings, other ferromagnetic implants, and claustrophobia.
2) Contraindications for TMS, including but not limited to: Intracranial devices susceptible to magnetic fields, pacemakers, intracardial defibrillators, artificial heart valves, uncontrolled epilepsy.
3) Known anamnestic neurological or psychiatric diseases.

3.3. MRI Acquisition

For the purpose of neuronavigation, each participant first underwent structural MRI imaging with a 3 T scanner (Achieva, Philips Healthcare, Best, Netherlands) and a 32 channel coil. The employed sequences included a T1-weighted, 3D acquired gradient-echo sequence with repetition time of 9 ms, echo time of 4 ms, flip angle of 8°, field of view of 240 mm x 252 mm x 200 mm, 1 mm isovoxel and sequence duration of 2 minutes 25 seconds. The imaging volume was adjusted to cover the entire head (Schramm et al., 2019).

All MRI images obtained for this thesis were screened for incidental findings by the local department of neuroradiology.

3.4. nTMS Protocol

The experiments conducted in the frame of this thesis were designed analogously to prior nTMS studies attempting to disrupt cognitive functions (Maurer et al., 2016; Sollmann, Fuss-Ruppenthal, et al., 2018). Specifically, we employed tasks designed to require SMA activity for optimal performance (see later sections), then let the participants perform the task first without stimulation (baseline), and afterwards during stimulation of six distinct stimulation targets (per hemisphere) located within the anatomical borders of the SMA. Since two hemispheres had to be mapped and rTMS aftereffects on neural activity have been demonstrated for multiple days after stimulation (Zhang et al., 2018), we decided to conduct two sessions, each for the mapping of one hemisphere, spaced apart by at least 14 days to ensure return to baseline neuronal activity (Schramm et al., 2019).

For all nTMS applications, the Nexstim NBS system was used (Nexstim eXimia NBS system, version 4.3; Nexstim Plc., Helsinki, Finland; Fig. 6).

Figure 6 – System setup. Figure 6 displays a typical navigated transcranial magnetic stimulation setup. 1) Stimulation coil; 2) Model head with headtracker (orange); 3) Tracking camera; 4) Navigation display; 5) Electromyography (EMG) cables; 6) EMG display; 7) Monitor and videocamera; 8) Trigger pedals.

3.4.1. Mapping Preparation and Image Registration

Before the appointment, the mapping room was prepared and randomization of the respective protocol parameters was conducted. The sagittal reconstruction of the 3D T1-weighted image was loaded into the stimulation system and an individual session was created. On the second appointment, the session generated on the first appointment was reused. On arrival, the participant was again informed about the upcoming procedure and consent was confirmed. The participant was then seated within the system chair and was outfitted with a headtracking device. Subsequently, the system's stereotactic tracking camera was positioned to register the headtracking device.

To co-register the virtual 3D head model with the physical patient head, three anatomical sites (left and right helicis crus of the outer ear and nasion) were marked both on the virtual headmodel as well as on the physical participant head (via a marking device tracked through infrared-reflecting markers). After this basic co-registration, 9 scalp points were marked in a similar manner. The process was only considered successful if the mismatch between virtual and physical head was lower than 5 mm. Otherwise, the procedure had to be repeated. Care was taken to not alter the position of the headtracker after successful co-registration. Afterwards, the motor mapping procedure began as detailed below.

3.4.2. Motor Mapping and Target Placement

As the first mapping step of each session, an nTMS mapping of primary motor cortex was conducted for two reasons:

1) Atypical representations of primary motor cortex within the premotor cortex have been demonstrated previously in patient cohorts (Bulubas et al., 2016; Moser et al., 2017). Despite this study only including healthy participants, we wanted to exclude the potential of motor representations within the premotor cortex, since these could interfere with our assessment of fine motor skills when stimulated.
2) The neuronal activation theshold for nTMS differs between individuals (Sollmann, Tanigawa, et al., 2017). Therefore, nTMS stimulation intensity has to be tailored to the individual day-specific rMT, the determination of which requires at least a rough motor mapping for the hotspot identification.

For the motor mapping and rMT determination, we followed existing guidelines as previously mentioned in the introductory section (Krieg et al., 2017). The participant was seated in the NBS system chair and instructed to relax their muscles. We recorded EMG activity from 4 upper extremity and 2 lower extremity muscles per side: abductor pollicis brevis, adductor digiti

minimi, flexor carpi radialis, biceps brachii, flexor carpi radialis and gastrocnemius. MEP-validity was assessed on the basis of amplitude (> 50 µV), latency (depending on muscle) and morphology. In a rough mapping, an overview over motor representations was generated and a hotspot selected. The optimal angulation for MEP elicitation was tested on the hotspot, and after identification used in the rMT determination process via the maximum likelihood algorithm (Awiszus, 2003). Afterwards, a fine resolution single-pulse motor mapping was conducted using 105% rMT as the stimulation intensity. Herein, special attention was paid to the premotor cortex (Schramm et al., 2019).

After the mapping process, the acquired data was first processed to filter out false positives and negatives, yielding the final motor map. Six stimulation targets were then placed anterior of the determined primary motor representations in a rectangular pattern within the anatomical area encompassing the SMA (Fig. 7).

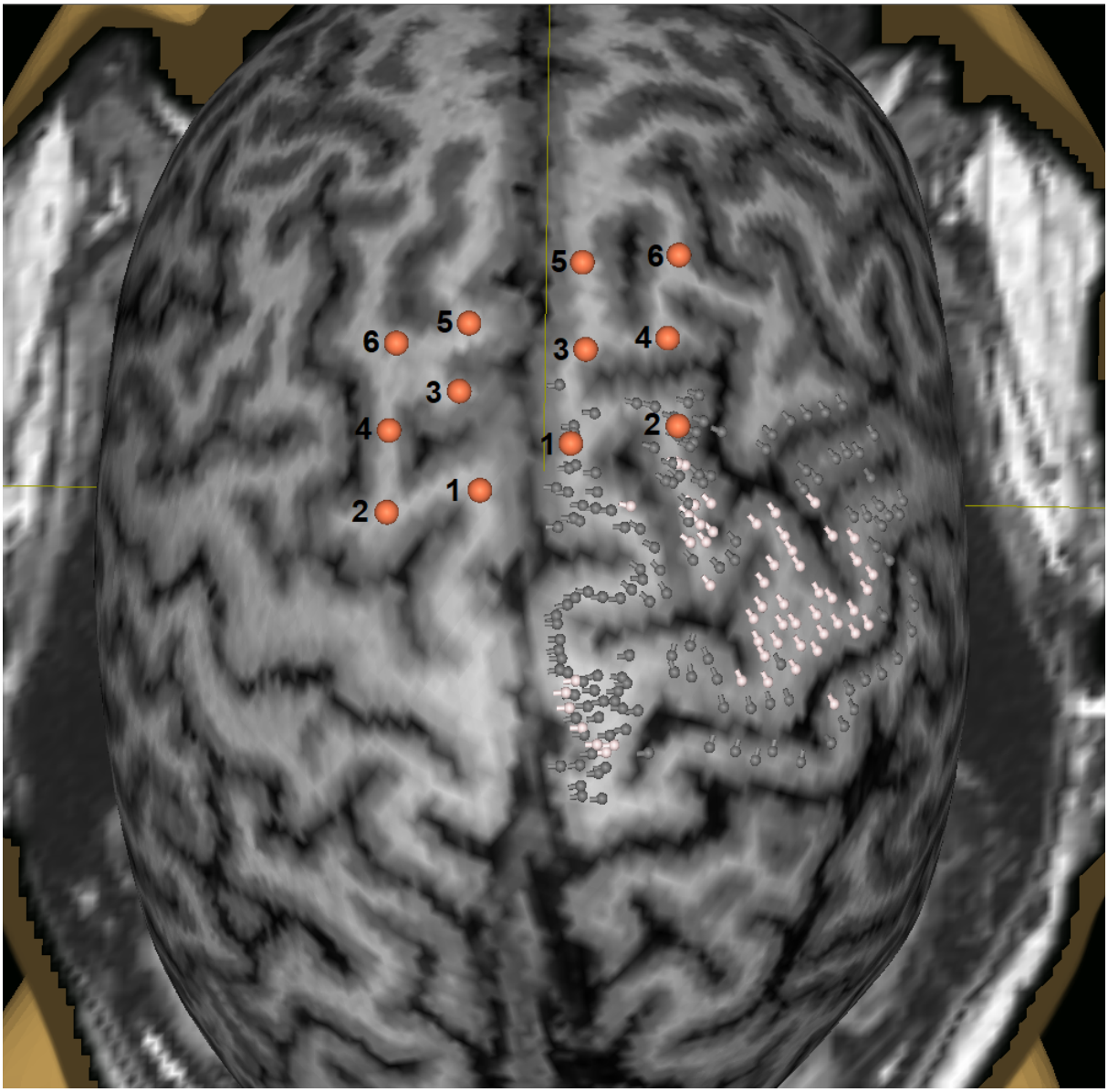

Figure 7 - Exemplary target placement. Figure 7 demonstrates the placement and denomination of supplementary motor area (SMA) stimulation targets in relation to mapped primary motor cortex. In this case, primary motor representations were found in premotor areas on the right hemisphere. Targets are depicted in orange and numbered in black lettering. Motor positive points are depicted as white pins, motor negative points as grey pins (Schramm et al., 2019).

3.4.3. Stimulation Protocols

Within the current literature, a wide variety of rTMS protocols are used for different purposes (Lefaucheur et al., 2014; Thut & Pascual-Leone, 2010). While many protocols have traditionally been separated into “inhibitory” and “excitatory” protocols (Thut & Pascual-Leone, 2010), newer research suggests that this categorization may be inadequate to fully model the complexity of rTMS effects (Goldsworthy et al., 2021). Additionally, the nature of the virtual lesion per se is currently not well understood (Silvanto & Cattaneo, 2017).

Since against this backdrop, a priori assumptions regarding the optimal suitability of any single stimulation protocol for a given purpose are difficult to justify, we decided to include three different rTMS protocols in our experiments.

1) 5 Hz rTMS (1500 pulses per burst, 1 burst), a high-frequency protocol which is applied in mappings of e. g. language function (Krieg et al., 2017).
2) 10 Hz rTMS (3000 pulses per burst, 1 burst), another high-frequency protocol which has shown promising results in the disruption of language processes (Sollmann, Fuss-Ruppenthal, et al., 2018).
3) cTBS; 3 pulses at 50 Hz per burst, 999 bursts, 0.16 s inter-burst interval), a protocol used for rapid neuromodulation which is widely presumed to cause local neuronal inhibition (Huang et al., 2005; Suppa et al., 2016).

With this selection, we have included two high-frequency protocols (5 Hz and 10 Hz), which in the classical understanding would be categorized as “excitatory”, as well as one “inhibitory” protocol in the form of cTBS.

3.5. Fine Motor Testing

Since the goal of this thesis relates to the elicitation of deficits in fine motor control, we employed a number of clinically established fine motor tests to objectify potential SMA disruptions as detailed in this section. The tests included the Jebsen-Taylor Hand Function Test (JHFT), Nine-Hole Peg Test (NHPT), pronator drift test (PDT), finger-nose test (FNT), finger tapping test (FTT) and flexion-extension test (FET) (Schramm et al., 2019).

At the beginning of each session, after the motor mapping, the participants were first instructed regarding each test. They then performed baseline trials (i. e. without stimulation) for each test and with each hand where applicable. Afterwards, the six stimulation targets of one hemisphere were stimulated sequentially while the patient continued to perform the tests, until one trial was recorded for each stimulation target, each hand, each hemisphere and each

stimulation protocol. The order of hemispheres, stimulation protocols, tests and hands were randomized. In cases where the performance or the stimulation was interrupted for any reason, another trial was recorded. In order to more precisely time the beginning of each trial, the patient was counted down towards the start ("3, 2 , 1, Go."). Stimulation began at the starting command and ended when the test was finished. In tests where specific times were recorded, a Matlab script was used to time the performance and record the values (see later section). In total, each session applied approximately 35,000 stimuli and took roughly 270 minutes. Each session was recorded on video for post-hoc analysis purposes. Timing of performances was measured and documented using a Matlab script (MATLAB, version R2018b; The MathWorks Inc., Natick, Massachusetts, USA) (Schramm et al., 2019).

3.5.1. JHFT

The JHFT is a commonly used test battery used to gauge upper limb fine motor skills in patients (Jebsen, 1969). Its assessment of hand function has been demonstrated to correlate with patient function in daily activities (Lynch & Bridle, 1989). In the presented experiments, we utilized a commercially available set containing all seven subtests of the JHFT (Sammons Preston, Bolingbrook, Illinois, USA; Fig. 8). The JHFT measures the test completion time (TCT), i. e. the time that a given participant needs to fulfill each subtask. Accordingly, we believed that SMA disruption would lead to increased TCTs compared to baseline. The JHFT is performed in a seated position after detailed instructions. For each hand, stimulation target and protocol, one trial of each subtest was acquired, corresponding to a total of 38 trials (two baseline trials plus 36 stimulation trials) per session and subtest. The entire JHFT is composed of (Fig. 8):

1) Writing: The participant is presented with a face-down card, on which a simple sentence is written. On command, the participant turns the card and writes down the sentence found on it.
2) Simulated page turning (SPT): The participant is presented with 5 paper cards arranged in front of him/her. On command, the participant sequentially turns the cards from face-down to face-up position, starting on the side of the executing hand (e. g. right-most card for right-handed trials).
3) Lifting small objects (LSO): The participant is presented with a selection of six objects (two paperclips, two coins, two bottle caps) arranged next to a metal can. On command, the participant sequentially moves each individual object into the metal can.
4) Simulated feeding (SF): The participant is presented with five beans, a metal can and is given a spoon. On command, the participant sequentially moves each bean into the can using the spoon, starting from the most distal bean in relation to the can.

5) Stacking checkers (SC): The participant is presented with four checkers pieces. On command, the participant stacks the pieces to form a small tower shape.
6) Lifting light objects (LLO): The participant is presented with five empty metal cans and a marked wood board. On command, the participant sequentially lifts each can onto the designated marking.
7) Lifting heavy objects (LHO): The participant is presented with five metal cans filled with sand and a marked wood board. On command, the participant sequentially lifts each can onto the designated marking.

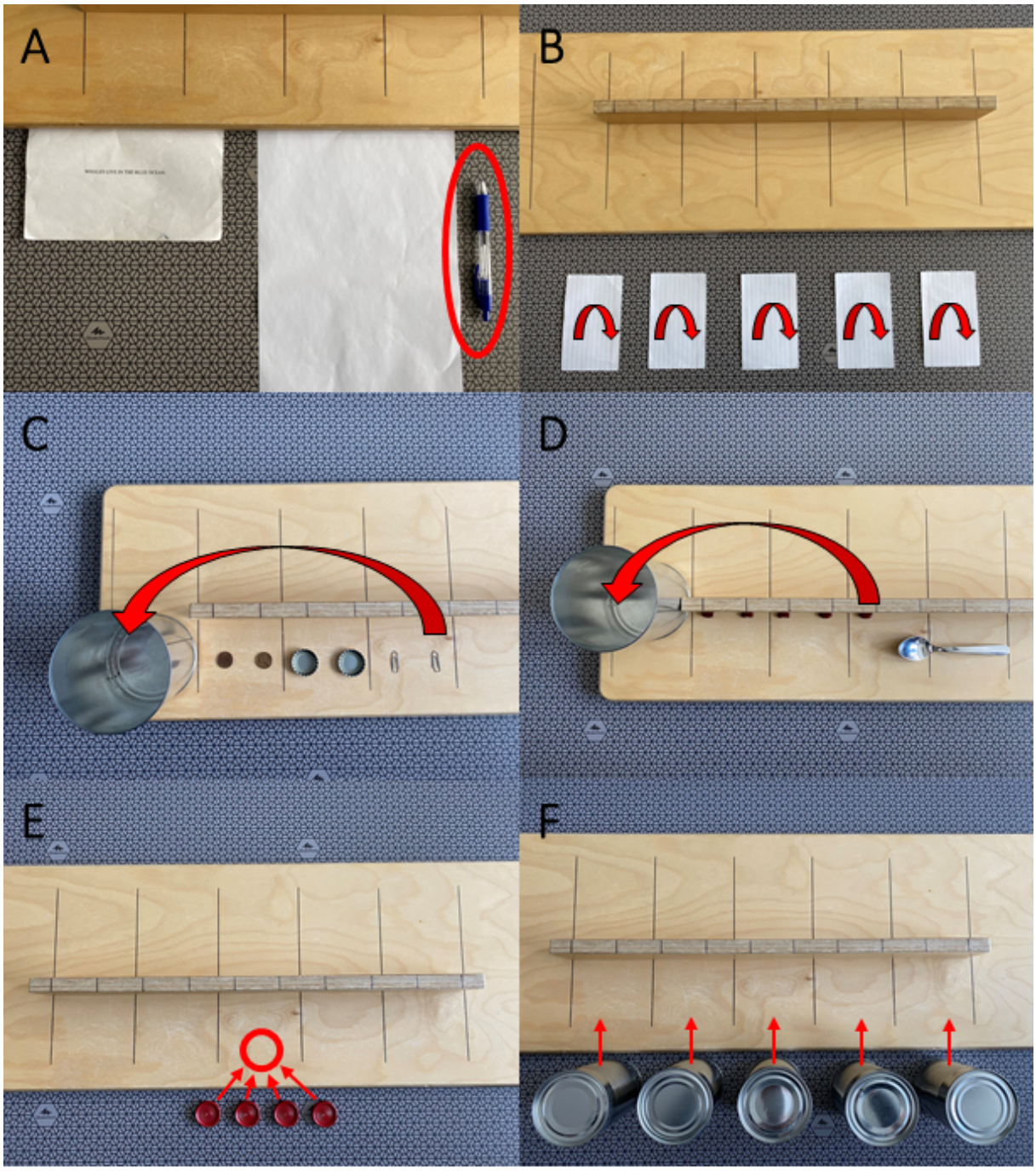

Figure 8 - Jebsen-Taylor Hand Function Test. Figure 8 shows the subtests of the Jebsen-Taylor Hand Function Test (JHFT) from the point of view of the participant. A: writing; B: simulated page turning; C: lifting small objects; D: simulated feeding; E: stacking checkers; F: lifting light objects and lifting heavy objects (test appears identical and only differs in can filling)

3.5.2. NHPT

The NHPT is a test widely used in occupational therapy to estimate upper limb fine motor skills (Mathiowetz et al., 1985). In the test, the subject is presented with a board containing nine holes arranged in a quadratic pattern and a small bowl in which 9 corresponding pins are located (Fig. 9). The subject is instructed to on command sequentially pick up the pins and place each one into a hole. After all pins have been placed, the participant then has to relocate each pin into the initial bowl. The time taken for the entire process is noted and used to gauge manual dexterity. In our setup, we employed a commercially available version (Patterson Medical, Bolingbrook, Illinois, USA). We hypothesized that, analogous to the JHFT, disrupted SMA activity would lead to increased TCTs.

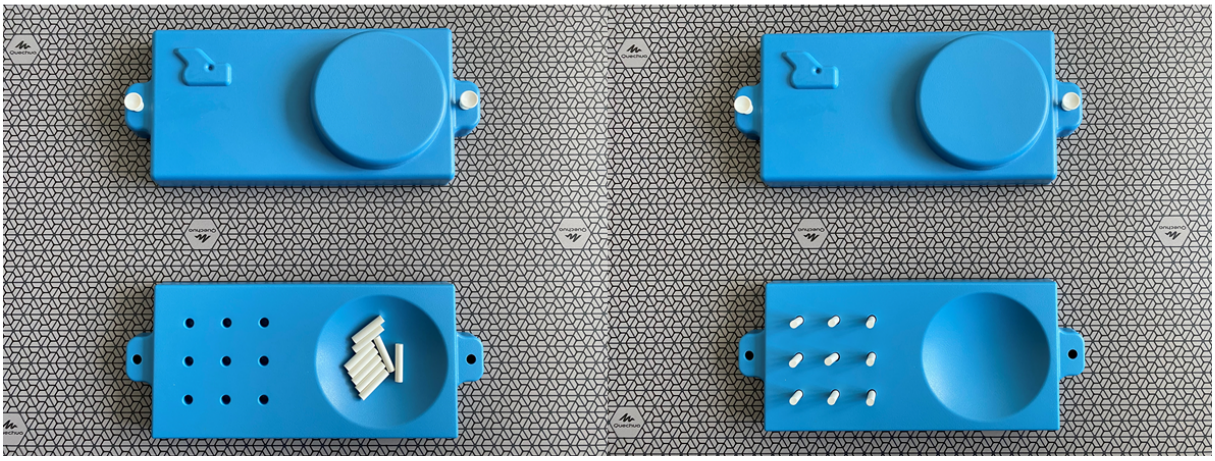

Figure 9 - Nine-Hole Peg Test. Figure 9 shows the Nine-Hole Peg Test (NHPT) from the point of view of the participant. The left side pictures the initial setup, the right side demonstrates the state after pin insertion. The test is completed after the participant has returned all pins, effectively returning to the initial condition.

3.5.3. PDT

The PDT is a staple in the field of neurological examinations. It is used to test for upper limb gross motor deficits, e. g. in the context of acute neurological injury. In this test, the participant is asked to elevate both arms 90° in front of him/her, fingers extended and palm directed upwards. The subject is then asked to hold the posture, and to close their eyes while doing so. For each target, 15 s of static posture were recorded. A pathological PDT can be observed when the patient is either unable to keeps their arms extended, or when the limbs go from the initial supination (palms upward) to a pronated position. We included this test to determine whether SMA disruption could also lead to gross motor deficits, e. g. a phenotype similar to a pathological PDT.

3.5.4. FNT

Similar to the PDT, the FNT is a commonly used test in neurological diagnostics, especially in the context of motor pathologies. In the FNT, the subject is asked to keep their arms extended laterally. The subject is then asked to close their eyes and put one of their index fingers on the tip of their nose. This movement is then alternated with both hands. In our specific setup, we adapted the FNT and asked the participant to alternate the movement in a 1 Hz rhythm generated via metronome. Per stimulation target, 15 s of performance were recorded. Pathological findings evident in the FNT include e. g. dysmetria (e. g. when the subject places its finger on the closed eye instead of the nose) or intention tremor (e. g. when the subject develops a tremor with stronger amplitude the closer they get to the nose). For this test, we hypothesized that SMA disruption could plausibly cause coordination problems resulting in problems in rhythm keeping or a phenotype similar to dysmetria.

3.5.5. FTT

Finger tapping paradigms have been previously used in attempts to visualize the SMA, and has in these studies served to successfully elicit neuronal activation in the SMA (Kokkonen et al., 2009). We therefore designed a rhythm guided finger tapping task for our experiments. In it, the subject is presented with a computer keyboard. The participant is instructed to tap two arbitrary keys in a 1 Hz rhythm with alternating hands. Per stimulation target, 20 taps were recorded. The timing of each tap was recorded and analyzed post-hoc. For consequences of SMA disruption, we hypothesized that the mean time interval between taps would be more variable during SMA disruption compared to baseline, resulting in a higher standard deviation of inter-tap intervals (SDIT) and reflecting a lessened ability to coordinate movement precisely

to a given timing. We decided to forego analysis of the mean inter-tap interval, since the mean interval is essentially determined by the time passed between first and last tap. Thus, deviations in mean inter-tap interval could relatively easily be corrected during the test.

3.5.6. FET

The SMA has previously been implicated in the coordination of rhythmic movements and bilateral limb coordination. Due to this, we have included the FET as a low-level task tailored to this aspect of SMA function. In the FET, we instructed the participant to extend both arms horizontally in front of them, with one arm fully extended and the other arm flexed 90 ° (i. e. forearm pointing upwards). The subject was then asked to alternate this position between both limbs according to a 1 Hz rhythm generated by a metronome. Per stimulation target, 15 s of performance were recorded. We hypothesized that SMA disruption could lead to dissociation of the performance from the generated 1 Hz rhythm.

3.6. Statistics

Depending on the respective test, we employed different statistical methods in our analyses. Testing for normal distribution with a Shapiro-Wilks test indicated that normal distribution was not met for SDITs and TCTs in multiple cases. Due to this, we decided against the usage of a parametric test for the analysis of the respective measures. Instead, a Wilcoxon rank-sum test was used in the primary analysis of TCTs and SDITs. Herein, the baseline performances were compared to performances during SMA stimulation. More specifically, the average of TCTs or SDITs obtained during stimulation of the six targets were compared to their respective baseline values. Due to the high number of parameters, specifically stimulated hemisphere, stimulation protocol and hand laterality, analyses were conducted according to the emerging subgroups (e. g. right hemispheric sessions; left-handed trials; baseline vs. 5 Hz means vs. 10 Hz means vs. cTBS means). Additionally, we performed analyses in which the data from both hands were pooled (e. g. right hemispheric session; values from both hands; baseline means vs. 5 Hz means vs. 10 Hz means vs. cTBS means) (Schramm et al., 2019).

In order to investigate potential regional differences of SMA stimulation, further analyses were performed with values grouped as either medial (1,3 and 5) or lateral (2,4 and 6) targets, as well as grouped as either anterior (5, 6), middle (3, 4) or posterior (1, 2) targets (Fig. 7). In these analyses, a Friedman test was employed to investigate potential group differences in the anterior-posterior group splits, while a Wilcoxon rank-sum test was utilized in the medio-lateral split. Analogously to the primary analyses, the comparisons were performed within their

specific subgroups according to hemisphere, hand and stimulation protocol (Schramm et al., 2019).

To investigate potential differences in rMT between both hemispheres, a paired t-test was utilized. Visible coordination problems (VCPs), by their nature, could not be analyzed in an elaborate analysis. Instead, the occurrences of VCPs were documented and their respective prevalence was documented. Herein, care was taken to ensure that no ambivalent events (such as e.g. simply dropped objects) were counted, and the focus was instead put towards more straightforward occurrences such as e.g. movement arrests or involuntary limb use (Schramm et al., 2019).

In order to further isolate the effect of the individual parameters (hemisphere, hand, stimulation target, stimulation protocol) on TCTs, a multi-level regression model was constructed. In this model, the TCT was coded as the dependent variable, while the aforementioned parameters were coded as the independent variables. This approach further allows for the modeling of interaction effects between hemisphere and hand. In this model, random effects were encoded to compensate for idiosyncrasies of the participants (Schramm et al., 2019).

For all tests, the level of statistical significance was set to 0.05. Statistical analyses were performed with two programs. Descriptive statistics and primary comparisons were computed in GraphPad Prism (version 7.0; GraphPad Software Inc., La Jolla, California, USA), while the regression models were run with the statistical software R (version 3.1.0; https://cran.r-project.org; The R Foundation for Statistical Computing, Vienna, Austria) (Schramm et al., 2019).

4. Publications

The publication „Application of Navigated Transcranial Magnetic Stimulation to Map the Supplementary Motor Area in Healthy Subjects“, published in the Journal of Clinical Neurophysiology, was chronologically the first publication in the context of this thesis, published online in October 2018 and having since appeared in print in March 2020 (Schramm, Sollmann, et al., 2020).

For this paper, we conducted a partial analysis of our hitherto collected data, specifically for a subset of 10 female participants, the JHFT and the 10 Hz stimulation protocol. We observed that compared to baseline performances, JHFT performances during stimulation were subject to heightened TCTs in three subtests, specifically SPT, LSO and SF. In three other subtests (LHO, LLO and SC), TCTs were not significantly different between baseline and stimulation, while one subtest (writing) demonstrated lowered TCTs during stimulation.

These findings were discussed in the context of the corresponding literature, with a focus on the novelty of the approach, the proof of concept regarding a potential mapping procedure, as well as discussions regarding the specific TCT alterations that were observed.

Regarding first author contributions, SS acquired the data, performed the statistical analyses, created the figures and drafted the manuscript with feedback from the coauthors.

Application of Navigated Transcranial Magnetic Stimulation to Map the Supplementary Motor Area in Healthy Subjects

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Purpose: The supplementary motor area is involved in the planning and coordination of movement sequences. This study investigates the potential of repetitive navigated transcranial magnetic stimulation for systematic mapping of the supplementary motor area by interfering with normal movement coordination processing.

Methods: Ten healthy females (median age: 23.5 years) performed the Jebsen–Taylor Hand Function Test, first without stimulation (baseline) and afterward during application of repetitive navigated transcranial magnetic stimulation with 10 Hz to 6 cortical sites located within the supplementary motor area of both hemispheres. The test completion times (TCTs) were then compared between baseline performances and performances during stimulation.

Results: We found significant slowing of TCTs in simulated page turning (baseline TCT 3.68 ± 0.67 seconds vs. stimulation TCT 4.04 ± 0.63 seconds, $P = 0.0136$), lifting small objects (baseline TCT 5.11 ± 0.72 seconds vs. stimulation TCT 5.47 ± 0.66 seconds, $P = 0.0010$), and simulated feeding (baseline TCT 6.10 ± 0.73 seconds vs. stimulation TCT 6.59 ± 0.81 seconds, $P = 0.0027$). Three other subtests were not affected, whereas one subtest was performed significantly faster (baseline TCT 17.09 ± 7.31 seconds vs. stimulation TCT 15.44 ± 5.72 seconds, $P = 0.0073$) under stimulation.

Conclusions: Repetitive navigated transcranial magnetic stimulation is capable of influencing the performance of healthy participants in a task relying on hand coordination. Our approach can serve as a mapping tool for the supplementary motor area, potentially relevant for preoperative diagnostics in patients with brain tumors, epilepsy, or other brain lesions to improve outcome and potentially predict clinical course and postoperative recovery.

Key Words: Brain stimulation, Fine motor skills, Functional mapping, Jebsen–Taylor Hand Function Test, Navigated transcranial magnetic stimulation, Supplementary motor area.

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In recent years, multiple studies have shown the feasibility of using navigated transcranial magnetic stimulation (nTMS) as a noninvasive method for performing functional brain mappings.[1–5] The data gained from these examinations can be used both to test theoretical constructs in neuroscience and to gain insights influencing clinical diagnostics. This is particularly relevant in subjects with altered intracranial anatomy due to brain tumors and/or chronic epilepsy, inducing functional reorganization.[6–8] Areas commonly investigated by nTMS mapping procedures are language areas or the primary motor cortex.[1–3] The necessity for mapping is further exemplified by findings showing that resection of nTMS-identified motor-positive points may lead to permanent postoperative paresis, even when located frontal of the precentral gyrus.[9]

The supplementary motor area (SMA) is a brain area anterior of the precentral gyrus, composed of an anterior (pre-SMA) and a posterior part (SMA proper).[10,11] It is involved in spatiotemporal cognitive tasks, most importantly movement coordination and sequencing.[12–15] Damage to the SMA, e.g., due to ischemic events or brain surgery, has been documented to cause clinical symptoms such as contralateral decline in motor functions.[16–21] The characteristic combination of contralateral hemiparesis and mutism, emerging after the affection of the SMA, is known as the SMA syndrome. Although regarded as a transient condition with symptoms typically disappearing within days to weeks, steps are undertaken to prevent the SMA syndrome from occurring. Prevention of even a transient syndrome would improve immediate outcome and facilitate postoperative care. Also, because the mechanism responsible for the usual recovery has not yet been satisfyingly explained, rare irreversible cases of the SMA syndrome as well as persisting fine motor deficits cannot be ruled out.[18]

Transcranial magnetic stimulation (TMS) has previously been applied to the SMA for various purposes. Transcranial

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S. Schramm and N. Sollmann contributed equally.

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magnetic stimulation studies exist on the nature of functional connections between the SMA and other motor-associated areas.[22–24] Furthermore, the role of the SMA in a broad spectrum of different cognitive processes has been explored using TMS.[25–29] However, applying TMS to the SMA has primarily been restricted to non-navigated TMS investigations, thus not allowing the precise allocation of function to the individual cortical anatomy, and the method has not yet been routinely considered in clinical diagnostics. This is, however, particularly necessary in subjects with altered brain anatomy due to brain tumors or chronic epilepsy, for instance. Data derived from nTMS to the SMA could give the neurosurgeon a clearer understanding of the patient's individual distribution of function preoperatively, possibly leading to improved surgical planning and better outcome in terms of reduced SMA-related deficits. In this study, we apply mapping of the SMA by repetitive nTMS (rTMS) in combination with the Jebsen–Taylor Hand Function Test (JHFT) with respect to the following hypotheses:

1. Application of rTMS is suitable to provoke differences in test performances compared to performances without the stimulus condition.
2. High-frequency rTMS can promote cortical excitability. Applied to the SMA, we expect one of the following effects:
   a. Facilitation of motor performance by excitation of functionally connected motor areas.
   b. Introduction of counterproductive cortical noise ("virtual lesion"), resulting in worsened test performance.

MATERIALS AND METHODS

Ethics

This study was approved by the local institutional review board (registration number: 293/17) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects.

Participants and Study Design

For this prospective study, we recruited 10 healthy female volunteers (median age: 23.5 years). Inclusion criteria were age of at least 18 years, informed consent, and right-handedness according to the Edinburgh Handedness Inventory.[30] Exclusion criteria were pregnancy, contraindications for MRI or rTMS (e.g., cochlear implants), and any history of neurological or psychiatric diseases.

Each participant first underwent anatomical MRI at 3T (Achieva; Philips Medical Systems, Best, The Netherlands) acquiring a T1-weighted gradient echo sequence (repetition time/echo time: 9/4 ms, 1 mm^3 isovoxel covering the whole head) needed for neuronavigation purposes during later rTMS. Mapping by rTMS (Nexstim eXimia NBS system, version 4.3; Nexstim Plc, Helsinki, Finland) then took place in the context of two sessions (separated by 14 days, one session per hemisphere) consisting of both motor mapping to delineate the primary motor cortex and systematic mapping of the SMA using the JHFT (for both hands in each session).

Determination of Mapping Targets

First, motor mapping of the upper extremity using single-pulse stimuli at 105% of the individual resting motor threshold was performed to delineate the primary motor cortex according to current practice.[31] This was done to prevent the attribution of any effects to accidental stimulation of the primary motor cortex during later mappings of the SMA because previous findings have demonstrated primary motor representations outside the confines of the precentral gyrus and located more anteriorly in frontal gyri.[9] Therefore, we considered it a crucial prerequisite for our targeting of the SMA to ensure that no primary motor cortex representations were located within the stimulation target area.

The extent of the primary motor cortex was completely mapped in all directions, with special attention to the superior frontal gyrus and middle frontal gyrus (Fig. 1). Then, six stimulation targets per hemisphere were placed outside the primary motor cortex according to previous motor mapping, either strictly anterior or, in cases where motor-positive points extended to the lateral premotor cortex, dorsal of the motor-positive spots, thus directly bordering on the primary motor cortex (Fig. 1). These points were manually placed within the posterior superior frontal gyrus, in some cases closely bordering on the middle frontal gyrus. The distance between single points was approximately 5 to 10 mm.

Mapping of the SMA

Baseline Assessment and Test Description

Subsequent to motor mapping and placement of the stimulation targets, baseline assessment (task performance without simultaneous stimulation) was conducted. We used the JHFT (Sammons Preston, Bolingbrook, IL), which consists of seven sequential subtests, namely writing, simulated page turning (SPT), lifting small objects (LSO), simulated feeding (SF), stacking checkers (SC), lifting light objects, and lifting heavy objects.[32]

The sequence of hands to be used was randomized at the start. For each subtest, instructions on the task to be performed were given orally and comprehension of the task was ensured by having the participant describe the task or do a test run. Participants were advised to perform each subtest as quickly and precisely as possible. The test completion times (TCTs) of the baseline performances were documented. If severe problems arose during the baseline (e.g., dropping of test objects to the floor), another baseline was conducted and considered instead of the previous one. Recordings of the entire baselines and the performances under stimulation were taken using a video camera.

Stimulation

The hemisphere stimulated in the first session was subject to randomization (coin toss). We used an rTMS protocol with a stimulus frequency of 10 Hz and a stimulation intensity of 100% of the resting motor threshold. Each stimulation train consisted of 3,000 stimulation pulses (total duration of the stimulation train: 5 minutes). Stimulation was started simultaneously with the start command for each trial and was applied until the trial was finished; thus, the individual duration of stimulation was different between subjects depending on the speed of subtest completion, but was <5 minutes in all subjects.

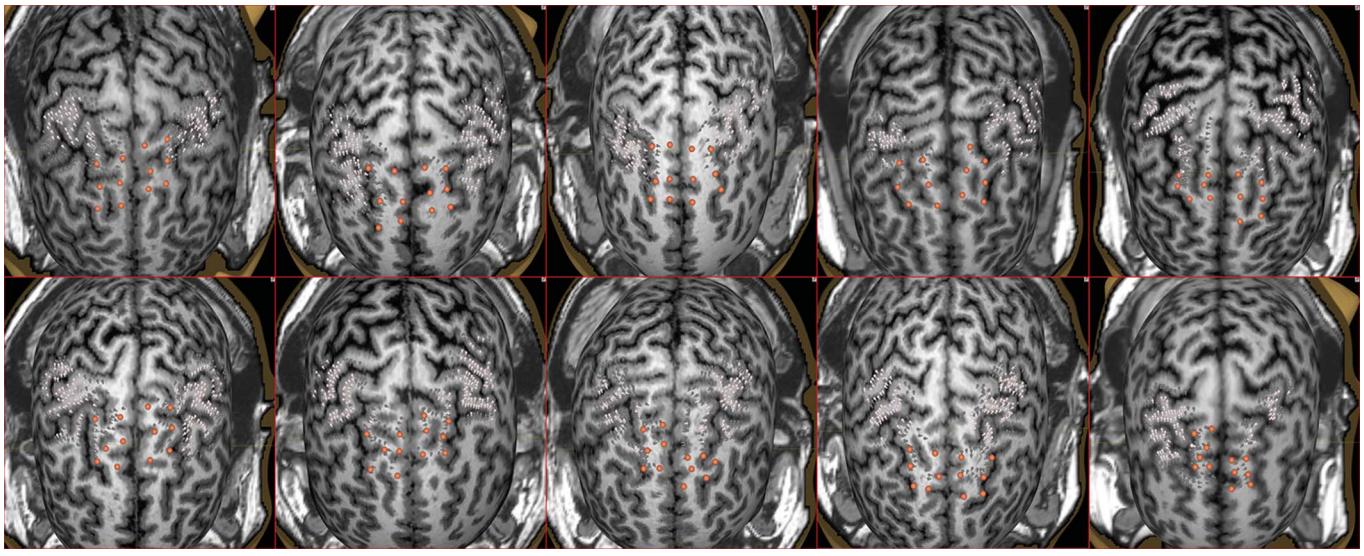

FIG. 1. Overview of stimulation targets. This figure shows the results of motor mapping by navigated transcranial magnetic stimulation, with targets (six per hemisphere, large points) for mapping of the supplementary motor area being placed at the borders of the motor-positive stimulation spots (white points). Three-dimensional reconstructions of the volunteers' cortex including stimulation spots and targets are shown, which stem from the 10 participants enrolled.

The course in which the predefined targets were stimulated was randomized. The coil was placed tangentially to the skull, with current flow directed orthogonally to the local gyrus orientation. The correct placement was aided by the neuronavigation.[33,34] After each trial, the test objects were rearranged into the starting position and the next target was stimulated until one performance was recorded for each target. Then, the subtest was repeated with the other hand. Parallel to testing, the TCTs were taken. In cases where the TCT could not be measured accurately during mapping, post hoc video analysis was performed to extract the TCT.

Statistical Analyses

GraphPad Prism (version 7.0; GraphPad Software Inc, La Jolla, CA) was used for statistics. The baseline TCTs of each subtest were compared with the respective TCTs under stimulation, considering stimulation over all stimulation targets together per hemisphere, using Wilcoxon rank–sum tests. The level of statistical significance was set at $P < 0.05$. Shapiro–Wilk test confirmed nonparametric distribution of TCT measurements in the majority of subtests.

For each subtest of the JHFT, we compared TCTs only for the hemisphere that they were collected from (i.e., baseline TCTs from left-hemispheric sessions were compared with stimulation condition TCTs from left-hemispheric sessions). We made three comparisons for each JHFT subtest regarding rTMS to the left hemisphere (LH) and right hemisphere (RH), respectively:

1. Left hand: baseline TCTs versus stimulation TCTs,
2. Right hand: baseline TCTs versus stimulation TCTs, and
3. Both hands pooled: baseline TCTs versus stimulation TCTs.

Furthermore, we performed subgroup analyses by comparing the stimulation TCTs between groups of medial versus lateral stimulation targets, and by comparing the stimulation TCTs between pairs of anterior versus middle versus posterior stimulation targets using Wilcoxon rank-sum and Friedman tests, respectively. This was again achieved for TCTs derived from rTMS to the LH and RH considering the different subtests and the performances of the left hand, right hand, and both hands together, respectively.

RESULTS

No subject reported adverse events during stimulation. The average resting motor threshold was 31.9 ± 4.6% (range: 26–41%) of the maximum stimulator output. The approximate time for each session, including motor mapping, baseline assessment, and mapping of the SMA, accounted for 90 minutes.

Writing

We found significantly faster TCTs under stimulation. Pooled TCTs for the LH (baseline TCT 17.09 ± 7.31 seconds vs. stimulation TCT 15.44 ± 5.72 seconds; $P = 0.0073$), left-handed TCTs for the RH (baseline TCT 21.45 ± 3.40 seconds vs. stimulation TCT 20.36 ± 3.49 seconds, $P = 0.0195$), and pooled TCTs for the RH (baseline TCT 16.20 ± 6.04 seconds vs. stimulation TCT 15.53 ± 5.63 seconds, $P = 0.0266$) showed significant dissociations of baseline and stimulation performance (Table 1 and Fig. 2).

In addition, the comparison of TCTs of medial versus lateral stimulation targets revealed a significantly slower performance during stimulation of lateral points for pooled TCTs for the RH (TCT of medial stimulation targets 15.32 ± 5.55 seconds vs. TCT of lateral stimulation targets 15.74 ± 5.74 seconds, $P = 0.0240$; see **Table 1**, **Supplement Digital Content 1**, http://links.lww.com/JCNP/A45).

TABLE 1. Comparison of Test Completion Times (TCTs) Between Baseline Performance and Performance Under Stimulation

Task	Hemisphere	Hand	Baseline TCT (s)	10-Hz TCT 6-Point Mean (s)	*P*	Significance
Writing	LH	Left	22.98 ± 5.78	20.37 ± 3.58	0.1055	—
		Right	11.19 ± 1.52	10.52 ± 1.52	0.0645	—
		Pooled	17.09 ± 7.31	15.44 ± 5.72	0.0073	**
	RH	Left	21.45 ± 3.40	20.36 ± 3.49	0.0195	*
		Right	10.94 ± 2.00	10.70 ± 1.73	0.6250	—
		Pooled	16.20 ± 6.04	15.53 ± 5.63	0.0266	*
SPT	LH	Left	4.12 ± 0.96	3.98 ± 0.44	0.9219	—
		Right	3.95 ± 0.58	3.73 ± 0.40	0.4316	—
		Pooled	4.03 ± 0.78	3.86 ± 0.43	0.4749	—
	RH	Left	3.76 ± 0.80	4.12 ± 0.68	0.0840	—
		Right	3.60 ± 0.54	3.97 ± 0.59	0.0840	—
		Pooled	3.68 ± 0.67	4.04 ± 0.63	0.0136	*
LSO	LH	Left	5.53 ± 0.78	5.52 ± 0.64	>0.9999	—
		Right	5.04 ± 0.59	5.19 ± 0.64	0.5566	—
		Pooled	5.28 ± 0.72	5.36 ± 0.64	0.7562	—
	RH	Left	5.24 ± 0.83	5.51 ± 0.70	0.1055	—
		Right	4.99 ± 0.62	5.44 ± 0.64	0.0039	**
		Pooled	5.11 ± 0.72	5.47 ± 0.66	0.0010	**
SF	LH	Left	6.73 ± 1.20	6.76 ± 0.39	>0.9999	—
		Right	6.15 ± 1.01	6.21 ± 0.44	0.8457	—
		Pooled	6.44 ± 1.12	6.48 ± 0.50	0.8124	—
	RH	Left	6.35 ± 0.83	7.01 ± 0.88	0.0195	*
		Right	5.85 ± 0.54	6.16 ± 0.46	0.1055	—
		Pooled	6.10 ± 0.73	6.59 ± 0.81	0.0027	**
SC	LH	Left	3.24 ± 0.58	3.16 ± 0.25	0.7695	—
		Right	2.98 ± 0.71	2.83 ± 0.29	>0.9999	—
		Pooled	3.11 ± 0.65	2.99 ± 0.31	0.7285	—
	RH	Left	3.14 ± 0.66	3.18 ± 0.35	0.5566	—
		Right	2.80 ± 0.52	2.95 ± 0.39	0.3223	—
		Pooled	2.97 ± 0.61	3.06 ± 0.38	0.2611	—
LLO	LH	Left	3.07 ± 0.42	2.90 ± 0.25	0.3223	—
		Right	2.99 ± 0.51	2.84 ± 0.18	0.7695	—
		Pooled	3.03 ± 0.46	2.87 ± 0.21	0.3118	—
	RH	Left	2.79 ± 0.34	2.80 ± 0.35	0.3750	—
		Right	2.69 ± 0.32	2.75 ± 0.19	0.3750	—
		Pooled	2.74 ± 0.32	2.78 ± 0.28	0.2305	—
LHO	LH	Left	3.00 ± 0.44	2.85 ± 0.29	0.2324	—
		Right	2.90 ± 0.46	2.75 ± 0.27	0.3223	—
		Pooled	2.95 ± 0.44	2.80 ± 0.28	0.1231	—
	RH	Left	2.89 ± 0.34	2.80 ± 0.41	0.6953	—
		Right	2.80 ± 0.40	2.75 ± 0.36	0.4316	—
		Pooled	2.84 ± 0.37	2.77 ± 0.37	0.8983	—

This table gives an overview of the results when comparing the test completion times (TCTs) between the baseline condition and the performance under stimulation (average of the TCTs of the six predefined targets stimulated per hemisphere). Results are split into subgroups determined by the specific subtests of the Jebsen–Taylor Hand Function Test (writing, simulated page turning [SPT], lifting small objects [LSO], simulated feeding [SF], stacking checkers [SC], lifting light objects [LLO], and lifting heavy objects [LHO]), the stimulated hemisphere (left hemisphere [LH] or right hemisphere [RH]), and manner in which hands were taken into account (left-handed TCTs, right-handed TCTs, and pooled TCTs).

Asterisks indicate statistical significance (*$P < 0.05$, **$P < 0.01$).

SPT

Only the comparison for pooled TCTs for the RH (baseline TCT 3.68 ± 0.67 seconds vs. stimulation TCT 4.04 ± 0.63 seconds, $P = 0.0136$) showed a statistically significant result. Contrasting the previous performance during the writing test, the stimulation led to slower performances (Table 1 and Fig. 3).

A significant difference in TCTs depending on the stimulation site along the anterior–posterior axis was observed for left-handed performances for the RH (TCT of anterior stimulation targets 3.97 ± 0.68 seconds vs. TCT of middle stimulation targets 4.12 ± 0.72 seconds vs. TCT of posterior stimulation targets 4.28 ± 0.74 seconds, $P = 0.0456$; see **Table 2**, **Suppplemental Digital Content 1**, http://links.lww.com/JCNP/A45).

LSO

We again found significantly slower TCTs when compared with baseline for both the right-handed TCTs for the RH (baseline TCT 4.99 ± 0.62 seconds vs. stimulation TCT 5.44

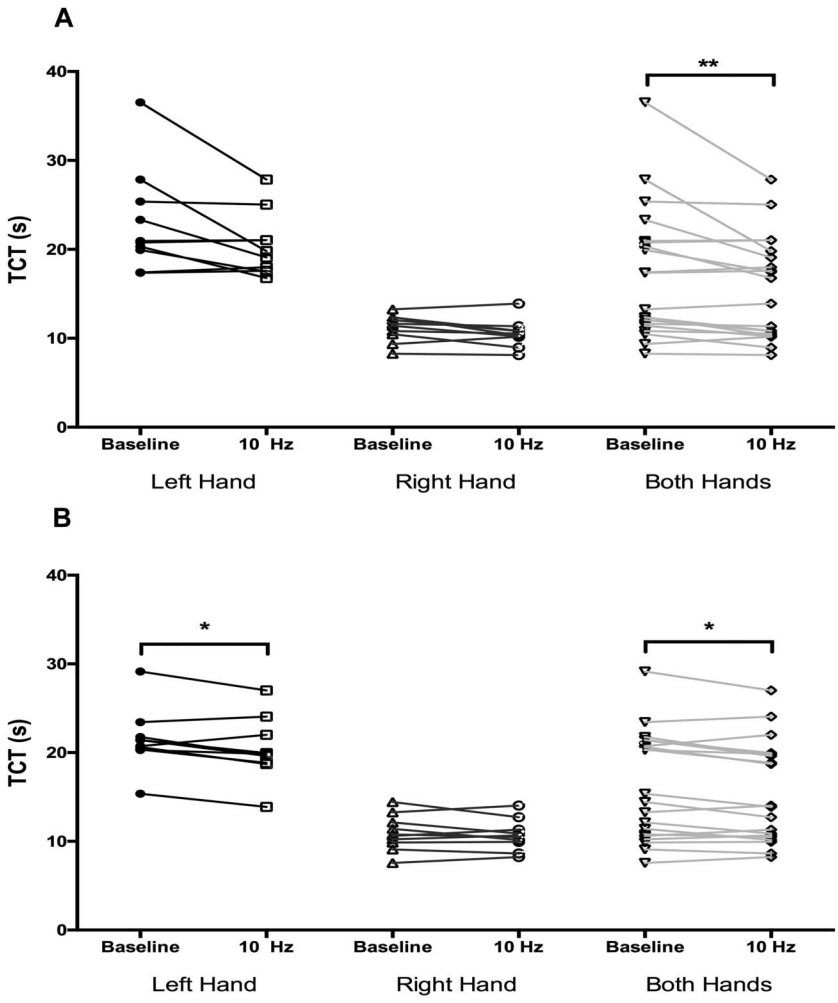

FIG. 2. Writing. Pictured are the comparison groups of baseline test completion times (TCTs) and stimulation TCTs for writing. **A**, The TCTs derived from stimulations on the left hemisphere. **B**, The TCTs of stimuli to the right hemisphere. Each symbol represents the TCT of a given participant (average of the six predefined targets stimulated per hemisphere), with a line connecting the TCTs of baseline assessments and respective TCTs under stimulation condition. Asterisks indicate statistical significance (*$P < 0.05$, **$P < 0.01$). The two plots farthest to the left represent the TCTs of left-handed performances (black). The two plots in the center represent the TCTs of right-handed performances (dark gray). The pair of plots on the right side represents the pooled TCTs of both hands (light gray).

± 0.64 seconds, $P = 0.0039$) and the pooled TCTs for the RH (baseline TCT 5.11 ± 0.72 vs. stimulation TCT 5.47 ± 0.66 seconds, $P = 0.0010$; Table 1 and Fig. 4).

SF

Two significant dissociations of baseline and stimulation TCTs were revealed, which were shown in comparisons of TCTs for the RH regarding the left hand (baseline TCT 6.35 ± 0.83 seconds vs. stimulation TCT 7.01 ± 0.88 seconds, $P = 0.0195$) and the pooled investigation (baseline TCT 6.10 ± 0.73 seconds vs. stimulation TCT 6.59 ± 0.81 seconds, $P = 0.0027$; Table 1 and Fig. 5).

SC, Lifting Light Objects, and Lifting Heavy Objects

No significant differences between baseline and stimulation TCTs were found in the comparisons for SC, lifting light objects, and lifting heavy objects ($P > 0.05$; Table 1).

However, during stimulation of the LH, the TCTs of the right-handed performances (TCT of anterior stimulation targets 2.78 ± 0.39 seconds vs. TCT of middle stimulation targets 2.94 ± 0.30 seconds vs. TCT of posterior stimulation targets 2.76 ± 0.27 seconds, $P = 0.0259$) as well as the pooled TCTs (TCT of anterior stimulation targets 2.94 ± 0.45 seconds vs. TCT of middle stimulation targets 3.16 ± 0.41 seconds vs. TCT of posterior stimulation targets 2.88 ± 0.30 seconds, $P = 0.0043$) varied, depending on the stimulation sites (see **Table 2**, **Supplemental Digital Content 2**, http://links.lww.com/JCNP/A45).

DISCUSSION

Feasibility of Mapping the SMA

The first of our hypotheses was that rTMS would influence our participants' performance regarding the JHFT. This proved to be correct because we were able to show slower TCTs in three of the seven subtests (SPT, LSO, and SF), whereas one subtest (writing) was subject to faster TCTs under stimulation (Table 1, Figs. 2–5). Overall, the presence of a general effect of rTMS on the SMA is unsurprising, considering the multitude of studies that have shown the SMA to be a target susceptible to TMS in general.[27–29] However, the approach of systematic mapping of the SMA using an electric-field–navigated system is novel.

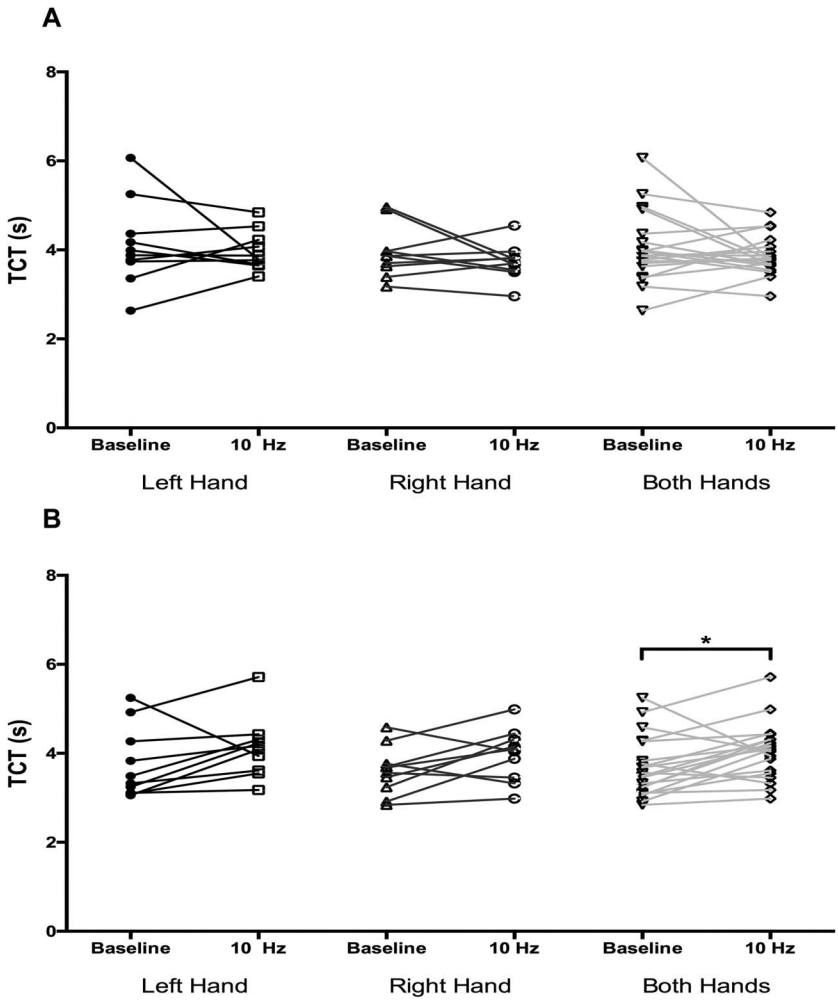

FIG. 3. Simulated page turning (SPT). This graph illustrates the baseline test completion times (TCTs) and stimulation TCTs for SPT. **A**, The TCTs derived from stimulations on the left hemisphere. **B**, The TCTs of stimuli to the right hemisphere. Each symbol represents the TCT of a given participant (average of the six predefined targets stimulated per hemisphere), with a line connecting the TCTs of baseline assessments and respective TCTs under stimulation condition. Asterisks indicate statistical significance (*$P < 0.05$). The two plots farthest to the left represent the TCTs of left-handed performances (black). The two plots in the center represent the TCTs of right-handed performances (dark gray). The pair of plots on the right side represents the pooled TCTs of both hands (light gray).

Facilitation of Writing

We observed faster TCTs during stimulation compared with baseline TCTs (Table 1, Fig. 2), contrasting other findings of an inhibiting effect of rTMS on writing-based tasks.[35] Although in this previous study a neuronavigated approach with high-frequency rTMS was used, the location of stimuli was more lateral in the premotor area, which might explain the different effect direction.[35] Notably, the area stimulated in our study distinctly aimed to spare primary motor areas. This does, however, not necessarily explain the facilitation experienced by our participants, which seems even more paradoxical when being compared with the increase in TCTs under stimulation found for the SPT, LSO, and SF. An explanation might be a certain level of practice effect. Writing within the JHFT has been found to profit from practice.[36] In the cited study, the TCTs constantly decreased over the course of three trials, whereas in our study practice extended over seven trials. This could mask an inhibiting effect of rTMS. Presently, the subtest is arguably not suited for a distinct rTMS mapping protocol.

Delaying of SPT, LSO, and SF

A significant slowing of TCTs was found for the SPT, LSO, and SF under stimulation (Table 1, Figs. 3–5). As to the neurophysiological cause of this effect, we would argue in favor of the virtual lesion, which posits that TMS applied with a certain frequency introduces unwarranted cortical excitation, thereby inhibiting correct computation of tasks.[37] This would lead to problems in coordination, resulting in slower TCTs, thus confirming our initial hypothesis. The fact that both hands were influenced corresponds to research demonstrating strong interhemispheric connections between the SMA and the contralateral primary motor cortex, allowing for impact on both the LH and RH.[13] We can, however, make no comment regarding the strength of contralateral versus ipsilateral projections using our momentary analyses.

Interestingly, only stimulation of the right-hemispheric SMA induced this slowing effect; regarding the executing hands, however, both the left and right hand were influenced (Table 1). This poses questions regarding lateralization of movement control. While the motor system seems to grow more lateralized in caudal direction, our study further points toward a difference in hemispherical dominance.[13,38] Our data might indicate that the SMA of the dominant—in our study left—hemisphere is either less involved in coordinating both limbs, less vulnerable to rTMS, or a combination of both. Because the dominant SMA is

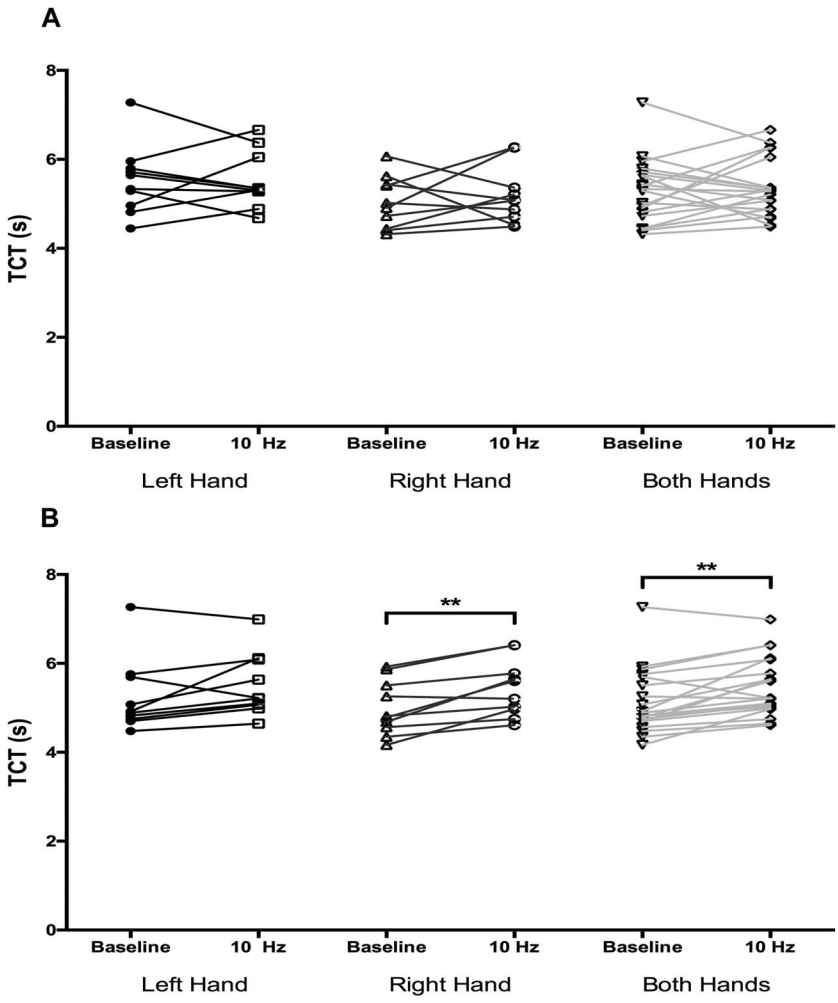

FIG. 4. Lifting small objects (LSOs). This graph plots the comparison of baseline test completion times (TCTs) and stimulation TCTs for LSO. **A**, The TCTs derived from stimulations on the left hemisphere. **B**, The TCTs of stimuli to the right hemisphere. Each symbol represents the TCT of a given participant (average of the six predefined targets stimulated per hemisphere), with a line connecting the TCTs of baseline assessments and respective TCTs under stimulation condition. Asterisks indicate statistical significance (**$P < 0.01$). The two plots farthest to the left represent the TCTs of left-handed performances (black). The two plots in the center represent the TCTs of right-handed performances (dark gray). The pair of plots on the right side represents the pooled TCTs of both hands (light gray).

known to be more implicated in speech production, it could be hypothesized that a more connected left-hemispheric SMA is either more resilient to focal stimuli or less integrated into extremity coordination due to its stronger parallel involvement in language.[19,39,40]

Absence of Effects during SC, Lifting Light Objects, and Lifting Heavy Objects

As to why the stimulation showed no effects on the last three subtests, multiple hypotheses are possible. The last three subtests were both the fastest and arguably least dependent on fine motor skills when comparing all subtests. As shown previously, the SMA is more involved in complex movement sequences than in simple ones, allowing for the hypothesis that a certain basal level of motor complexity is required for TMS-based disruption.[29] Furthermore, it has been hypothesized that the SMA is crucial for sequencing multiple movement components over time.[12,41] This would suggest that shorter tasks are less susceptible to disturbance because there are less upcoming movement encodings present for stimulation influence. An inverse correlation between TCTs and stimulation effect sizes would seem possible.

Limitations and Significance

First, our sample size should be addressed as a relevant limitation. Because our data stem from a comparatively small sample of ten participants, it may be optimistic to assume full generalizability of our confirmed hypotheses. Second, we did not evaluate parameters other than TCTs. We can therefore not exclude the fact that more subtle influences of stimulation (e.g., changes in writing form during the writing subtest) have escaped our notice. In defense of our approach, we wanted to use objective measurements, which certainly speaks for the use of TCTs. Third, the application of the JHFT, a test relying on one-handed performances, does not allow for the evaluation of possible stimulation influences on bimanual movements, which previous studies point toward.[28,42] The usage of other tests should be considered in upcoming studies on the matter; however, for the purpose of distinct mappings of the SMA, the use of a standardized and easy test such as the JHFT seems justified.

With these limitations in mind, we nevertheless consider our study a successful first step toward the development of a systematic mapping approach of the SMA by rTMS, which has not yet been undertaken according to the authors' knowledge. Our study benefits from a wide variety of subtests evaluated, and the

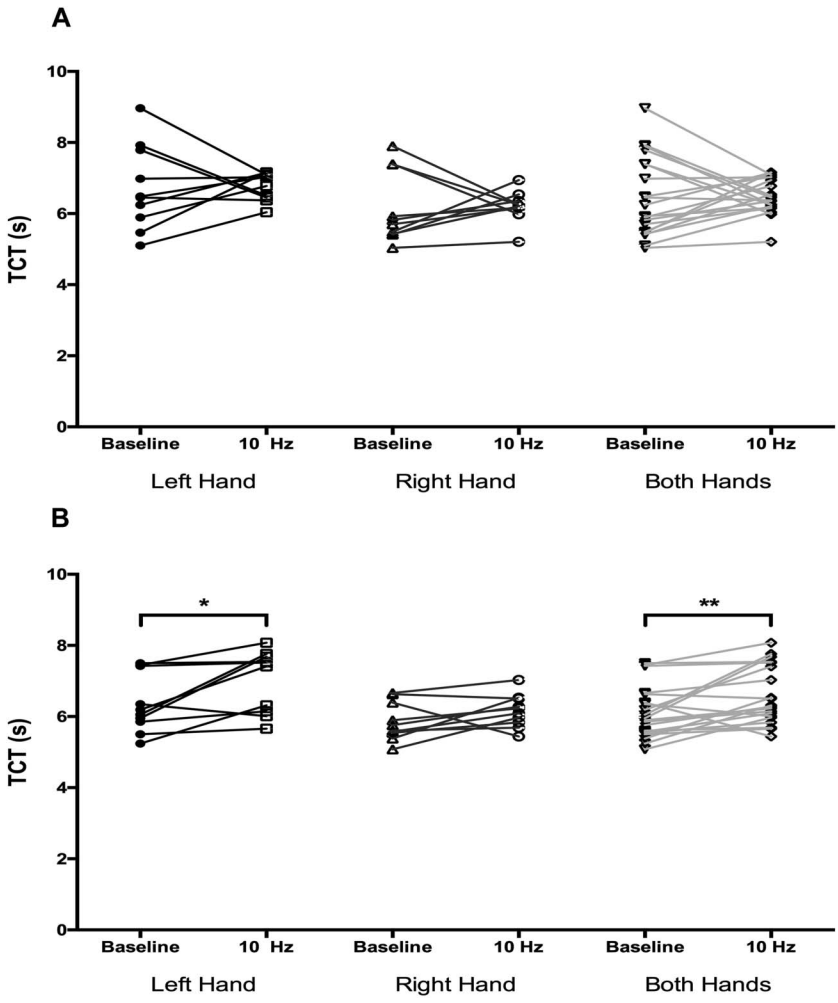

FIG. 5. Simulated feeding (SF). Pictured are the comparison groups of baseline test completion times (TCTs) and stimulation TCTs for SF. **A**, The TCTs derived from stimulations on the left hemisphere. **B**, The TCTs of stimuli to the right hemisphere. Each symbol represents the TCT of a given participant (average of the six predefined targets stimulated per hemisphere), with a line connecting the TCTs of baseline assessments and respective TCTs under stimulation condition. Asterisks indicate statistical significance (*$P <$ 0.05, **$P < 0.01$). The two plots farthest to the left represent the TCTs of left-handed performances (black). The two plots in the center represent the TCTs of right-handed performances (dark gray). The pair of plots on the right side represents the pooled TCTs of both hands (light gray).

included investigation of not only one-sided but also bihemispheric mappings of the SMA considering task performance with both hands represent a strength of our work. After the proof of concept that this study can be taken to be, future research should focus on a comparison of different rTMS protocols to maximize measurable effects of mappings of the SMA. Herein, especially theta-burst stimulation should be considered due to its presumed inhibition of cortical activity.[43]

Furthermore, a bigger group of participants, also including males, is needed to ensure generalizability of the results. The study of stimulation effects on the SMA in left-handed subjects could further be object to investigation. To ensure that the stimulation is applied to valid targets, we recommend neuronavigated approaches with prior mapping of primary motor cortex for future research. Because we found isolated representations of the primary motor cortex in presumed premotor areas of our participants (Fig. 1), the exclusion of accidentally stimulating these representations is a key factor in upcoming study designs.

Compiling a protocol for systematic rTMS mapping of the SMA could be beneficial for future studies to gain better insights into the motor system or yield benefits regarding outcome after brain surgery. Improved outcome has already been reported in terms of preoperative nTMS motor mapping, including reduced rates of postoperative deficits.[44–47] Thus, the SMA syndrome, sometimes even found to be a lasting condition, might be prevented from occurring. It is therefore an important objective to investigate rTMS mapping of the SMA in patients harboring brain tumors within this region. If a reliable preoperative mapping procedure for the SMA can be established, the next step should be to conduct studies that investigate the distinct impact on clinical outcome when data derived from such mapping are considered during surgical planning and intraoperative guidance. Moreover, the effects of rTMS on TCTs depending on the stimulation location within the SMA, as observed in the context of subgroup analyses in this study (see **Tables 1 and 2**, **Supplemental Digital Content 1 and 2**, http://links.lww.com/JCNP/A45), should be investigated in more detail during upcoming studies. In addition, sham-controlled designs could further help to separate possible bias effects from actual rTMS influence.

CONCLUSION

In our study, a high-frequency rTMS protocol was applied to the SMA, which proved to be capable of eliciting slowing of

performance in multiple subtests of the JHFT. Notably, the effects were more prevalent when stimulating the RH, which implicates either a stronger rTMS resilience or a smaller involvement in motor control of the dominant hemisphere. For the goal of establishing a reliable protocol for SMA mappings, further stimulation protocols should be examined. Systematic investigation of the SMA by rTMS mappings could also enhance our understanding of processes underlying movement control if more variables (e.g., interactions of hand and stimulated hemisphere, non-TCT-based mistakes) are analyzed in future studies.

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Application of Navigated Transcranial Magnetic Stimulation to Map the Supplementary Motor Area in Healthy Subjects

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BACK CLOSE WINDOW

The article „Navigated transcranial magnetic stimulation of the supplementary motor cortex disrupts fine motor skills in healthy adults", published in Scientific Reports in November 2019 was the second publication based on our healthy volunteer data (Schramm et al., 2019).

In this article, we have analyzed the entirety of our data, including all conducted tests, participants and stimulation protocols. We observed a high number of instances of heightened TCTs for test performances during SMA stimulation compared to baseline for all applied stimulation protocols. SDITs were similarly found to be heightened during stimulation compared to baseline. Additionally, many instances of often stereotypical (between participants) VCPs were observed and documented. Videomaterial of both the VCPs as well as contrasting baseline performances was added to the publication and can be viewed online on the publisher homepage (https://www.nature.com/articles/s41598-019-54302-y; last checked 24th of February 2022). As in the previous publication, writing TCTs were observed to be lowered during stimulation compared to baseline and some tests appeared to not be impacted by the stimulation (see discussion below).

The findings were discussed in the context of the respective literature, with a focus on the potential development of a clinically applicable SMA mapping protocol derived from the observations made. Potential causes for specific test results and implications for their use in a mapping protocol were discussed.

Regarding first author contributions, SS acquired the data, performed the statistical analyses, created the figures and videos and drafted the manuscript with feedback from the coauthors.

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Navigated transcranial magnetic stimulation of the supplementary motor cortex disrupts fine motor skills in healthy adults

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Navigated transcranial magnetic stimulation (nTMS) over the supplementary motor area (SMA) may impact fine motor skills. This study evaluates different nTMS parameters in their capacity to affect fine motor performance on the way to develop an SMA mapping protocol. Twenty healthy volunteers performed a variety of fine motor tests during baseline and nTMS to the SMA using 5 Hz, 10 Hz, and theta-burst stimulation (TBS). Effects on performance were measured by test completion times (TCTs), standard deviation of inter-tap interval (SDIT), and visible coordination problems (VCPs). The predominant stimulation effect was slowing of TCTs, i.e. a slowdown of test performances during stimulation. Furthermore, participants exhibited VCPs like accidental use of contralateral limbs or inability to coordinate movements. More instances of significant differences between baseline and stimulation occurred during stimulation of the right hemisphere compared to left-hemispheric stimulation. In conclusion, nTMS to the SMA could enable new approaches in neuroscience and enable structured mapping approaches. Specifically, this study supports interhemispheric differences in motor control as right-hemispheric stimulation resulted in clearer impairments. The application of our nTMS-based setup to assess the function of the SMA should be applied in patients with changed anatomo-functional representations as the next step, e.g. among patients with eloquent brain tumors.

The supplementary motor area (SMA) is a cortical region located in the premotor cortex, overlapping with Brodmann area 6. It can be divided into two subregions, the pre-SMA, located more anteriorly, as well as the SMA-proper, bordering on the primary motor cortex[1,2].

Regarding functional aspects of the SMA, long lines of research have demonstrated its involvement in a variety of cognitive and motor-related processes. Multiple reviews exist in this regard[2,3]. Traditionally, its most noted role is the preparation and simulation of complex movement chains[2–4]. This is confirmed by lesion studies after ischemic events and by studies among patients who have undergone resections of brain lesions, which revealed a characteristic constellation of symptoms if the SMA is damaged: the so-called SMA syndrome usually presents as hemiparesis accompanied by varying degrees of mutism[5–7]. The SMA syndrome is usually considered to exist only temporarily and typically resolves over the course of weeks to months, which is likely associated with contralateral functional compensation[8–10]. The exact mechanism, however, remains largely unknown, and it is important to be aware of the fact that more detailed clinical examinations may be capable of detecting lasting deficits related to SMA damage, thus questioning the mere transient character of the SMA syndrome[11–14]. In addition, rare motor-related phenomena, such as the alien-limb syndrome, have also been reported in the past resulting from damage to the SMA. The alien-limb syndrome is characterized by a loss of conscious control of the afflicted limb, which may then move counter to the actual intent[12,15]. Furthermore, the role of the SMA in different cognitive processes such as mental object rotation, perception of effort, grip force scaling, and controlled coordination

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of movements has been explored repeatedly[16–19]. Other studies found evidence for projections interpreted to be associated with motor learning processes[20,21].

Attempts at spatio-functional SMA delineation have been made using techniques such as functional magnetic resonance imaging (fMRI), magnetoencephalography (MEG), and positron emission tomography (PET)[22–25]. In one case study, MEG activity corresponding to voluntary movement preparation was recorded in a stroke patient possessing only one active SMA[23]. The study, however, mentions that MEG may at times be unable to record SMA activity due to both hemispheres canceling out each other's recordable signal[23]. Whereas in two studies on 18 and 66 participants, fMRI has been used to some success in the localization of the SMA, there are reports of variable visibility in identification by resting-state fMRI[22,25].

A modality to test or modulate SMA-related function is represented by transcranial magnetic stimulation (TMS). In therapeutic approaches a mild beneficial effect on the motor symptoms of Parkinson's disease has been demonstrated (studies including 26 and 106 patients), seemingly arising from modulation of SMA excitability via repetitive stimulation, such as for example theta-burst stimulation (TBS)[26,27]. Other small-scale TMS studies (10 to 21 participants) exist on the study of functional connections between the SMA and (pre)motor areas[28–30]. A paired-pulse approach was used to create evidence for projections from the dorsal premotor area to the contralateral primary motor cortex[28]. Another study implies a difference in circuitry between premotor areas and the primary motor cortex[29]. Repetitive TMS has also been used over the SMA to heighten motor-evoked potentials[30].

However, most of these studies used non-navigated TMS. Thus, correlations between measured effects and the exact spatial location of stimulation remained largely unclear. For multifarious TMS applications it has repeatedly been suggested that accurate neuronavigation of the stimulation, including optimal positioning and angulation of the stimulating coil with respect to cortical architecture, is important and may enhance precision and impact of stimulation[31,32]. Thus, particularly during preoperative application in modern neurosurgery, functional mapping by navigated TMS (nTMS) has emerged as a technology suited for mappings of sites including the motor cortex, language-related areas, or areas responsible for arithmetic processing[33–35]. Regarding further applications, the SMA has recently emerged as a potential new target structure for nTMS mappings; however, evidence is currently limited to one small series[36]. Yet, the need for mapping is clearly present in light of the questionable mere transient character of the SMA syndrome[11–14]. In the mentioned small series, a proof of concept was provided, showing that nTMS to the SMA can principally impact the performance of healthy adults in the Jebsen-Taylor Hand Function Test (JHFT) when delivering repetitive nTMS (rTMS) with 10 Hz[36]. However, whether other motor-related tasks or stimulation protocols are favorable for potential application of nTMS for mapping of the SMA has been beyond the scope of this previous investigation.

Against this background, the present study aims for systematic testing of nTMS effects on a variety of motor-related tasks by applying multiple stimulation protocols within healthy adults. In this framework, we decided to investigate the effects of stimulation with 5 Hz, 10 Hz, and TBS. Generally, repetitive stimulation is recommended over single-pulse TMS for the disruption of cortical processes in which precise timelines of activation are unknown[37]. 5-Hz stimulation has previously been shown effective in the mapping of various cortical functions. For example, multiple studies exist on its use in the mapping of cortical language function[38–41]. Furthermore, 10-Hz stimulation and TBS were chosen due to their different effects on cortical activity. 10-Hz stimulation is considered a paradigm leading to heightened neuronal activity[42]. On the other hand, TBS may have different neuromodulatory effects based on the exact mode of application, specifically with respect to the interval between bursts of stimulation. While continuous TBS is associated with dampening of cortical activity, intermittent TBS is believed to have facilitatory effects[43]. To identify possible stimulation-related effects, this study compares baseline task performance with performance under stimulation. The conclusions drawn from the present approach are supposed to aid in establishing an SMA mapping procedure analogous to previously used paradigms (e.g., mapping of language or calculation functions) and to better understand SMA functionality, its bilateral coordination, and its subcortical connectivity patterns.

Results

Cohort and mapping characteristics. This study was performed in twenty healthy volunteers (8 males and 12 females, median age: 22.5 years, age range: 19–30 years), who were right-handed according to the Edinburgh Handedness Inventory (mean score: 74.5 ± 16.6 points). Motor and SMA mappings by nTMS were successfully performed in all participants during two separate appointments without technical problems or adverse events. Participants did not self-report any side effects of stimulation. According to randomization, the left hemisphere was stimulated during the first appointment in twelve participants. The average resting motor threshold (rMT) was 32.6 ± 5.5% (range: 23–42%) of the maximum stimulator output for the left hemisphere and 32.0 ± 4.2% (range: 25–42%) of the maximum stimulator output for the right hemisphere ($p = 0.4967$). Six stimulation targets per hemisphere were placed anteriorly of the primary motor cortex as determined by nTMS motor mapping (Fig. 1).

Jebsen-Taylor Hand Function Test. *Writing.* Left hemisphere: Our analyses revealed significantly faster performances during stimulation in the majority of comparisons ($p < 0.05$; Table 1). Notably, the only comparisons not yielding significant dissociations were right-handed performances during stimulation with TBS (baseline test completion time [TCT] 10.8 ± 1.3 s; TBS TCT 10.5 ± 1.3 s [$p = 0.1650$]; Table 1). For right-handed performances during 10-Hz stimulation, stimulation to lateral targets led to slower TCTs than medial stimulation (medial group TCT 10.2 ± 1.3 s; lateral group TCT 10.5 ± 1.4 s [$p = 0.0215$]).

Right hemisphere: Faster performances during stimulation were revealed for analyses of the left hand and both hands pooled ($p < 0.05$; Table 1). Right-handed runs were not significantly different between baseline and stimulation (baseline TCT 10.6 ± 1.6 s; 5 Hz TCT 10.5 ± 1.2 s [$p = 0.9273$]; 10 Hz TCT 10.4 ± 1.4 s [$p = 0.5958$]; TBS TCT 10.5 ± 1.3 s [$p = 0.5706$]). For left-handed performances, lateral stimulation resulted in slower TCTs

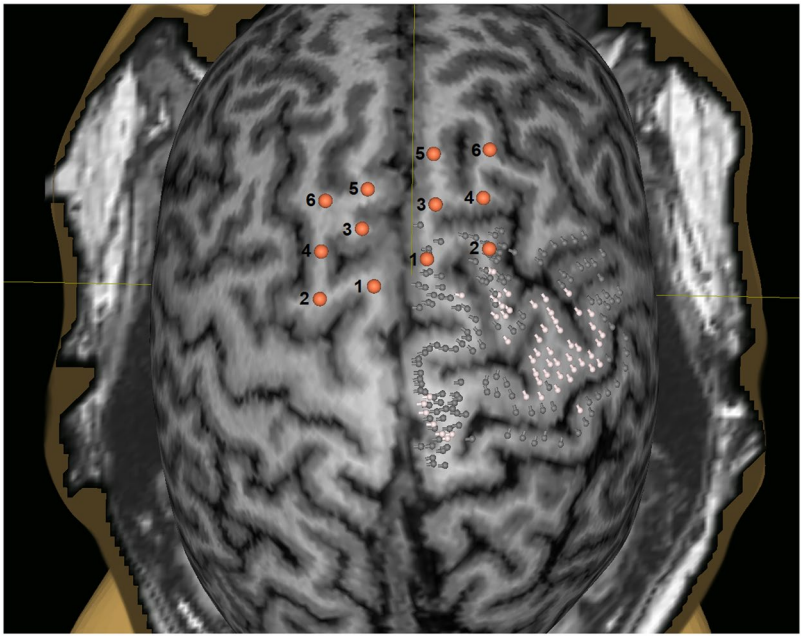

Figure 1. Showcase for stimulation target placement. This figure depicts the stimulation targets for one participant (six stimulation targets per hemisphere). On the right hemisphere, for additional information, the primary motor cortex as determined by motor mapping by navigated transcranial magnetic stimulation (nTMS) is shown in relation to the stimulation targets. Motor-positive points are displayed as white pins, motor-negative points as dark grey pins.

compared to medial stimulation for both stimulation with 5 Hz (medial group TCT 20.1 ± 3.6 s; lateral group TCT 20.7 ± 3.5 s [$p = 0.0064$]) and 10 Hz (medial group TCT 19.7 ± 3.0 s; lateral group TCT 20.5 ± 3.3 s [$p = 0.0073$]).

Regression and visible coordination problems: The regression model revealed that writing was performed with on average 10.4 s (95%-confidence interval [CI] = [−10.7; −10.1]) shorter TCTs for the right hand compared to the left hand ($p < 0.0005$). All stimulation protocols seemed to significantly shorten the TCTs by 1.4 to 1.1 s compared to the baseline TCT for writing in the model ($p < 0.0005$ for all protocols; Table 2). No visible coordination problems (VCPs) were detected.

Simulated page turning. Left hemisphere: Opposite effects for simulated page turning were predominantly revealed when compared to writing. For the left hemisphere, we found significant slowing for right-handed performances during TBS (baseline TCT 3.8 ± 0.7 s; TBS TCT 4.1 ± 0.7 s [$p = 0.0362$]; Table 1).

Right hemisphere: For the right hemisphere, every comparison obtained showed significant slowing of performances ($p < 0.05$), except for right-handed performances during TBS (baseline TCT 3.7 ± 0.6 s; TBS TCT 3.9 ± 0.7 s [$p = 0.0583$]; Table 1).

Regression and visible coordination problems: For simulated page turning, the stimulation of the right hemisphere (independent of the executing hand) seemed to result in slightly shorter TCT of on average 0.1 s (95%-CI = [−0.2; −0.0], $p = 0.0050$) compared to stimulation of the left hemisphere in the model. All stimulation protocols seemed to slow the TCTs by 0.2 to 0.3 s compared to baseline ($p < 0.0005$ for all protocols; Table 2). In total, four instances of VCPs occurred (Supplementary Videos S1 and S2).

Lifting small objects. Left hemisphere: Our analysis discovered significant slowing during stimulation of the left hemisphere with 10 Hz and TBS for right-handed performances (baseline TCT 5.2 ± 0.6 s; 10 Hz TCT 5.7 ± 0.9 s [$p = 0.0266$]; TBS TCT 5.7 ± 0.9 s [$p = 0.0083$]) as well as for both hands pooled (baseline TCT 5.4 ± 0.7 s; 10 Hz TCT 5.8 ± 0.8 s [$p = 0.0224$]; TBS TCT 5.8 ± 0.8 s [$p = 0.0029$]; Table 1 and Fig. 2).

Right hemisphere: The comparisons focusing on the right hemisphere showed significant slowing in all analyses ($p < 0.05$; Table 1 and Fig. 2).

Regression and visible coordination problems: For lifting small objects, the regression model showed an in average 0.2 s (95%-CI = [−0.3; −0.1], $p < 0.0005$) faster performance with the right hand compared to the left hand. All stimulation protocols seemed to slow the TCT by 0.3 to 0.4 s compared to baseline in this test ($p < 0.0005$ for all protocols; Table 2). Three VCPs were detected (Supplementary Videos S3 and S4).

Simulated feeding. Left hemisphere: The statistical analysis showed no significant effect on TCTs through stimulation of the left hemisphere compared to baseline ($p > 0.05$; Table 1). Within the stimulation paradigm, left-handed performances of 5-Hz stimulation differed between medial and lateral stimulation targets (medial group TCT 6.9 ± 0.8 s; lateral group TCT 7.2 ± 1.1 s [$p = 0.0215$]). Moreover, for TBS, a rostro-occipital difference

			Writing		Simulated page turning		Lifting small objects		Simulated feeding		Stacking checkers		Lifting light objects		Lifting heavy objects	
			TCT (s)	p-value to baseline	TCT (s)	p-value to baseline	TCT (s)	p-value to baseline	TCT (s)	p-value to baseline	TCT (s)	p-value to baseline	TCT (s)	p-value to baseline	TCT (s)	p-value to baseline
Left Hemisphere	Left hand	Baseline	22.7 ± 5.4		4.0 ± 0.8		5.6 ± 0.8		6.8 ± 1.1		3.3 ± 0.6		3.0 ± 0.4		2.9 ± 0.4	
		5 Hz	20.3 ± 4.1	0.0012	4.1 ± 0.5	0.2611	5.7 ± 0.6	0.8124	7.1 ± 1.0	0.3118	3.1 ± 0.5	0.0637	2.8 ± 0.3	0.0240	2.8 ± 0.3	0.0897
		10 Hz	19.9 ± 3.1	0.0001	4.1 ± 0.6	0.3683	5.8 ± 0.7	0.2943	7.0 ± 0.8	0.4749	3.3 ± 0.6	0.6742	2.8 ± 0.3	0.1650	2.8 ± 0.3	0.2774
		TBS	20.7 ± 4.3	0.0037	4.0 ± 0.6	0.7841	5.9 ± 0.7	0.1536	6.9 ± 0.8	0.5459	3.3 ± 0.5	0.8695	2.9 ± 0.4	0.3300	2.8 ± 0.4	0.6742
	Right hand	Baseline	10.8 ± 1.3		3.8 ± 0.7		5.2 ± 0.6		6.0 ± 0.8		3.2 ± 0.7		2.9 ± 0.4		2.8 ± 0.4	
		5 Hz	10.4 ± 1.3	0.0240	4.0 ± 0.7	0.2162	5.5 ± 0.7	0.1140	6.3 ± 0.6	0.1769	3.0 ± 0.4	0.5706	2.7 ± 0.3	0.1327	2.7 ± 0.3	0.5217
		10 Hz	10.4 ± 1.3	0.0441	4.0 ± 0.9	0.3683	5.7 ± 0.9	0.0266	6.4 ± 0.8	0.1429	3.0 ± 0.6	0.5459	2.7 ± 0.3	0.4091	2.7 ± 0.3	0.1650
		TBS	10.5 ± 1.3	0.1650	4.1 ± 0.7	0.0362	5.7 ± 0.9	0.0083	6.3 ± 0.5	0.1429	3.1 ± 0.5	0.6477	2.8 ± 0.4	0.9854	2.7 ± 0.3	0.9854
	Pooled hands	Baseline	16.8 ± 7.2		3.9 ± 0.7		5.4 ± 0.7		6.4 ± 1.0		3.2 ± 0.7		2.9 ± 0.4		2.8 ± 0.4	
		5 Hz	15.3 ± 5.9	<0.0001	4.0 ± 0.6	0.0747	5.6 ± 0.7	0.1922	6.7 ± 0.9	0.0892	3.1 ± 0.5	0.0817	2.8 ± 0.3	0.0056	2.7 ± 0.3	0.0842
		10 Hz	15.1 ± 5.4	<0.0001	4.0 ± 0.7	0.2064	5.8 ± 0.8	0.0224	6.7 ± 0.8	0.1181	3.1 ± 0.6	0.4680	2.8 ± 0.3	0.0918	2.7 ± 0.3	0.1000
		TBS	15.6 ± 6.0	0.0016	4.0 ± 0.7	0.1000	5.8 ± 0.8	0.0029	6.6 ± 0.7	0.1214	3.2 ± 0.5	0.7750	2.9 ± 0.4	0.5099	2.8 ± 0.3	0.5992
Right Hemisphere	Left hand	Baseline	22.4 ± 3.8		3.7 ± 0.7		5.5 ± 1.0		6.5 ± 0.9		3.1 ± 0.6		2.8 ± 0.3		2.8 ± 0.3	
		5 Hz	20.4 ± 3.5	0.0027	3.9 ± 0.7	0.0056	5.8 ± 0.7	0.0240	7.3 ± 1.1	0.0062	3.3 ± 0.6	0.0094	2.9 ± 0.3	0.3683	2.8 ± 0.3	>0.9999
		10 Hz	20.1 ± 3.1	<0.0001	4.0 ± 0.6	0.0009	5.8 ± 0.7	0.0240	7.1 ± 1.1	0.0094	3.3 ± 0.5	0.0192	2.8 ± 0.3	0.4304	2.8 ± 0.3	0.5958
		TBS	20.4 ± 3.7	0.0009	4.0 ± 0.7	0.0172	5.8 ± 0.6	0.0037	7.1 ± 0.8	0.0020	3.4 ± 0.6	0.0136	2.8 ± 0.2	0.3884	2.8 ± 0.3	0.6477
	Right hand	Baseline	10.6 ± 1.6		3.7 ± 0.6		5.1 ± 0.7		6.0 ± 0.7		2.9 ± 0.6		2.7 ± 0.3		2.7 ± 0.4	
		5 Hz	10.5 ± 1.2	0.9273	4.0 ± 0.7	0.0266	5.4 ± 0.7	0.0172	6.6 ± 0.9	0.0446	3.0 ± 0.4	0.2024	2.7 ± 0.3	0.7562	2.7 ± 0.3	0.4980
		10 Hz	10.4 ± 1.4	0.5958	4.0 ± 0.5	0.0296	5.6 ± 0.6	<0.0001	6.3 ± 0.9	0.0266	3.1 ± 0.5	0.1893	2.7 ± 0.2	>0.9999	2.7 ± 0.4	>0.9999
		TBS	10.5 ± 1.3	0.5706	3.9 ± 0.7	0.0583	5.4 ± 0.6	0.0037	6.4 ± 0.5	0.0136	3.1 ± 0.5	0.0696	2.8 ± 0.3	0.3488	2.7 ± 0.3	0.9854
	Pooled hands	Baseline	16.5 ± 6.6		3.7 ± 0.6		5.3 ± 0.9		6.2 ± 0.8		3.0 ± 0.6		2.8 ± 0.3		2.8 ± 0.4	
		5 Hz	15.5 ± 5.7	0.0079	4.0 ± 0.7	0.0006	5.6 ± 0.7	0.0006	6.9 ± 1.1	0.0003	3.2 ± 0.5	0.0073	2.8 ± 0.3	0.4280	2.8 ± 0.3	0.6753
		10 Hz	15.3 ± 5.5	0.0002	4.0 ± 0.6	<0.0001	5.7 ± 0.6	<0.0001	6.7 ± 1.0	0.0004	3.2 ± 0.5	0.0136	2.7 ± 0.3	0.6463	2.7 ± 0.3	0.1831
		TBS	15.4 ± 5.7	0.0025	4.0 ± 0.7	0.0012	5.6 ± 0.7	<0.0001	6.7 ± 0.8	<0.0001	3.2 ± 0.5	0.0020	2.8 ± 0.3	0.8264	2.8 ± 0.3	0.5900

Table 1. Test completion times (TCTs) for the Jebsen-Taylor Hand Function Test (JHFT). This table depicts the TCTs of baseline performances and the TCTs measured during stimulation of the supplementary motor area (SMA). The TCTs are sorted by hemisphere, respective protocol, as well as hand (left/right/both pooled). The p-values refer to comparisons of the specific stimulation TCT to the respective baseline evaluation.

could be demonstrated (anterior group TCT 6.7 ± 0.8 s; middle group TCT 6.9 ± 0.8 s; posterior group TCT 7.0 ± 1.1 s [p = 0.0429]).

Right hemisphere: Every comparison during right-hemispheric stimulation demonstrated a significantly slower performance compared to respective baselines ($p < 0.05$; Table 1).

Regression and visible coordination problems: The regression model also revealed an independent effect for the hand and the stimulation protocols, with 0.7 s (95%-CI = [−0.8; −0.5], $p < 0.0005$) faster TCT for the right hand than for the left hand, and on average 0.4 to 0.5 s slower TCTs for the stimulation protocols compared to baseline ($p < 0.0005$ for all protocols; Table 2). Two VCPs occurred in total (Supplementary Videos S5 and S6).

Stacking checkers. Left hemisphere: No significant TCT dissociations for left-hemispheric stimulation were revealed ($p > 0.05$; Table 1).

Right hemisphere: Concerning right-hemispheric stimulation, we found significant slowing of TCTs in all comparisons of left-handed performances (baseline TCT 3.1 ± 0.6 s; 5 Hz TCT 3.3 ± 0.6 s [p = 0.0094]; 10 Hz TCT 3.3 ± 0.5 s [p = 0.0192]; TBS TCT 3.4 ± 0.6 s [p = 0.0136]), no significant slowing for right-handed performances ($p > 0.05$), and only slowing for the comparisons for pooled hands (baseline TCT 3.0 ± 0.6 s; 5 Hz TCT 3.2 ± 0.5 s [p = 0.0073]; 10 Hz TCT 3.2 ± 0.5 s [p = 0.0136]; TBS TCT 3.2 ± 0.5 s [p = 0.0020]); Table 1).

Regression and visible coordination problems: The TCTs for the right hand seemed to be slightly shorter than for the left hand (0.2 s, 95%-CI = [−0.2; −0.1], $p < 0.0005$). Only for the TBS protocol a significant difference in the TCTs compared to baseline was observed in the model with on average slightly slower TCT of 0.1 s (95%-CI = [0.0; 0.2], p = 0.0210; Table 2). Six VCPs were identified (Supplementary Videos S7 and S8).

Lifting light objects. Left hemisphere: For left hemisphere performances, only stimulation with 5 Hz resulted in a significant effect, both in left-handed performances (baseline TCT 3.0 ± 0.4 s; 5 Hz TCT 2.8 ± 0.3 s [p = 0.0240]; Table 1) and in comparisons for pooled hands (baseline TCT 2.9 ± 0.4 s; 5 Hz TCT 2.8 ± 0.3 s [p = 0.0056]; Table 1).

Right hemisphere: No statistically significant dissociations in TCTs emerged for this subtest ($p > 0.05$; Table 1).

Regression and visible coordination problems: Lifting light objects was performed slightly slower when the right hemisphere was stimulated (0.04 s, 95%-CI = [−0.0; 0.1], p = 0.0100) and faster when the right hand was used (0.1 s, 95%-CI = [−0.1; −0.0], $p < 0.0005$). Stimulation with 5 Hz and with 10 Hz seemed to slightly shorten the TCT by 0.1 s, respectively (p = 0.0010 and $p < 0.0005$; Table 2). During this task, the highest total number of VCPs out of all tests occurred, namely twelve errors (Supplementary Videos S9 and S10).

			Jebsen-Taylor Hand Function Test							
			Writing	Simulated page turning	Lifting small objects	Simulated feeding	Stacking checkers	Lifting light objects	Lifting heavy objects	Nine-hole Peg Test
Right hemisphere (compared to left hemisphere)		TCT difference in s [95%-CI]	−0.09 [−0.38; 0.2]	−0.11 [−0.18; −0.03]	−0.05 [−0.15; 0.04]	0.07 [−0.07; 0.21]	0.04 [−0.04; 0.12]	0.04 [−0.04; 0.12]	0 [−0.03; 0.03]	0.12 [−0.08; 0.31]
		p	0.562	0.005	0.278	0.358	0.332	0.01	0.938	0.241
Right hand (compared to left hand)		TCT difference in s [95%-CI]	−10.39 [−10.68; −10.1]	−0.04 [−0.12; 0.03]	−0.23 [−0.32; −0.13]	−0.68 [−0.82; −0.54]	−0.15 [−0.23; −0.07]	−0.08 [−0.12; −0.04]	−0.08 [−0.11; −0.04]	−1.28 [−1.47; −1.08]
		p	<0.0005	0.252	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
Interaction hemisphere and hand		TCT difference in s [95%-CI]	0.5 [−0.33; 0.49]	0.12 [−0.08; 0.13]	0.14 [−0.24; 0.03]	0.14 [−0.21; 0.19]	0.11 [−0.19; 0.03]	0.06 [−0.06; 0.04]	0.06 [−0.07; 0.02]	0.31 [−0.38; 0.18]
		p	0.699	0.671	0.131	0.932	0.172	0.697	0.36	0.493
Stimulation target (reference first target)	2	TCT difference in s [95%-CI]	0.16 [−0.2; 0.51]	0.06 [−0.03; 0.15]	−0.03 [−0.14; 0.09]	0.01 [−0.16; 0.19]	0.05 [−0.05; 0.15]	0.01 [−0.03; 0.06]	0.03 [−0.01; 0.07]	0.33 [0.08; 0.57]
		p	0.381	0.227	0.632	0.864	0.33	0.615	0.152	0.008
	3	TCT difference in s [95%-CI]	0.03 [−0.32; 0.39]	0.01 [−0.08; 0.1]	−0.09 [−0.21; 0.03]	−0.05 [−0.22; 0.12]	−0.01 [−0.11; 0.08]	0.03 [−0.02; 0.07]	0.01 [−0.03; 0.05]	0.17 [−0.07; 0.41]
		p	0.853	0.812	0.126	0.571	0.805	0.264	0.58	0.167
	4	TCT difference in s [95%-CI]	0.18 [−0.17; 0.54]	−0.01 [−0.1; 0.08]	−0.05 [−0.17; 0.06]	−0.04 [−0.21; 0.13]	0.02 [−0.07; 0.12]	0.03 [−0.02; 0.07]	0.04 [0; 0.08]	0.21 [−0.03; 0.45]
		p	0.312	0.895	0.368	0.639	0.621	0.256	0.083	0.092
	5	TCT difference in s [95%-CI]	0.02 [−0.34; 0.37]	−0.01 [−0.1; 0.08]	0 [−0.12; 0.12]	−0.11 [−0.28; 0.07]	0.01 [−0.08; 0.11]	0.02 [−0.02; 0.07]	0.02 [−0.03; 0.06]	0.23 [−0.01; 0.48]
		p	0.923	0.767	0.988	0.231	0.781	0.355	0.462	0.057
	6	TCT difference in s [95%-CI]	0.06 [−0.3; 0.41]	0.02 [−0.07; 0.11]	−0.05 [−0.17; 0.06]	−0.1 [−0.27; 0.08]	0.03 [−0.07; 0.12]	0.02 [−0.03; 0.06]	0.01 [−0.03; 0.05]	0.05 [−0.19; 0.29]
		p	0.748	0.653	0.372	0.278	0.598	0.527	0.659	0.687
Stimulation protocol (reference baseline)	5 HZ	TCT difference in s [95%-CI]	−1.23 [−1.52; −0.94]	0.23 [0.16; 0.3]	0.27 [0.17; 0.36]	0.48 [0.34; 0.62]	0 [−0.08; 0.08]	−0.06 [−0.1; −0.03]	−0.06 [−0.1; −0.03]	0.79 [0.6; 0.99]
		p	<0.0005	<0.0005	<0.0005	<0.0005	0.922	0.001	<0.0005	<0.0005
	10 Hz	TCT difference in s [95%-CI]	−1.44 [−1.73; −1.15]	0.28 [0.2; 0.35]	0.38 [0.29; 0.48]	0.38 [0.24; 0.52]	0.03 [−0.05; 0.11]	−0.09 [−0.13; −0.05]	−0.07 [−0.11; −0.04]	0.44 [0.25; 0.64]
		p	<0.0005	<0.0005	<0.0005	<0.0005	0.426	<0.0005	<0.0005	<0.0005
	TBS	TCT difference in s [95%-CI]	−1.12 [−1.41; −0.83]	0.24 [0.16; 0.31]	0.38 [0.29; 0.48]	0.35 [0.21; 0.49]	0.09 [0.01; 0.17]	−0.03 [−0.07; 0.01]	−0.05 [−0.08; −0.02]	0.74 [0.54; 0.93]
		p	<0.0005	<0.0005	<0.0005	<0.0005	0.021	0.126	0.003	<0.0005

Table 2. Test completion time (TCT) differences for stimulation-related parameters in the multi-level regression analyses. This table depicts the regression model based on TCTs gained during baseline performance and stimulation of the supplementary motor area (SMA). The calculated influence on TCT of variables such as hemisphere, hand, hemisphere x hand, stimulation target, and stimulation protocol is given in the form of average difference in seconds with the corresponding 95% confidence interval (CI) and p-values.

Lifting heavy objects. Left hemisphere: The analyses showed no significant difference between baseline and the varying stimulation conditions in any comparisons ($p > 0.05$; Table 1).

Right hemisphere: No significant differences between baseline and stimulation were present ($p > 0.05$; Table 1). Our analysis of stimulation region however showed that lateral TBS was associated with higher TCTs than medial stimulation for left-handed performances (medial group TCT 2.77 ± 0.29 s; lateral group TCT 2.81 ± 0.27 s [$p = 0.0362$]).

Regression and visible coordination problems: The average TCT seemed to be shorter when the right hand was used as shown in the regression model (0.1 s, 95%-CI = [−0.1; −0.0], $p < 0.0005$). For all stimulation protocols the model revealed a slightly faster performance than at baseline (0.1 s, $p < 0.0005$ for 5 Hz and 10 Hz, respectively, and $p = 0.0030$ for TBS; Table 2). A relatively high number of eight VCPs was identified (Supplementary Videos S11 and S12).

Nine-hole Peg Test. *Left hemisphere.* For the Nine-hole Peg Test (NHPT), we found significantly slower TCTs during left-hemispheric stimulation in comparisons of right-handed performances (baseline TCT 16.8 ± 1.3 s; 5 Hz TCT 17.5 ± 1.6 s [$p = 0.0328$]) and comparisons for pooled hands (baseline TCT 17.5 ± 1.9 s; 5 Hz TCT 18.0 ± 1.6 s [$p = 0.0422$]; TBS TCT 18.0 ± 1.8 s [$p = 0.0394$]; Table 3 and Fig. 3).

Right hemisphere. For stimulation of the right hemisphere, every comparison showed significant slowing during stimulation ($p < 0.05$; Table 3 and Fig. 3). In left-handed performances, lateral stimulation with 10 Hz corresponded to higher TCTs than medial stimulation (medial group TCT 18.3 ± 1.5 s; lateral group TCT 19.0 ± 1.8 s [$p = 0.0136$]).

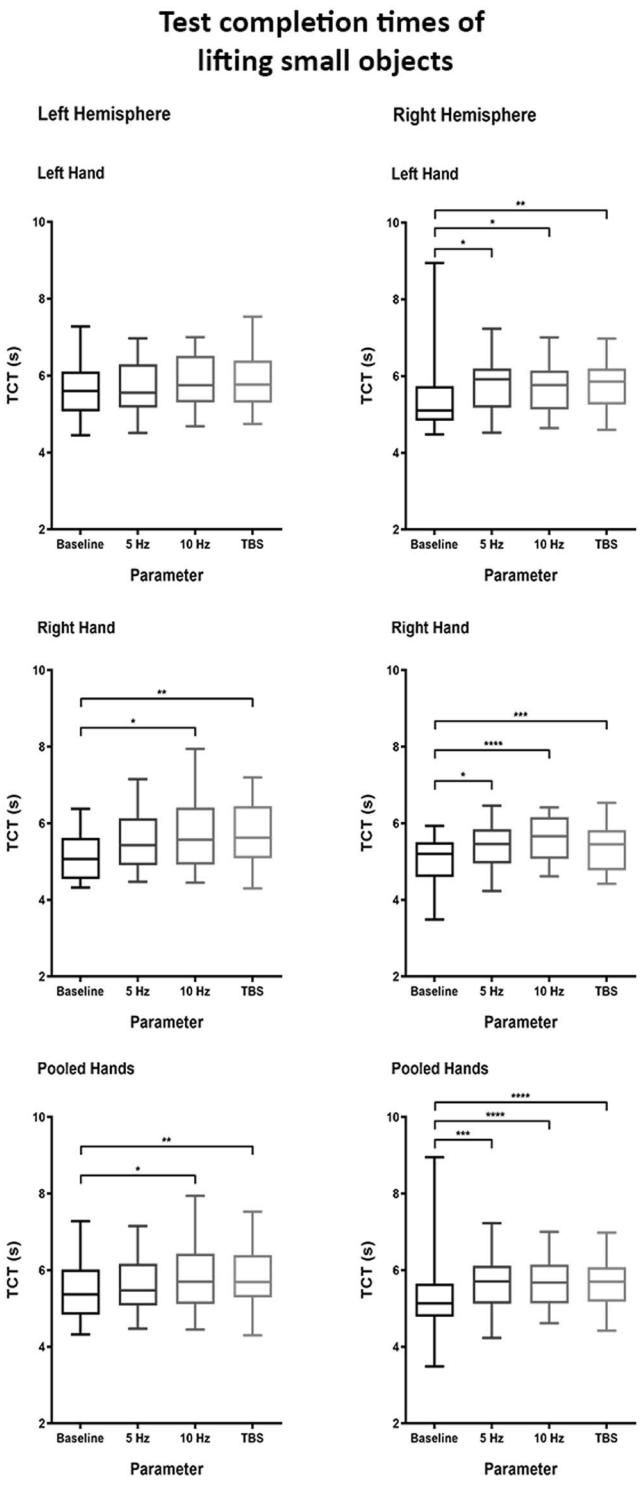

Figure 2. Test completion times (TCTs) of lifting small objects. Boxplots depicting the distribution of TCTs in the task of lifting small objects, separated according to the various analysis pools. For the plots pertaining to left hand and right hand, each single boxplot indicates the distribution of 20 values (one per participant). For the plots pertaining to pooled hands, each single boxplot indicates the distribution of 40 values (the collection of the values from each hand). Whiskers indicate the range of values, boxes depict the two middle quartiles of values. The median is shown by the line inside the box. Testing was done using Wilcoxon rank-sum tests. Statistical significance of differences is indicated by asterisks (cutoffs at $p < 0.05$, <0.01, <0.001, and < 0.0001 for *, **, *** and ****, respectively).

			Nine-hole Peg Test	
			TCT (s)	p-value to baseline
Left Hemisphere	Left hand	Baseline	18.3 ± 2.1	
		5 Hz	18.6 ± 1.4	0.3884
		10 Hz	18.2 ± 1.3	0.8983
		TBS	18.7 ± 1.6	0.1429
	Right hand	Baseline	16.8 ± 1.3	
		5 Hz	17.5 ± 1.6	0.0328
		10 Hz	17.1 ± 1.6	0.5217
		TBS	17.3 ± 1.8	0.1140
	Pooled hands	Baseline	17.5 ± 1.9	
		5 Hz	18.0 ± 1.6	0.0422
		10 Hz	17.7 ± 1.6	0.7146
		TBS	18.0 ± 1.8	0.0394
Right Hemisphere	Left hand	Baseline	17.7 ± 1.7	
		5 Hz	18.9 ± 1.4	0.0012
		10 Hz	18.6 ± 1.6	0.0049
		TBS	19.0 ± 2.1	0.0006
	Right hand	Baseline	16.6 ± 1.8	
		5 Hz	17.6 ± 1.8	0.0073
		10 Hz	17.2 ± 1.8	0.0484
		TBS	17.4 ± 1.6	0.0014
	Pooled hands	Baseline	17.2 ± 1.8	
		5 Hz	18.2 ± 1.7	<0.0001
		10 Hz	17.9 ± 1.8	0.0005
		TBS	18.2 ± 2.0	<0.0001

Table 3. Test completion times (TCTs) for the Nine-hole Peg Test (NHPT). This table depicts the TCTs of baseline performances and the TCTs measured during stimulation of the supplementary motor area (SMA). The TCTs are sorted by hemisphere, respective protocol, as well as hand (left/right/both pooled). The p-values refer to comparisons of the specific stimulation TCT to the respective baseline evaluation.

Regression and visible coordination problems. A significant effect was demonstrable within the regression model for the right hand with a shorter TCT of in average 1.3 s (95%-CI = [−1.5; −1.1], $p < 0.0005$). This was the only test where an independent effect for a stimulation target was observed. In this context, stimulation target 2 seemed to have an in average 0.3 s slower TCT compared to stimulation target 1 (95%-CI = [0.1; 0.6], $p = 0.0080$). All stimulation protocols seemed to slow the performance for this test (by 0.4 to 0.8 s) compared to baseline ($p < 0.0005$ for all protocols; Table 2). The analysis revealed nine VCPs in total (Supplementary Videos S13 and S14).

Finger tapping test. Analysis of the standard deviation of inter-tap intervals (SDITs) during the finger tapping test showed significantly more variable inter-tap intervals during stimulation in all comparisons ($p < 0.05$; Table 4), except for TBS over the right hemisphere (baseline SDIT 67.7 ± 41.3 ms; TBS SDIT 73.8 ± 19.0 ms [$p = 0.0826$]; Table 4). No VCPs were registered.

Pronator drift test, finger-nose test, and flexion-extension test. No difficulties during performance of the pronator drift test, finger-nose test, or flexion-extension test during baseline assessments or during the stimulation conditions were observed.

Comparison of stimulation parameters. Comparison of the CIs of the TCT differences for the three stimulation protocols to baseline revealed no clearly superior protocol since CIs were overlapping for all tests (Table 2).

Discussion

We hypothesized that nTMS to the SMA causes measurable effects on the task performance, which we documented via TCTs, SDITs, and video recordings of VCPs. We were able to demonstrate significant differences of TCTs between baseline and stimulation condition ($p < 0.05$) in six of seven subtests of the JHFT (except for lifting heavy objects) and in the NHPT, which are both established tests to assess fine motor skills. SDITs were significantly higher under stimulation in the finger tapping test. VCPs were detected most prominently during lifting light objects, lifting heavy objects, and during the NHPT. No effects of stimulation in the pronator drift test, flexion-extension test, or finger-nose test were detected. Our multi-level regression model did not demonstrate one stimulation protocol to be clearly more effective when compared to the other protocols based on the analysis

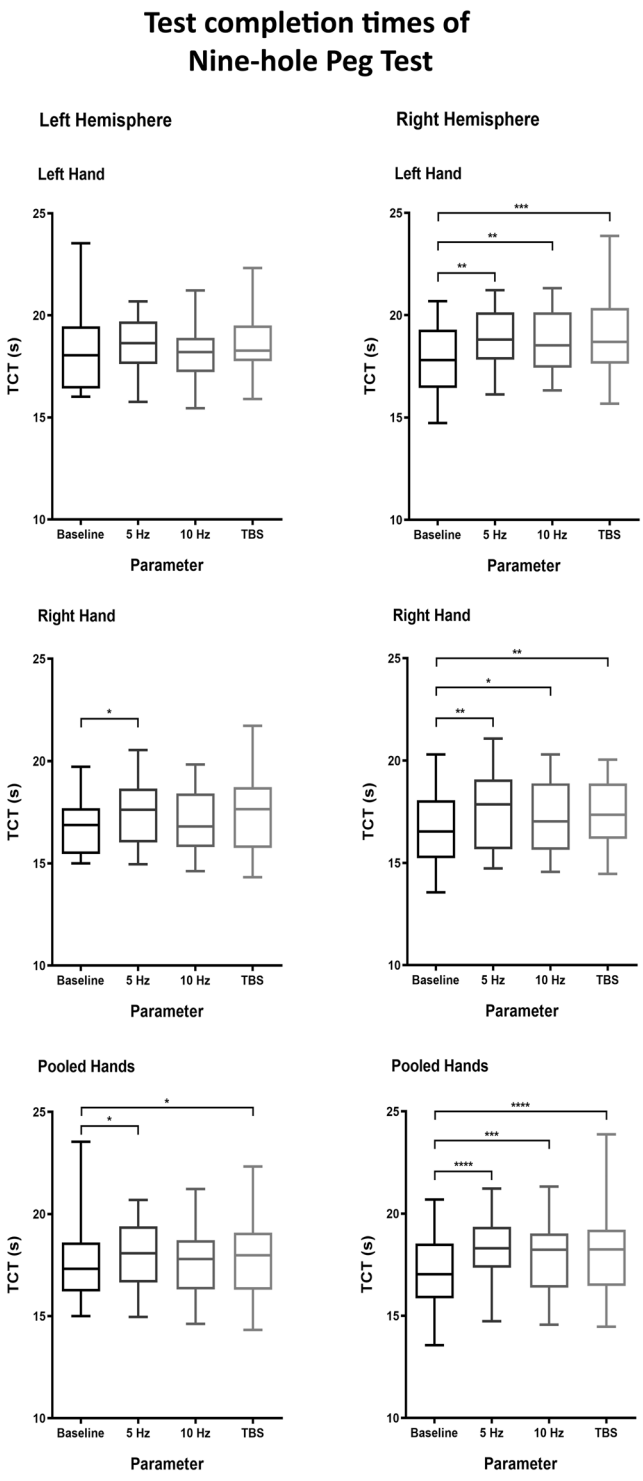

Figure 3. Test completion times (TCTs) for the Nine-hole Peg Test (NHPT). Boxplots depict the distribution of TCTs in the NHPT, separated according to the various analysis pools. For the plots pertaining to left hand and right hand, each single boxplot indicates the distribution of 20 values (one per participant). For the plots pertaining to pooled hands, each single boxplot indicates the distribution of 40 values (the collection of the values from each hand). Whiskers indicate the range of values, boxes depict the two middle quartiles of values. The median is shown by the line inside the box. Testing was done using Wilcoxon rank-sum tests. Statistical significance of differences is indicated by asterisks (cutoffs at $p < 0.05$, <0.01, <0.001, and < 0.0001 for *, **, *** and ****, respectively).

of TCTs. Also, our model did not show clear differences of effect when comparing individual stimulation sites. We were able to determine six instances of lateral targets leading to stronger slowing of TCTs than medial targets. Notably, stimulation of the right hemisphere was able to influence the performance of both executing hands.

		Finger tapping test	
		SDIT (ms)	p-value to baseline
Left Hemisphere	Baseline	63.1 ± 40.2	
	5 Hz	77.5 ± 29.2	0.0484
	10 Hz	73.3 ± 32.2	0.0266
	TBS	79.6 ± 36.5	0.0441
Right Hemisphere	Baseline	67.7 ± 41.3	
	5 Hz	88.1 ± 35.2	0.0064
	10 Hz	86.5 ± 45.7	0.0400
	TBS	73.8 ± 19.0	0.0826

Table 4. Standard deviation of inter-tap intervals (SDITs) of finger tapping test. This table represents an overview regarding the SDITs calculated from the finger tapping test in baseline and under stimulation of the supplementary motor area (SMA). The SDITs are sorted by hemisphere and respective baseline/stimulation condition, with p-values indicating statistical significance.

To explain the observed results, we would like to point out the concept of the virtual lesion. It assumes that stimulation pulses are able to interfere with physiological computing inside the target structures, thereby eliciting momentary deficits[44]. Analogously, SMA disruption via TMS has been shown in other incarnations, e.g. as degradation of bimanual movement or decline of force control[16,19]. The challenge for the clinical approach with regards to these objective criteria is now to infer a rule for classification of a given point as "SMA-positive". In this regard, we would argue for a combination of a time-based classification with the more accessible classification via VCPs.

In certain tasks, most notably lifting light objects, lifting heavy objects, and the NHPT, VCPs became apparent (Supplementary Table 1). This again may relate to cognitive control (Supplementary Videos S9–S14). In lifting light objects and lifting heavy objects, we frequently noticed participants using the wrong limb to lift the last can when nTMS was applied (Supplementary Videos S9–S12). All of these occurred seemingly unconscious. The VCPs of the NHPT took a different form. Here, the disruption seems to show itself either as problems in selecting and executing an appropriate movement (Supplementary Videos S13 and S14) or as akinesia, where participants did not initiate any movement for the second part of the task. The former mistakes might resemble the alien-limb syndrome, a condition known to be associated with SMA damage, in which conscious control of a limb is lost[15,45]. The latter could probably reflect acute inability to accommodate to the new part of the task, which is congruent with some studies involving the SMA in attention and performance monitoring[46–48]. Due to this correlation, we consider the occurrence of these VCPs as a positive sign for SMA disruption. This would in turn clearly mark a given target as "SMA-positive". Due to the ease of detection of these mistakes, we suggest including them in future approaches of nTMS-based SMA mapping.

In our study, the most notable instances of seeming performance facilitation under stimulation occurred during the JHFT subtest of writing (Table 1). Our regression model showed a significant acceleration of TCTs during all three stimulation protocols, which was independent of the stimulated hemisphere (Table 2). However, we are hesitant to interpret this as a verified sign of performance amelioration through nTMS. Looking at studies that examined the practice effect during multiple run-throughs of the JHFT, a quickening in TCTs seems more likely to be due to a practice effect[49]. While we did not implement any specific measurements or corrections for practice effects in this study, their presence remains an important factor. However, studies on practice effects in the JHFT are rare and limited to only one study including 20 women, which also only tested practice over the course of three runs[49]. Meanwhile, our study does contain at least 19 (baseline + 6 targets * 3 protocols) repetitions per hand. In this context, our data might gain new aspects. Since during right-hemispheric stimulation no significant acceleration of TCTs was observed for the right hand, the possibility of right-hemispheric stimulation preventing a learning effect emerges. This would fit the current body of research, which puts emphasis on the role of the SMA in the learning of motor tasks[2,50]. Interference with this function could explain the observed lack of a practice effect. This interpretation does, however, not explain the potentially persisting practice effect for writing with the left hand.

Our data may also be regarded as a qualitative finding regarding lateralization of movement control. Different approaches have led to the model of a rostro-caudal gradient of rising movement lateralization towards occipital direction[1–3]. Our data might affirm this because stimulation of one hemisphere was in some cases able to influence both right-handed as well as left-handed performances (Tables 1 and 3). Furthermore, the absence of a clear interaction between the stimulated hemisphere and hand regarding TCTs can as well be interpreted in this line of thought (Table 2). If there was strong lateralization on the level of the SMA, we should have been able to observe specific effects of hemisphere-hand relations. However, this was not the case, potentially indicating a less strict lateralization.

Furthermore, we would like to point out the difference between the hemispheres. Stimulation of the right hemisphere was more likely to significantly slow down performances than stimulation of the left hemisphere and resulted in stronger slowing in general (Tables 1 and 3). Within the current literature, the body of research investigating interhemispheric differences between the SMA of both hemispheres is small. Some studies found a strong lateralization of inhibitory control functions toward the right hemisphere with a pronounced activation of right SMA in cognitive control tasks[51,52]. While our data fits this model, it is conflicting with other studies. Resection of the SMA in the left or right hemisphere can rather easily be compensated by the respective contralateral SMA[9].

Our data in turn would imply a more severe deficit when resecting SMA of the right hemisphere. A relevant factor in this regard is our focus on right-handed individuals, which would suggest left-hemispheric dominance. Hemispheric dominance has been linked to the apparition of aphasia following SMA resection. This again conflicts with the importance our data places on the right-hemispheric SMA. One might hypothesize that the cognitive control aspect has to be viewed separately from the pure motor aspect. To further shed light on this issue, a study focusing on left-handed individuals might prove to be useful in determining the influence of hemispheric dominance.

Out of the seven significant analyses in which the effect of target location (anterior / middle / posterior targets and medial / lateral targets, respectively) was examined, six showed a stronger effect of lateral stimulation compared to medial stimulation. This could potentially relate to the complex of SMA somatotopy. Many studies point to a somatotopic organization, specifically a rostro-occipital sequence of orofacial, upper extremity, and lower extremity movements[53,54]. The present study was unable to demonstrate this gradient. Instead, we observed a difference between medial and lateral stimulation targets. In preclinical experiments on monkeys, mesial areas have been demonstrated to contain more movement representations than the convexities of the hemispheres[54]. There could be the possibility that by stimulating laterally, mesial parts of the SMA are costimulated. However, the mentioned difference was observed only rarely and without consistent relation to hemisphere, hand, or stimulation parameter. With all this in mind, we are hesitant to assume a stronger stance on the topic of SMA somatotopy within the context of this study. However, this finding may be taken into account when looking for the most effective way to influence SMA activity in general.

No clear difference in effect between the stimulation protocols could be objectively determined (Table 2). Current literature indicates that our protocols should lead to different modulations of activity. TBS is known to lessen activity over time while higher frequencies should rather heighten activation[42]. Considering this, we cannot rule out the possibility of a nocebo effect taking place. However, due to both the frequency of similar VCPs and their correlation with current models of SMA function, we consider this to be unlikely. Taking our findings together, our observations lead us to presume that both inhibiting and activating protocols are able to induce transient and measurable effects on the SMA. One factor possibly elevating TBS over other protocols could be the fact that it has specifically been used for rapid affection of neuronal activity via comparatively high stimulus frequency and has performed slightly better than the other protocols in eliciting VCPs. Due to the very slight differences though, more research regarding 5-Hz and 10-Hz stimulation protocols is still required.

Regarding the study's limitations, several points have to be raised. First, the lack of a control condition (e.g., targeted stimulation to a region outside of the SMA and primary motor area) has to be considered a relevant shortcoming. Inclusion of such a control condition might have allowed to assign observed effects to the distinct effect of stimulation with more certainty. While we cannot entirely rule out the influence of confounding variables such as the sensation of stimulation, the strong parallels to symptoms connected to SMA dysfunction make an unspecific effect seem unlikely. Both in monkeys and humans, failed coordination of limbs and involuntary movements, similar to the observed VCPs of this study, have arisen out of damage or dysfunction to the SMA[45,55,56]. Furthermore, a recent study applying direct cortical stimulation to premotor areas during awake craniotomy found that the employed stimulation was able to disrupt coordination of hand muscle groups[57]. Thus, real effects of stimulation on SMA-related function seem evident, and stimulation-induced effects have been found consistently using the same electric-field-navigated TMS system regarding other brain functions. For the second limitation, our sample size includes only 20 participants. This limits the generalizability of our findings to some degree. Our statistical analyses, however, imply that the effects we found are robust and more participants would not necessarily add statistical value. Third, our participants were exclusively right-handed according to the Edinburgh Handedness Inventory. We can therefore not expand our findings to the total population and must await more research into the aspects of hemispheric dominance. Fourth, our participants give us data only for the conditions in healthy brains. We can currently make no statements as to the applicability in patients suffering from brain tumors, epilepsy, or taking any kind of neuroactive medication. Fifth, while looking at data from both hemispheres and both hands is necessary in investigating SMA-related phenomena, this also introduces a high amount of complexity to the valid interpretation of data. Nevertheless, we are presently able to demonstrate that there are no difficulties regarding general feasibility of an nTMS-based SMA mapping procedure. While the entirety of the applied tasks would likely prove too overbearing for clinical usage, a reduction to the most promising tests would be very usable.

In conclusion, this study adds to the growing number of investigations of SMA function. Specifically, identification and selective manipulation of SMA via nTMS mapping could enable new approaches in neurophysiological settings to investigate the area's involvement in its many supposed functions[16,17,52,58]. Moreover, this study contributes evidence to a hemisphere-dependent bilateral motor influence of the SMA by showing stronger disruptions arising from the stimulation of the right hemisphere. Furthermore, we found and statistically confirmed multiple instances of impacted fine motor function during nTMS. These are expressed by higher TCTs and higher SDITs to VCPs. This further builds up the viability of an nTMS-based mapping protocol.

Materials and Methods

Ethics. The present study was approved by the local institutional review board (Ethics Committee of Technical University Munich) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all subjects. All participants gave explicit informed consent to publication of any video material collected within the context of this study including identifying information/images in an online open-access publication.

Participants and study design. Twenty healthy volunteers (8 males and 12 females, median age: 22.5 years, age range: 19–30 years) participated in this study. For inclusion criteria, we defined age of at least 18 years, informed consent, and right-handedness according to the Edinburgh Handedness Inventory[59]. Exclusion criteria were pregnancy, contraindications for magnetic resonance imaging (MRI) or TMS (e.g., metallic implants), and any history of neurological or psychiatric diseases. In our analysis, we partly used data previously published within a smaller study in which we focused on ten female volunteers and exclusively on the effects of 10-Hz stimulation regarding the performance during execution of the JHFT[36].

Each participant first underwent anatomical MRI at 3 Tesla (Achieva; Philips Healthcare, Best, The Netherlands) to acquire a three-dimensional T1-weighted gradient echo sequence (repetition time/echo time: 9/4 ms, 1 mm^3 isovoxel covering the whole head), used for neuronavigation during later nTMS. Procedures by nTMS were then performed in the context of two separate appointments, which were scheduled at least 14 days apart in each participant. Each appointment was dedicated to motor and SMA mappings of one hemisphere, with the sequence of hemispheres stimulated, single tests, hands (in case of tests for unilateral performance), and order of stimulation of predefined targets being subject to randomization. Apart from these randomizations, the approach of motor and SMA mappings as well as the performance and analyses of tests applied during stimulation were identical during both appointments.

For all nTMS procedures, an electric-field-navigated TMS system was used in order to provide the highest possible accuracy (Nexstim eXimia NBS system, version 4.3; Nexstim Plc., Helsinki, Finland)[60].

Motor mapping and determination of targets for SMA mapping. Prior to SMA mappings, motor mapping by nTMS using single-pulse stimulation was performed to delineate the primary motor cortex according to current practice[61]. First, the rMT was determined considering electromyography (EMG) recordings of either the abductor pollicis brevis muscle (APB) or abductor digiti minimi muscle (ADM) using pregelled surface electrodes (Neuroline 720; Ambu, Ballerup, Denmark). The cortical motor hotspot was first identified and then utilized for rMT determination using the built-in procedure considering the maximum likelihood algorithm[62–64]. After the rMT was determined, motor mapping took place considering EMG recordings from electrodes attached to the APB, ADM, flexor carpi radialis muscle, and biceps brachii muscle, with a stimulus intensity of 105% of the individual rMT[61]. The motor mapping of each hemisphere took place directly before SMA mapping, as did the determination of the respective rMT, which was individually assessed in each appointment per participant.

Motor mapping was used to spatially enclose the whole extent of primary motor representations particularly within the superior frontal gyrus (SFG) and middle frontal gyrus (MFG). During analysis of motor mapping data, manual review of mapped points took place, with points marked as motor-positive when the corresponding EMG recording showed plausible latency for upper extremity muscles (15 to 30 ms) and amplitudes of at least 50 μV[61]. Stimulated points not fulfilling these criteria were defined as motor-negative and not considered as part of the primary motor cortex in this study.

For SMA mapping, six stimulation targets per hemisphere were manually placed into the presumed SMA outside of the determined motor cortex delineated by nTMS motor mapping, bordering next to the most anteriorly located motor-positive stimulation spot (Fig. 1)[36]. This was done to ensure that induced motor impairment during SMA mapping could be attributed to SMA stimulation without being confounded by possible stimulation of very anterior parts of primary motor cortex representations[36,65,66]. Prior reports have indicated that the primary motor cortex can extend far anteriorly and beyond the precentral gyrus, with resection of very anterior motor-positive stimulation spots causing postoperative motor deficits related to the primary motor cortex[65]. The number of targets was chosen to allow for complete extension over the anatomical region corresponding to the SMA, while at the same time remaining far enough apart to allow for allocation of effects to each specific target (without presumed stimulation overlap). The targets were generally placed within the posterior SFG, in some cases bordering on the posterior MFG, thus corresponding to the location of pre-SMA and SMA proper as reported in the literature[2]. Inter-target distance was 5 to 10 mm (Fig. 1)[36]. For analysis purposes, the targets were named as follows: posterior targets were targets 1 (medial) and 2 (lateral), middle targets were targets 3 (medial) and 4 (lateral), and anterior targets were targets 5 (medial) and 6 (lateral).

SMA mapping. *Test descriptions and baseline assessments.* During initial baseline assessments and the SMA mappings, we used the following standardized batteries and tests of movement and coordination:

- JHFT (Sammons Preston, Bolingbrook, Illinois, USA), consisting of seven subtests: writing, simulated page turning, lifting small objects, simulated feeding, stacking checkers, lifting light objects, and lifting heavy objects,
- NHPT (Patterson Medical, Bolingbrook, Illinois, USA),
- Pronator drift test (participants were instructed to lift and hold their arms horizontally in front of them),
- Finger-nose test (participants were instructed to, with their eyes closed, touch the tip of their noses with alternating hands),
- Finger tapping test (participants had to reproduce a simultaneously metronome-generated rhythm of 1 Hz by pressing a key on a keyboard, alternating between the left and right hand), and
- Flexion-extension test (participants had to perform alternating anti-phasic flexing and extending of their arms in a 1-Hz rhythm as given by a metronome).

Baseline assessments (performance without simultaneous stimulation) for these tests were carried out shortly before SMA mappings and subsequent to precise instructions by the examiner and one practice run for each of the above-mentioned tests. For each subtest of the JHFT and for the NHPT, one baseline performance is included

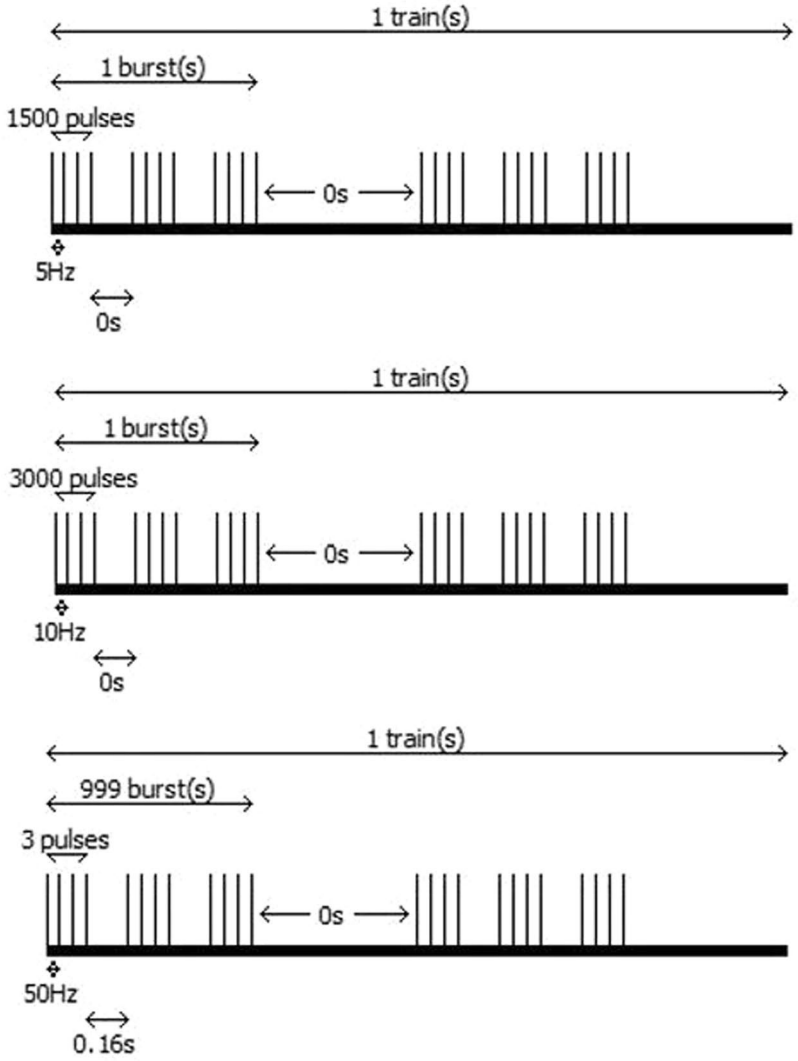

Figure 4. Schematic presentation of stimulation protocols. This figure shows the applied stimulation protocols. From top to bottom, 5-Hz stimulation, 10-Hz stimulation, and theta burst stimulation (TBS) are schematically illustrated with corresponding frequencies and relevant timing details. Stimulation was only applied for the duration of each test performance.

in the supplementary videos to this study for the ease of understanding (Supplementary Videos S1–S14). Each test performance was started on command of the examiner. Participants were further told to aim for both a fluent and precise performance, keeping the given rhythm in tests where a metronome was used. All tests were audio- and video-recorded for further detailed analysis after the test procedures.

Stimulation of the SMA. After baseline assessments, SMA mapping was carried out using three different stimulation protocols (Fig. 4):

- 5 Hz (100% rMT, delivered with 1,500 pulses per burst, 1 burst per train, 1 train per sequence),
- 10 Hz (100% rMT, delivered with 3,000 pulses per burst, 1 burst per train, 1 train per sequence), and
- TBS (100% rMT, delivered with 50 Hz, 3 pulses per burst, 160 ms between bursts, 999 bursts per train, 1 train per sequence)[43,67].

The selected parameters fall under current safety guidelines for stimulation with conventional and patterned TMS outside the motor cortex, where, however, currently no universal limit for safe application has been published[68]. The total duration of each protocol was set so that the stimulation was long enough to cover the full test performance for each test (length >3 min of each protocol). The coil was hand-held during stimulations. Through real-time neuronavigation we ensured optimal conditions for stimulation, keeping the stimulating coil perpendicular to the skull and maintaining a 90° angle of the induced electric field to local gyrus orientation, while at the same time keeping the maximum of the electric field fixed on the respective stimulation target during the task

performance[60,69,70]. After each performance, test objects were rearranged into starting constellation, and the coil was moved to the next stimulation target.

After acquisition of one performance for each target, the test was repeated in similar fashion, but executed with the other hand before continuing with the next test. For the entire procedure of SMA mapping including prior motor mapping and baseline acquisition, approximately 270 minutes were needed per participant and appointment, including several breaks to minimize fatigue effects. Total number of stimuli applied was about 35,000 per session (count estimation depending on chosen stimulation protocols and the individual time needed for completion of the tasks).

Evaluation of test performances. All test performances under the baseline and stimulation conditions were recorded as video files using the integrated camera of the nTMS system, which allows recording time-locked to the nTMS pulse onset. The camera was placed to allow for full view of the participant performing the tasks, including the test equipment. The following criteria were documented for single tests and considered during later post-hoc evaluation of performances:

- TCTs; time between start command and finishing of a test – JHFT and NHPT,
- SDITs; spread of different inter-tap intervals as gauge for rhythm-keeping ability – finger tapping test, and
- VCPs; qualitative indicators of SMA disruption, such as forgetting the task, inability to move, less fluid movements, significantly worse rhythm keeping – JHFT, NHPT, finger tapping test, flexion-extension test, finger-nose test, and pronator drift test (Supplementary Table 1). VCPs were noted immediately after occurrence by the conducting personnel and with the help of video material.

Statistics. GraphPad Prism (version 7.0; GraphPad Software Inc., La Jolla, California, USA) was used to calculate descriptive statistics of the cohort and stimulation-related parameters and to generate graphs. Shapiro-Wilk tests confirmed non-normal distribution of TCTs and SDITs.

We compared the rMT between hemispheres by a paired t-test. Wilcoxon rank-sum tests were used to compare the TCTs (JHFT, NHPT) and SDITs (finger tapping test) of the baseline performances to the performances during stimulation. In this context, we compared the average TCTs or SDITs under stimulation (mean of the data gained from all six stimulation targets) to the respective TCTs or SDITs of the baseline condition, which was achieved separately for the mapping of the left and right hemisphere considering the three different stimulation protocols and test conductions with the left and right hand, respectively. Furthermore, we formed additional analyses for pooled data of both hands per stimulated hemisphere and stimulation protocol.

Additional analyses were performed to investigate possible TCT differences between stimulated regions. To this end, we formed three analysis groups based on rostro-occipital orientation (anterior [targets 5 & 6], middle [targets 3 & 4], and posterior [targets 1 & 2], Fig. 1) and two groups based on medio-lateral orientation (medial [targets 1, 3 & 5] and lateral [targets 2, 4 & 6], Fig. 1). We then used Friedman tests to detect possible TCT differences between anterior, middle, and posterior groups. For comparisons of medial to lateral groups, Wilcoxon rank-sum tests were utilized. Within these analyses, stimulation frequency, stimulated hemisphere, and tested hand were always compared correspondingly (e.g., medial left-hemispheric, left-handed 5-Hz stimulation compared to lateral left-hemispheric, left-handed 5-Hz stimulation).

Regarding VCPs (JHFT, NHPT, finger tapping test, flexion-extension test, finger-nose test, and pronator drift test), absolute frequencies were determined by counting such errors, with no statistical method being applied for further evaluation. We did not automatically include instances of dropped test objects, but focused on clear events of movement arrest, limb confusion, or visible decrease in fine motor skills.

For each test a multi-level regression model was generated, with the TCT as the dependent variable and the stimulated hemisphere, hand, stimulation target, and stimulation protocol as the independent variable. An interaction term between hemisphere and hand was added to the model to test for effect modification. To account for dependencies between different test settings for one patient, random effects for patients were added. The regression models were run with the statistical software R (version 3.1.0; https://cran.r-project.org; The R Foundation for Statistical Computing, Vienna, Austria). The corresponding results are given within a 95%-CI.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

S.S., S.I., A.S. and N.S. generated the data. S.S., L.A. and N.S. performed statistical analyses and generated the tables and figures. N.S., B.M. and S.K. were responsible for the study design and conception. All authors reviewed and aided in the writing of the manuscript and approved the final version.

Competing interests

B.M. and S.K. are consultants for Brainlab AG (Munich, Germany). S.K. is consultant for Nexstim Plc (Helsinki, Finland), N.S. received honoraria from Nexstim Plc (Helsinki, Finland). The study was financed by institutional grants from the Department of Neurosurgery and the Department of Diagnostic and Interventional Neuroradiology.

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Navigated transcranial magnetic stimulation of the supplementary motor cortex disrupts fine motor skills in healthy adults

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5. Discussion

In the present thesis, we hypothesized that specific nTMS protocols can disrupt physiological SMA activity through the induction of a virtual lesion, and that this disruption can be objectified through tests of fine motor skills, thereby opening an avenue for an nTMS-based SMA mapping similar to mappings of other cognitive functions. To test this hypothesis, we have conducted a prospective study in 20 healthy, right-handed volunteers who performed specific established fine motor tests both without and during nTMS of the SMA with varying stimulation parameters. In the analysis of our data, we have found significant performance differences in a number of tests. Further, we have observed that in multiple cases, VCPs of varying forms could be elicited through stimulation. The following section will discuss these findings in detail and contextualize them in the framework of related literature.

5.1. Performance Disruption in JHFT and NHPT

We observed reduced fine motor skills during stimulation in multiple subtests of the JHFT and the NHPT, indicated by significantly heightened TCTs during stimulation. This was most notably the case for the subtests SPT, LSO, SF and SC, as well as for the NHPT. This finding confirms our initial hypothesis, in which we posited that the induction of a virtual lesion could lead to a phenotype of reduced fine motor control.

Heightened TCTs during stimulation fit well into the framework of the SMA literature: As detailed in the introductory section, some of the primary functions of the SMA appear to be related to fine motor control, such as e. g. coordination of movement chains or control of grip strength. In a review published in 2001, Tanji summarizes convergent evidence from a number of different studies in humans and animal models which point towards the SMA's relevance specifically for the concatenation of multiple consecutive movements (Tanji, 2001). On a related, but different topic, White et al. observed that TMS of the SMA can interfere with the targeted application of grip force (White et al., 2013). Given these findings, we interpret the observed prolongation of TCTs under stimulation as the genuine results of heightened unphysiological neural activity interfering with the coordination of movement, resulting in suboptimal motor sequences and thereby negatively impacting TCTs (Schramm et al., 2019).

In order to more closely examine the contribution of individual variables such as stimulation target, hand and stimulated hemisphere to TCTs, we constructed regression models for each test. In the SMA literature, evidence exists that points towards a somatotopic organization of the SMA. Specifically, evidence from monkeys and from neurosurgical procedures in humans indicates that orofacial structures are represented more anteriorly, the upper limb in the middle

and the lower limb in the posterior parts of the SMA (Fontaine et al., 2002; Mitz & Wise, 1987). While we did not include tests directed at orofacial or lower limb coordination, we nonetheless tested for differences between the targets. Regarding individual stimulation targets, no clear pattern emerged in our models. Only stimulation target two in the NHPT passed our threshold for significance, implicating that this target caused stronger impairment than target one. In our grouped analyses based on target groups (anterior/middle/posterior and medial/lateral), we observed that in six comparisons, lateral stimulation led to significantly slower TCTs than medial stimulation. Here it should be noted that this difference followed no clear pattern as far as hands, hemisphere, test and protocol is concerned. It should therefore be considered with caution in our opinion, especially since the established somatotopic models mostly refer to anterior-posterior topology. One speculative interpretation could relate to the fact that lateral stimulation might be more suited to costimulate mesial parts of the SMA (Schramm et al., 2019).

Our regression models did not demonstrate any meaningful difference between the three stimulation protocols. This might on first view be considered surprising, since two of the protocol would in the classical sense be categorized as high-frequency and thus “excitatory” protocols (5 Hz and 10 Hz), while cTBS would be considered a “inhibitory” protocol (Thut & Pascual-Leone, 2010). Current literature however often takes a more differentiated stance on the expectable outcomes of different rTMS protocols, since studies have shown variable results following the application neuromodulatory protocols (Goldsworthy et al., 2021). Additionally, the relation between neuromodulation and the virtual lesion is unclear, since the virtual lesion is a momentary and not prolonged phenomenon unlike e. g. the suppressive effect cTBS has demonstrated on MEPs (Suppa et al., 2016). Since the current literature on the virtual lesion remains inconsistent (Silvanto & Cattaneo, 2017), framing our finding of relative equivalence in virtual lesion induction between the three protocols could only be speculative. More research into the virtual lesion model would be required in order to generate optimal hypotheses with which to conduct optimizations for high rates of function disruption (Schramm et al., 2019).

Despite not being among the statistically validated results, the pattern of significant TCT dissociations might also be of interest for future investigations. While our study was not specifically tailored to a respective analysis (since the regression models were conducted within and not across each test) inspection of Schramm et al., 2019, Table 1 reveals that stimulation of the right hemispheric SMA appears to have led to a notably higher rate of significant TCT heightening compared to stimulation of the left hemisphere. This appears to be the case for both left- and right-handed performances. Within the current literature, differences between left- and right sided SMA have often been investigated in the context of handedness. One 2014 study by Pool et al. described that functional connectivity measured

by fMRI differed between the SMA of right-handed subjects and left-handed subjects, with right-handed subjects possessing a higher effective connectivity between the “motor dominant” (left) SMA and other motor areas than left-handers (“motor dominant” hemisphere defined as right) during movement of the dominant hand (Pool et al., 2014). Another fMRI study, conducted by Solodkin et al., demonstrated that complex movements caused higher and farther distributed BOLD signal in left-handed compared to right-handed individuals in motor areas including the SMA (Solodkin et al., 2001).

While both studies provide interesting results and serve as a precedent for functional SMA-asymmetry, only right-handed individuals took part in our experiments. Therefore, a perhaps more adequate parallel to our situation can be drawn to a study conducted by Dinomais et al., who in a seed-based rs-fMRI study demonstrated that the right SMA-proper was functionally connected to more brain areas than the left SMA-proper in healthy right-handed volunteers (Dinomais et al., 2016). Additionally, a resection study in the admittedly more distant model of mice has shown that the animals performed skilled movements worse after right-sided hemispherectomy compared to left-sided hemispherectomy (Paes-Branco et al., 2012).

Although these findings fit well with our data, namely by implying a functional dominance to right sided motor areas, it should be noted that other studies such as Yan et. al similarly employed resting state fMRI functional connectivity analyses and found a stronger connectivity of the left SMA compared to the right SMA (albeit only in relation to the corresponding contralateral middle frontal gyrus) (Yan et al., 2012). Additionally, task-based fMRI results of Rogers et al. emphasized the dominance of the left-sided SMA in coordinating unilateral movements of both hands, while the right-sided SMA appeared to contribute exclusively to left-handed movements (Rogers et al., 2004). Furthermore, in the neurosurgical context, the function of a resected SMA appears to be compensated by the respective healthy hemisphere independent of which side is damaged (Krainik et al., 2004).

Taken together, the literature on SMA asymmetry currently appears to be too inconsistent to properly frame our observations regarding hemispheric laterality. Thus, we can at best serve as a motivating factor for future attempts at investigating hemispheric differences between the SMA.

Despite these open questions, our main hypothesis remains confirmed: nTMS can be used to momentarily degrade fine motor skills. While this confirmation is an important prerequisite for an SMA mapping in the manner we proposed, it yields no intuitive heuristic for the classification of given stimulated points into categories of “SMA-positive” and “SMA-negative”. For this, a cutoff-value would be required, which at this point would have to be determined arbitrarily, e. g. on the basis of standard deviations from the individual norm. Since the collection of representative normal data or each subtest would however be a logistical challenge in the

clinical setting (e. g. regarding time constraints or practice effects), other phenomena such as VCPs (see later section) might hold more promise, analogous to e. g. language mappings (Schramm et al., 2019).

5.2. Performance Disruption in FTT

In the FTT, we observed a significant difference in SDITs between the baseline and nTMS conditions, with SDITs being significantly higher during stimulation in five out of six comparisons and approaching statistical significance in the sixth (Schramm et al., 2019, Table 4). Analogous to our findings for TCTs, this confirms our initial hypothesis of SMA disruption via nTMS.

The SMA's role for internal time monitoring has been studied with a wide range of modalities, including e.g. EEG and fMRI, and has been put forth as a form of neuronal temporal accumulator (Casini & Vidal, 2011). Studies conducted within animals that possess analogous areas to the human SMA (such as e. g. macaques) create convergent evidence regarding the accumulator hypothesis (Nickl, 2017). In our FTT, the subjects were tasked to consistently tap keyboard keys in a 1 Hz rhythm generated by a nearby metronome. Our results indicate that during stimulation, the subjects were less able to keep their rhythm regular, and instead produced a mix of shorter and longer intervals compared to the baseline condition. In light of the previously mentioned evidence, this could plausibly be explained as a failure of the SMA accumulator to keep internal timing consistent with outside timing.

Our observation could however also be seen in a different light, namely under the aspect of bimanual coordination. If internal time estimation was not disturbed by our stimulation, the heightened SDITs might be instead explained by a failure to properly execute the necessary movement, similar to our explanation of heightened TCTs. This option becomes even more plausible when considering that our FTT task specifically necessitated the proper direction of bilimbic movement, which the SMA in turn has a prominent role in (Serrien et al., 2002).

While the distinction between movement execution problem and time keeping problem can not be made on the basis of our data, the resulting hypotheses could nonetheless motivate future studies. One could for example imagine an experiment in which internal timekeeping is measured in a way other than by movement output, e. g. by presenting a rhythm to a subject, stopping it for a specific interval and then asking the participant how many ticks should have passed in the paused interval. This task could then be performed with and without SMA stimulation, similar to our setup. The results could help to further elucidate the role of the SMA in internal timekeeping.

5.3. Performance Facilitation in JHFT

We observed multiple cases of seeming performance boosts under stimulation, most notably for the subtest of writing in the JHFT. While the raw TCT data seems impressive in this regard (Schramm et al., 2019, Table 1-2), this result should be regarded with caution.

Over the course of the experiment, the participants performed the writing subtest 38 times per appointment. With such a high number of repetitions, the consideration of practice effects becomes highly important.

Systematic investigations into practice effects for the JHFT are not widely found in the literature. One study by Stern et al. investigated the stability of JHFT results across three test sessions in a cohort of 20 right-handed women (Stern, 1992). In this publication, the authors report that the writing subtest went from a mean time of 10.03 s in the first run to 9.06 s in the third run. In the other subtests, a practice effect was similarly observable (Stern, 1992). Since in our experiments, the baseline TCTs were always taken before the actual mapping in order to exclude the potential of confounding neuromodulatory effects, it is not surprising that the subsequent performances are heavily skewed towards lower TCTs (Schramm et al., 2019).

From this perspective, the priorly discussed performance disruptions in the other subtests become in fact more impressive since they occurred despite the natural tendency towards performance optimization. One possible contributing factor in this regard could be speculated to be the SMA's apparent import in the learning of movement programs (Nachev et al., 2008; Nakamura et al., 1998, 1999). Nakamura et al. demonstrated that the injection of muscimol into and thereby inactivation of the pre-SMA in monkeys caused an increase of errors for new movement sequences, but not already learned sequences, emphasizing the role of the pre-SMA in the acquisition of motor programs (Nakamura et al., 1999). A virtual lesion in this area could plausibly lead to a similar effect, since it would disrupt regulated neuronal activity (Schramm et al., 2019).

5.4. Lack of Disruption in FNT, PDT and FET

We did not observe conclusive signs of SMA disruption in the FNT, PDT and FET. In the context of the relevant literature, this is not a very surprising finding for a number of reasons.

The PDT for one is a static test, which does not require a high amount of fine motor control. Prior studies have demonstrated that the SMA is less involved in the generation of simple

compared to complex movements (Gerloff et al., 1997; Solodkin et al., 2001). Thus, the PDT was likely the task the least suited to test SMA activity out of our test battery.

While the FET, similar to FTT, required consistent timekeeping according to external pacing, it relied both on larger, more proximal muscle groups (especially biceps and triceps brachii), which again might not underly the same fine motor control as more distal hand musculature. Additionally, in contrast to our procedure for the FTT, we did not exactly time each movement and instead only screened for unambiguous visually identifiable correlates of SMA dysfunction. This in turn prohibits a more detailed statistical workup of our FET data, and thus might mask an actual timing-related effect.

Out of these three tests, the lack of movement problems in the FNT are potentially the most interesting. Variants of the FNT are staples of neurological examination (Fugl-Meyer et al., 1975). One of its main applications is in the assessment of cerebellar damage, which is often apparent as dysmetria (on the basis of ataxia) in the FNT (Krishna et al., 2019). The validity of FNT usage for upper limb coordination assessment has recently been confirmed in a detailed study measuring the kinematics involved in the test, at least in patients suffering from stroke (Rodrigues et al., 2017). Despite being sensitive to cerebellar function, based on this literature one would assume that the test should involve the SMA to the degree necessary to make it vulnerable to nTMS-based disruptions.

Clinical reports on patients with SMA damage after stroke however indicate that even when strong clinical signs of SMA dysfunction such as an AHS are present, the FNT may not show signs of ataxia (Kim, 2001). This calls into question the sensitivity of the FNT as related to SMA activity.

As the literature appears inconclusive on this issue, our findings can again only inform future investigations. One could for example imagine that a more detailed investigation into the FNT kinematics under stimulation, such as performed by Rodrigues et al. might be helpful to unmask potential non-obvious FNT disruptions during stimulation (Rodrigues et al., 2017). Other modalities such as fMRI could potentially be applied as well, if limitations such as movement artifacts can be adequately controlled.

5.5. Interpretation of VCPs

The most notable, and potentially most helpful finding in developing an applicable mapping procedure was the identification of VCPs. These were visually identifiable momentary motor deficits which had a detrimental effect on task performance. VCPs were observed in almost every test we conducted and manifested in a number of different ways. Notably, multiple participants showed the same type of VCP in the same test (Schramm et al., 2019).

The best example for this is found in the tests LLO and LHO, which involved the controlled grasp, lifting and placement of metal cans in specific positions. In multiple cases, the subjects lifted the last can in the row using not the hand that was demanded for the task, but the contralateral limb (e. g. the participants lifted 4 cans with the right hand and switched to the left hand at the fifth can). The mistake appeared to arise unconsciously, and the subjects often expressed annoyance over their mistake. This can be related to existing literature in a number of ways (Schramm et al., 2019).

Firstly, it is interesting that only the last can in the row was affected. There are multiple explanations for this. One aspect is the organization of movement chains in the SMA. Prior research demonstrated that SMA disruption interferes with movement elements downstream in a chain of motor sequences, with a latency of about 1.8 s from stimulation onset (Gerloff et al., 1997). Thus, the last can could simply be the first possible candidate for disruption due to the time required for the effects of stimulation to appear.

While this can not be ruled out, LHO and LLO required about 3 s for their performance. Thus, if we were to fully apply Gerloff's findings to our admittedly different setup, it is unlikely to only affect the last can in the row, since at 2 s, the fourth can would also be a viable target.

A different interpretation could be found in considering the SMA's role in executive control, relating back to the AHS. As previously detailed, the AHS can occur after damage to the SMA and results in the loss of conscious control over the specific limb, which instead performs involuntary actions (Assal et al., 2007; Scepkowski & Cronin-Golomb, 2003). The SMA has been implicated in playing a critical role for the inhibition of undesired movement programs (Filevich et al., 2012). In extreme cases, such as with bilateral SMA damage, utilization behavior can arise, which is characterized by the compulsion to use specific objects when they are presented (e.g. compulsive scribbling when presented with a pen) (Boccardi et al., 2002; Iaccarino et al., 2014).

If we assume that the SMA is in fact important for the suppression of unwanted motor programs, then this could plausibly explain the VCPs observed in LLO and LHO: The last can is by definition the one closest to the non-executing hand. Thus, the external stimulus of "can close to hand" would naturally result in the participant developing the intuitive program of "use closest hand for task". In a physiological setting, the SMA would suppress this program, and instead prepare the execution of the sequence with the desired hand. When subject to a virtual lesion however, this function can not be adequately fulfilled, resulting in the execution of the more "natural" motor program.

Another form of VCP was observed in participants during the NHPT. Here, participants either struggled to select an adequate motor program (as seen in the supplementary material of

Schramm et al., 2019), or instead stopped the task midway-through (e. g. when all pegs were placed in the NHPT, the participant did not begin to return them). Instead of relating to impulsive behavior or the AHS, these VCPs could potentially relate back to cognitive control aspects of the SMA, where the subject is unable to adapt to the changed motor requirements for the second part of the task (Nachev et al., 2005; Schramm et al., 2019; Stuphorn et al., 2000; Wager et al., 2004).

Irrespective of the exact neuropsychological mechanism, we interpret the observed VCPs as analogous to language errors elicited in nTMS language mappings. This opens up not only more avenues into investigations of varying aspects of SMA function, but also yields an intuitive and easy to apply heuristic for the analysis of mapping results. If stimulation of a given point leads to the occurrence of VCP, then, given a normal baseline performance, the specific point could be classified as “SMA-positive”, and could then be used in advanced surgical planning.

5.6. Implications for Future Research

Our findings serve as a proof of principle regarding the potential of an nTMS based mapping of the SMA. Naturally, more research is needed to determine key aspects such replicability, accuracy as compared to DCS, and reduction to the optimal parameters. In the long term, the goal would be to find a standardized, easily applicable, both sensitive and specific combination of tests and stimulation parameters. If this could be achieved, it would be plausible that this mapping could add useful information to the perioperative diagnostics of SMA resections, and that it could thereby ameliorate the eventual outcome in terms of e. g. postoperative deficits or other surgery outcome measures, similar to what has been demonstrated for motor mappings (Krieg et al., 2014). Future research in this direction should also aim to include actual patients suffering from SMA lesions, since our current experiments were conducted in healthy subjects only.

Aside from neurosurgical applications, nTMS based disruption of SMA function could also potentially be interesting to investigate the functions of the SMA itself. As previously described, the SMA has a variety of functional domains attributed to it, some of which are more closely linked to the pre-SMA, while others are referenced more in the context of the SMA proper. nTMS could aid in endeavors to precisely characterize SMA function in a key way: It allows the accurate and replicable stimulation of individually selected targets based on high-resolution MRI. Thus, one could imagine that a specific site in the anatomical area of pre-SMA is stimulated repeatedly while an arbitrary number of tests related to the functions of interest are conducted. In this way, one could narrow down which exact cognitive functions are disrupted

by stimulation, and in this way get a better understanding of the exact cognitive import of each SMA subsection.

Additionally, the mapping of SMA function to cortical anatomy is not only interesting in resection planning, but might also be of interest for researchers attempting therapeutic neuromodulation of the SMA. Prior studies have investigated the potential of SMA neuromodulation in e. g. Parkinson's disease or psychiatric diseases such as gambling disorder (Eggers et al., 2015; Marras et al., 2019). Herein, a prior mapping of the SMA could for example help in identifying optimal stimulation targets.

5.7. Limitations

Naturally, there are a number of limitations which need to be discussed.

First, our study was limited to a sample of 20 participants, who moreover were exclusively right-handed. This carries with itself obvious caveats in terms of potential limits to the generalizability of our results. The in part highly significant results in our statistical analyses in conjunction with the functional plausibility of our observations however leave us confident that we were able to demonstrate a real effect that should extend beyond the limits of our cohort. Investigations including left-handed individuals might contribute to the literature investigating neural correlates of handedness (Schramm et al., 2019; Solodkin et al., 2001).

Second, we were unable to demonstrate superiority of a given stimulation protocol over the others. Here, we might indeed have profited from a higher sample size, in which less obvious differences in performance between the three protocols could potentially have been separated more clearly. At this time, the virtual lesion is unfortunately not understood in the necessary detail to make justified assumptions about which protocol could emerge as the most suited. However, for the immediate use case of SMA mappings, it should be noted that all protocols were at the least able to disrupt SMA function to a degree where VCPs were observable, which is at the very least a starting point for future protocol optimization (Schramm et al., 2019).

Third, our cohort consisted exclusively of healthy participants. We can at this time make no statements as to the applicability of SMA mappings in actual patients. This however will be the next step of our investigations (Schramm et al., 2019).

Fourth, our experiment did not include a control condition outside the baseline (e. g. sham stimulation), which is a important weakness. It could be argued that nonspecific factors such as the sensation of stimulation could also affect test performances. Again however, we believe that the strong parallels between clinical symptoms of SMA dysfunction such as the AHS and

the observed VCPs speak against our results occurring only due to unspecific effects (Schramm et al., 2019).

6. Conclusion

In the present thesis, we have demonstrated that nTMS over the SMA can detrimentally influence healthy subject's ability to perform clinically established fine motor tasks. The resulting motor deficits ranged from heightened TCTs and higher SDITs to VCPs similar to clinical phenomena of SMA deficits such as e. g. the AHS. We argue that this phenomenon can be exploited to ascribe SMA function to specific cortical sites, similar to the principle used in nTMS mappings of other cognitive functions. Thus, we present an avenue towards a clinically applicable nTMS-based SMA mapping for the use in neurosurgical diagnostics. Future research could use the present findings to optimize the presented protocol for the application in patient populations.

7. Summary

The SMA is a premotor brain area involved in the coordination of complex movements. Damage to the SMA, such as e. g. in the context of surgery, can lead to a number of clinical phenomena, such as hemiparesis, mutism or the alien hand syndrome. nTMS is a method for the noninvasive stimulation of brain tissue. nTMS has a history of usage in the mapping of function to cortical anatomy, such as with motor cortex mappings or mappings of language function. This has been used in neurosurgery to identify and protect eloquent areas, thereby minimizing patient risk. We hypothesize that nTMS could be applied in an analogous manner in the mapping of SMA function onto the cortical anatomy. A cohort of 20 right-handed, healthy volunteers was subjected to a battery of clinically established fine-motor tests before and during stimulation of six individual targets per hemisphere located within the anatomical SMA. The task performances under stimulation were then analyzed and compared to baseline performances. We observed statistically significant worsening of test performances during stimulation compared to baseline in multiple of the tests conducted. Additionally, we recorded the occurrence of characteristic VCPs, which showed similarity to clinical signs of SMA damage. nTMS of the SMA can detrimentally affect fine motor skills in healthy, right-handed subjects. Analogous to other mapping procedures, this could allow for inferences in regard to local cortical function. This phenomenon could be refined into a clinically applicable SMA mapping protocol for the use in perioperative diagnostics in e. g. brain tumor surgery.

Das SMA ist ein prämotorisches Hirnareal, welches in der Koordination komplexer Bewegungen involviert ist. Schäden am SMA, z.B. im Kontext einer Operation, können zu einer Vielzahl klinischer Phänomene führen, wie z. B. Hemiparese, Mutismus oder dem Alien Hand Syndrom. nTMS ist eine Methode zur noninvasiven Stimulation von Hirngewebe. nTMS wird bereits seit Längerem zur Zuweisung bestimmter Funktionen zu kortikaler Anatomie verwendet, z. B. im Rahmen von Motorkortexkartierungen oder Kartierungen der Sprachfunktion. Dies wird in der Neurochirurgie genutzt, um eloquente Areale zu identifizieren und zu schützen, wodurch Risiken für den Patienten minimiert werden. Wir stellen die Hypothese auf, dass man nTMS auf eine analoge Weise anwenden kann, um funktionelles SMA auf dem Kortex abzubilden. Eine Kohorte von 20 rechtshändigen, gesunden Freiwilligen wurde vor und während der Stimulation sechs einzelner Ziele pro Hemisphäre einer Batterie aus klinisch etablierten Feinmotoriktestungen unterzogen. Die Ausführungen während der Stimulation wurden anschließend analysiert und mit der Ruhekondition verglichen. Wir konnten eine statistisch signifikante Verschlechterung der Testdurchführung während Stimulation verglichen mit der Ruhekondition beobachten. Zudem dokumentierten wir das Auftreten charakteristischer VCPs, welche sich ähnlich klinischer Zeichen von SMA Schäden

*äußerten. nTMS des SMA kann die Feinmotorik gesunder Rechtshänder*innen negativ beeinträchtigen. Analog zu anderen Kartierungsprotokollen könnte dies eine Inferenz hinsichtlich lokaler kortikaler Funktion ermöglichen. Auf Basis dieses Phänomens könnte ein klinisch applikables SMA Kartierungsprotokoll zur perioperativen Diagnostik z.B. in der Hirntumorchirurgie entwickelt werden.*

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